Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity

A Thesis

Submitted towards the Requirements for the Award of Degree of

Doctor of Philosophy

In

Pharmacy

Under the Faculty of Pharmacy

By

MOHIT SAINI

(Enrollment no. 161522516252)

Under the Supervision of

Dr. JITENDER KUMAR MALIK

Professor

P.K. UNIVERSITY

2024

P.K. UNIVERSITY

NH- 27, Vill. Thanra (P.O. Dinara)

Shivpuri, M.P. India - 473665

www.pkuniversity.edu.in

P.K.UNIVERSITY
SHIVPURI (M.P.)

University Established Under section 2F of UGC ACT 1956 Vide MP Government Act No 17 of 2015

CERTIFICATE OF THE SUPERVISOR

This is to certify that the work entitled **"Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity"** is a piece of research work done by **Mr. Mohit Saini** under my Guidance and Supervision for the degree of Doctor of Philosophy of **Faculty of Pharmacy, P.K. University (M.P) India.**

I certify that the candidate has put an attendance of more than 240 day with me. To the best of my knowledge and belief the thesis:

I – Embodies the work of the candidate himself/herself.

II – Has duly been completed.

III – Fulfill the requirement of the ordinance relating to the Ph.D. degree of the University.

18/11/24

Date: / /

Signature of the Supervisor

.....

DECLARATION BY THE CANDIDATE

I declare that the thesis entitled "**Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity**" is my own work conducted under the supervision of **Prof. (Dr.) Jitender Kumar Malik** at **Faculty of Pharmacy, P.K. University, Shivpuri, M.P.** Approved by Research Degree Committee. I have put more than 240 days of attendance with supervisor at the center.

I further declare that to the best of my knowledge the thesis does not contain my part of any work has been submitted for the award of any degree either in this University or in any other University.

Without proper citation.

Signature of the candidate

Date: 18/11/2024

Place: Shivpuri (M.P.)

P.K.UNIVERSITY
SHIVPURI (M.P.)

University Established Under section 2F of UGC ACT 1956 Vide MP Government Act No 17 of 2015

FORWARDING LETTER OF HEAD OF INSTITUTION

The Ph.D. thesis entitled "**Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity**" Submitted by **Mr. Mohit Saini** is forwarded to the university in six copies. The candidate has paid the necessary fees andthere are no dues outstanding against him/her.

Name..... **Seal**..... **Date:** .....

Place: .....

(Signature of Head of Institution where the Candidate was registered for Ph.D. degree)

Principal
Faculty of Pharmacy
P.K. University, Shivpuri (M.P.)

Signature of the Supervisor Date:

Date: 18/11/24..... **Address:** .....

Place: ....

ACKNOWLEDGEMENT

The Acknowledgement of a research endeavor is an opportunity to express appreciation for the joint effort of those who have been sources of motivation and support, and who have contributed invaluable knowledge towards achieving success in the pursuit of knowledge with utmost humility and pride, I reflect on the inspiring individuals who have propelled my work forward, and I am filled with immense pleasure, gratitude and thanks for their unwavering dedication.

First for most I would like to thank almighty god for perservence, courage and strength bestowed on me to undertake this project.

For most, I extend my deepest appreciation to my esteemed Supervisor, **Prof. (Dr.) Jitendra Kumar Malik,** Dean of Faculties, P.K University Shivpuri (M.P) without whom this work would not have come to fruition. His exceptional guidance, unwavering support, kind cooperation and provision of a conductive work environmental have been invaluable beyond measure. I remain indebted to her instilling in me a relentless pursuit of excellence, a strong sense of honesty, and respect for ethical principles that govern our profession.

Furthermore, I extend my sincere thanks to the **Prof. (Dr.) Shri Jagdeesh Prasad Sharma** Chancellor, P.K University Shivpuri (M.P) for providing the necessary infrastructure and facilities that were essential during my research work. My profound sense of gratitude, faith, and awe also goes to **Prof. (Dr.) Yogesh Chandra Dubey** Vice Chancellor, P.K University Shivpuri (M.P) for his generous and valuable support throughout my research.

I take this pleasant opportunity to express my deep sence of gratitude and reverencs to his holiness **Prof. (Dr.) Jitendra Kumar Mishra** Director; P.K University Shivpuri (M.P) for his constant encouragement and providing me with the required facilities that enabled me to complete my project work

I would also like to extend my gratitude to and deep appreciation to respected **Prof (Dr). Bhaskar Nalla,** Dean Reasearch of P.K University M.P, I/c Ph.D cell of P.K University M.P, **Prof (Dr.) Deepesh Naamdev** Registrar P.K University Shivpuri (M.P) who has inspired me with his encouraging guidance and support right from the beginning of this work in spite of his busy schedule . I am short of words to thank him for his affectionate behavior and patience throughout the duration of research work.

I express my gratitude to **Prof. Dr. Aiman Fatima,** Dean Accadmics of P.K University Shivpuri M.P and **Prof Dr. G Pawan Kumar** Exam controller of P.K University Shivpuri M.P.

I owe a great deal of application and gratitude to entire faculty members department of pharmacy P.K University, **Prof.(Dr) Vivek Gupta** Head of the Deptt of Faculty of Pharmacy P.K University Shivpuri (M.P) **Mr.sunil kumar (Asso.Prof), Dr.Surendra Pratap Singh (Prof),Mr Gyan Singh (Asso.Prof), Mr.Vinay Kumar Siroliya (Asst.Prof) ,Mr.Bhavesh Gupta (Asst.Prof)** and all non-teaching staff of department of Pharmacy without their support it would have been difficult to shape my research work.

I also express my gratitude to **Miss Nisha Yadav,** Librarian of P.K University along with Mr. Anand Kumar, Assistant Librarian and other staff of P.K University. I am also thankful to Mr. Kamlesh Yadav and Mr. Alok Khare, I.T. Cell of P.K. University.

I am grateful **Dr. (Prof) Vijendra Singh**. Group Director ATMS Group of Institutions Achheja, Hapur U.P for invaluable cooperation during my project work.

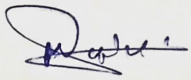
I am grateful to **Dr. Nita Mondal** Former Professor and HOD Pulla Reddy Institute of Pharmacy, Hyderabad for their kind help and cooperation during my research work.

I have no words to express my gratitude to my beloved parent's dear brother's and my lovely wife for their love, support and constant encouragement during the entire course of my research work.

I also wish to express my appreciation and heartful thanks to my entire classmate's **Amit kumar**, **Saurabh Gupta**, **Vivek singh** and **Stuti Pandey** for their kind co-Operation in the smooth running my of research work

Last but not least I would like to honestly appreciate the fact that it is my pleasure to have an opportunity to pursue my higher education at P.K University, Shivpuri (M.P). I owe my permanent debt towards it. This is having the special place in my heart for shaping my care.

Lastly, I am highly grateful to God, the almighty, for giving me courage and strength to face the most difficult times of life and for answering my prayers in a special way. I remain grateful for all that I have achieved in my life thus far.



Date: 18/11/2024

Place: Shivpuri, (M.P)

(Mohit Saini)

LIST OF ABBREVIATIONS

Abbreviations	Indication
g	Gram
mol	Molecular weight
w/v	Weight by volume
w/w	Weight by weight
mm	Millimeter
°C	Degree centigrade
M.p.	Melting point
h	Hour/s
ml	Mili litre
pH	Negative Logarithm of hydrogen ion concentration
min	Minute/s
%	Percentage
nm	Nanometer
ml	Mili litre
µg	Microgram
cm	Centimeter
mg	Milligram
w/v	Weight by volume
q.s	Quantity sufficient
rpm	Revolution per minute
mV	Millivolt
keV	Kilovolt

TEM	Triethanolamine
MP	Methylparaben
PP	Propylparaben
PVP	Polyvinylpyrrolidone
NaBH4	Sodium borohydride
AgNO3	Silver nitrate
H2SO4	Sulfhuric acid
NaOH	Sodium hydoxide
HCl	Hydro chloric acid
DMSO	Dimethyl sulphoxide
MIC	Minimum inhibitory concentration
FT-IR	Fourier Transform Infrared
SEM	Scanning electron microscope
EDS	Energy dispersive spectrometer
XRD	X-ray Diffraction
Uv	Ultra-violet
SI. No.	Serial Number
ICH	International conference onharmonization
RH	Routh-Hurwitz
SD	Standard deviation
CDR	Cumulative percentage drug release

LIST OF TABLES

.

LIST OF FIGURES

COURSE WORK CERTIFICATE

P.K.University
Shivpuri (M.P.)

Enrolment Number 1611516252

Select Course Ph.D. in Pharmacy (Course Work) Select Semester 1

Result

Enrolment Number : 161522516252
Candidate Name : MOHIT SAINI **Course :** Ph.D. in Pharmacy (Course Work)
Father's Name : MAHENDRA SINGH **Year/Sem :** 1
Mother's Name : SAVTA DEVI **Session :** 2021-22

Subject Name	Internal T	Internal T	Internal P	Internal P	External T	External T	External P	External P	
Research Methodology	N/A	N/A	N/A	N/A	50	31	N/A	N/A	31 / 50
Subject Specialization	N/A	N/A	N/A	N/A	100	69	N/A	N/A	69 / 100

Marks Obtained 100 Result Pass Max Marks 150

- Student must pass in Theory and Practical separately.
- For pass the candidate is required to obtain 40% marks in each paper and 50% marks in aggregate.
- For pass the Ph.D candidate is required to obtain 65% marks in aggregate.

P.K.UNIVERSITY
SHIVPURI (M.P.)

UniversityEstablished Under section 2F of UGC ACT 1956 Vide MP Government Act No 17 of 2015

CENTRAL LIBRARY

Ref. No. PKU/C.LIB /2024/PLAG. CERT./ 172 Date: 09.11.2024

CERTIFICATE OF PLAGIARISM REPORT

1. Name of the Research Scholar	:	Mohit Saini
2. Course of Study	:	Doctor of Philosophy (Ph.D.)
3. Title of the Thesis	:	Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti- Proliferative Activity
4. Name of the Supervisor	:	Prof. (Dr.) Jitender Kumar Malik
5. Department	:	Pharmacy
6. Subject	:	Pharmacy
7. Acceptable Maximum Limit	:	10% (As per UGC Norms)
8. Percentage of Similarity of Contents Identified	:	9%
9. Software Used	:	Drillbit
10. Date of Verification	:	10.09.2024

(Librarian, Central Library)
P.K.University, Shivpuri (M.P.)

LIBRARIAN
P.K. University
Shivpuri (M.P.)

ADD: VIL: THANRA, TEHSIL: KARERA, NH-27, DIST: SHIVPURI (M.P.) -473665
MOB: 7241115902, Email: library.pku@gmail.com

DrillBit

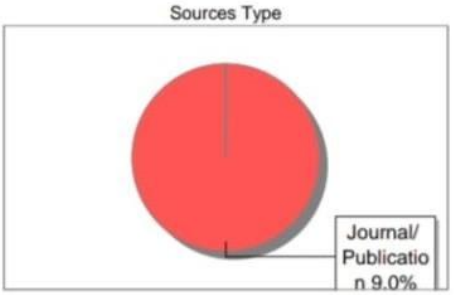
The Report is Generated by DrillBit Plagiarism Detection Software

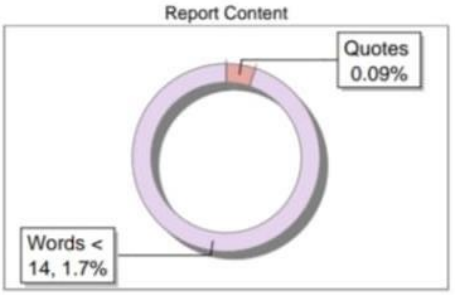
Submission Information

Author Name	Mohit Saini
Title	Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity
Paper/Submission ID	2297926
Submitted by	library.pku@gmail.com
Submission Date	2024-09-10 12:02:35
Total Pages, Total Words	123, 28193
Document type	Thesis

Result Information

Similarity **9 %**





Exclude Information

Quotes	Not Excluded
References/Bibliography	Not Excluded
Source: Excluded < 14 Words	Not Excluded
Excluded Source	**0 %**
Excluded Phrases	Not Excluded

Database Selection

Language	English
Student Papers	Yes
Journals & publishers	Yes
Internet or Web	No
Institution Repository	No

A Unique QR Code use to View/Download/Share Pdf File

P.K.UNIVERSITY
SHIVPURI (M.P.)

University Established Under section 2F of UGC ACT 1956 Vide MP Government Act No 17 of 2015

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: **"Formulation and Evaluation of an Herbal Novel Drug Delivery System for Anti-Proliferative Activity"**

Candidate's Name: **Mr. Mohit Saini**

COPYRIGHT TRANSFER

The undersigned hereby assigns to the P.K. University, Shivpuri all copyrights that exist inand for the above thesis submitted for the award of the Ph.D. degree.

Date: 18/11/2024

(Mr. Mohit Saini)

abstracts.

ABSTRACT

The disease of cancer affects people significantly on a global scale. Science and research are paying more attention to natural chemicals because it is believed that they have less dangerous side effects than conventional therapies like chemotherapy. Innovative technology that controls the release of the molecule and investigates alternate delivery methods in an effort to enhance the anticancer effects of substances derived from plants. In folk medicine, glycyrrhiza is still frequently used to treat tremors, peptic ulcers, respiratory infections, and gastritis. Improving the targeted delivery of anticancer medications is one of the objectives that is being intensively pursued in anticancer chemotherapy. Microspheres are tiny, spherical particles that typically have a diameter between 1 and 1000 m. This study's objective was to isolate glycyrrhizin from the root of G. glabra, which was followed by the formulation, improvement, and assessment of microspheres loaded with the compound. The *G. glabra* root underwent a preliminary physiochemical and phytochemical investigation. Following that, glycyrrhizin was extracted from an aqueous root extract of *G. glabra* and identified using modern analytical techniques. Glycyrrhizin undergoes preformulation studies prior to formulation, and the microspheres are produced by using gluteraldehyde as a cross-linking agent during the emulsification cross-linking process. By employing (FT-IR) drug - excipient interaction was carried out. It was possible to assess the yield, drug loading efficiency, particle size distribution, and *in-vitro* drug release studies of the formulated microspheres. Because of its high drug efficiency, formulation (F2) was chosen for *in-vitro* testing. AURKA and COX_2 interaction with the selective inhibitor was compared in this *in-silico* investigation, and the results were extremely encouraging in terms of binding affinity and molecular dynamics to the validated inhibitor. Further *in-vitro* testing to ascertain the antiproliferative efficacy was planned to be connected to the *in-silico* validation. The findings of the experiment demonstrated that this substance had a potent cytotoxic impact and effectively led these cells to colonise. Additionally, research has demonstrated that glycyrrhizin can prevent MCF-7 cancer cells from invading in a concentration-dependent way. These results are encouraging since glycyrrhizin can stop the behaviour of breast cancer, which is known to be one of the most invasive malignancies. Glycyrrhizin, in our opinion, may show to be a crucial lead molecule for anti-proliferation. The complete glycyrrhizin-microsphere combination was a multistep targeted drug delivery system that increased drug accumulation in tumours, hence increasing anti-cancer efficacy and minimising side reactions.

TABLE OF CONTENTS

CHAPTER - 1
INTRODUCTION

Herbal medicine was a prominent form of health care in the early 20th century, when modern medical treatments such as antibiotics and pain relievers were not readily available. Botanical therapy medicine is gradually losing popularity among people as a result of the development of allergy medicine systems. This type of medicine is predicated on the rapid therapy action of synthetic drugs. We have seen the limitations of the allergy medicine system in the nearly a century that has passed since its development. Herbal medicine has recently been gaining popularity, which is not surprising. In point of fact, certain herbal remedies have reached the same level of effectiveness as their synthetic counterparts. We are able to draw the conclusion based on our familiarity with Alternative and Complementary Systems. Ayurveda, botany, pharmacology and phytochemistry, biochemistry, ethnography, and pharmacology and toxicology are all essential components of the field of phytotherapy. The utilization of herbal medicine has witnessed a significant surge in recent years. Numerous studies and meta-analyses have consistently indicated a rising trend of individuals seeking therapeutic guidance from herbalists. This positive development aligns with the World Health Organization's recognition of the value of herbal medicine. A study conducted in the United States revealed that between 60 and 70 percent of rural patients rely on herbal remedies to manage their chronic health conditions.

It is generally accepted that the pharmacological activities of medicinal plants are the result of trial and error, but in order for a new drug to be developed that satisfies the criteria of modern treatment, the pharmacological activities of medicinal plants need to be thoroughly researched. The goals of research in this field include, in particular:

- Determining the active components of medicinal plants and examining their extracts to ascertain whether or not they are risk-free, efficient, and maintain their level of activity over time.
- Determine the structures of the active ingredients so that they can be synthesized, modified, and extracted with greater efficiency. Isolate the active ingredients (Mukharjee PK,2002)

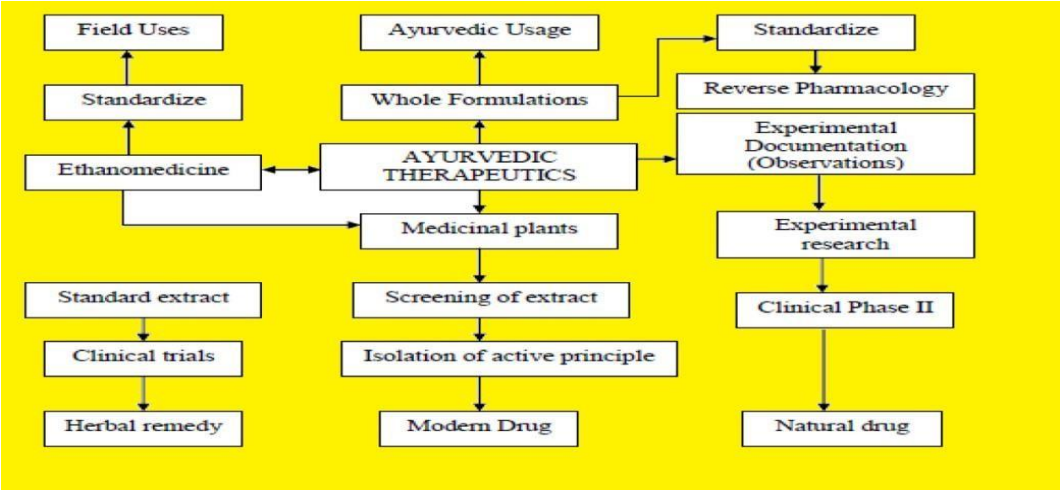


Fig. 1 Research approach to Herbal Products (Soni H *etal*;2012)

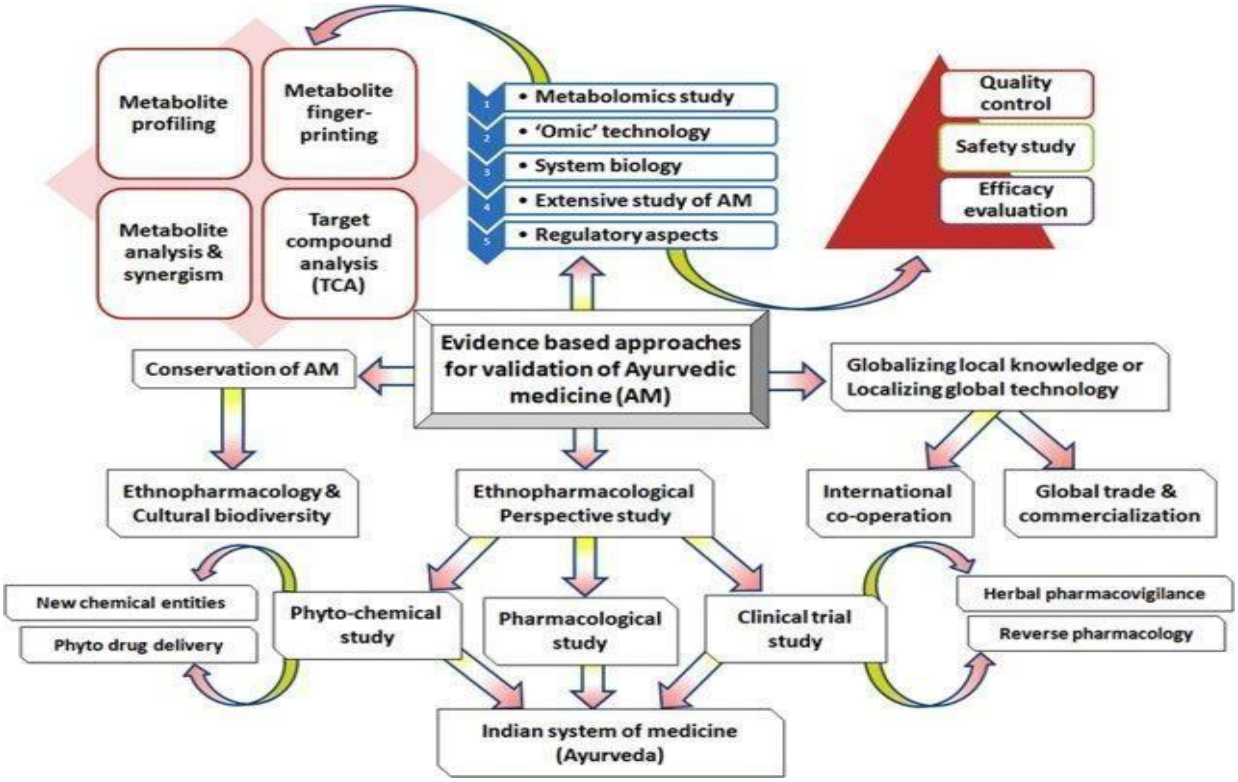


Fig. 2 Recent Approaches of Herbal Drug Research

1.1 Predominance of Cancer

Cancer is a disease that frequently cannot be treated and is regarded as an intolerable and painful condition for which there is no cure. This perspective on cancer is widespread, but it is oversimplified and overbroad. Cancer is a sickness that offers a considerable risk to its patients and is also a life-threatening condition. This is something that cannot be denied. Specifically, it is the top cause of death for individuals in the United States who are younger than 85 years old, and it is the second highest cause of death for individuals in the United States who are older than 85 years old. It is estimated that more than 570,000 individuals will lose their lives to cancer in the United States, while 1.5 million new instances of the illness will be identified inside the country. It is important to note that this does not take into consideration basal and scaly skin cancers, which have not been recorded but have the potential to generate an extra 2 million cases annually (ACS, 2010). It is a fallacy, on the other hand, to assume that there is no therapy available for cancer and that the disease would ultimately lead to death.

The reality is that there are many different kinds of cancer, and the good news is that many of these cancers can be effectively treated in the present day to eliminate, lessen, or postpone the effects of the disease on the life of the patient. Even though receiving a cancer diagnosis incapacitates the patient and takes control of their life away from them, there is reason for hope rather than despair in many situations in today's world. Damage to cancer cells' DNA, which is the intracellular genetic material that determines cell characteristics and function, is the root cause of the abnormal growth and division that is characteristic of cancer cells. There are a number of different mechanisms that can cause a cell's DNA to become flawed or damaged. For example, environmental variables such as cigarette smoke can set off a chain reaction that eventually results in cancer-causing DNA alterations in a person’s cells. This can result in cancer. It is also possible that faulty DNA will be inherited from one's parents. When cancer cells divide and multiply, the result is frequently the formation of a mass of cancer cells that is known as a tumour. Tumors are responsible for many of the symptoms of cancer because they compress, crush, and destroy the noncancerous cells and tissues that are surrounding them. There are two distinct types of tumours:

Benign and malignant

Benign tumours are those that do not have the potential to become cancerous and do not grow or spread as quickly as cancerous tumours. In most cases, benign tumours do not pose a threat to the patient's life. In contrast, malignant tumors are characterized by their uncontrolled growth and ability to spread to distant parts of the body. This process, known as metastasis, involves the migration of cancer cells from the primary tumor site to other organs or tissues.

Metastasis (Mental Help.net.).

In general, cancer causes disruptions in the normal relations between cells and leads to the malfunction of essential genes. This disruption has an effect on the cell cycle, which ultimately results in abnormal cell proliferation. Under normal circumstances, the proto-oncogene is responsible for the division and growth of cells. However, it transforms into an oncogene when a gene tic mutation occurs, which is the mutation that poses the greatest threat to the existence of cells. In addition, the absence of genes or proteins that suppress tumours causes the division of cells without control. In a normal situation, repair genes are normally translated into proteins and enzymes that possess repairing properties, and there have been detected to be more than 30 different types of repair proteins. When uracil is removed from DNA, DNA damage can be prevented, and major UV-induced DNA damage can be repaired. In essence, this is the role that the repair gene plays in the process of repairing DNA (Hassanpour SH etal; 2017).

1.2 Overview of Cancer

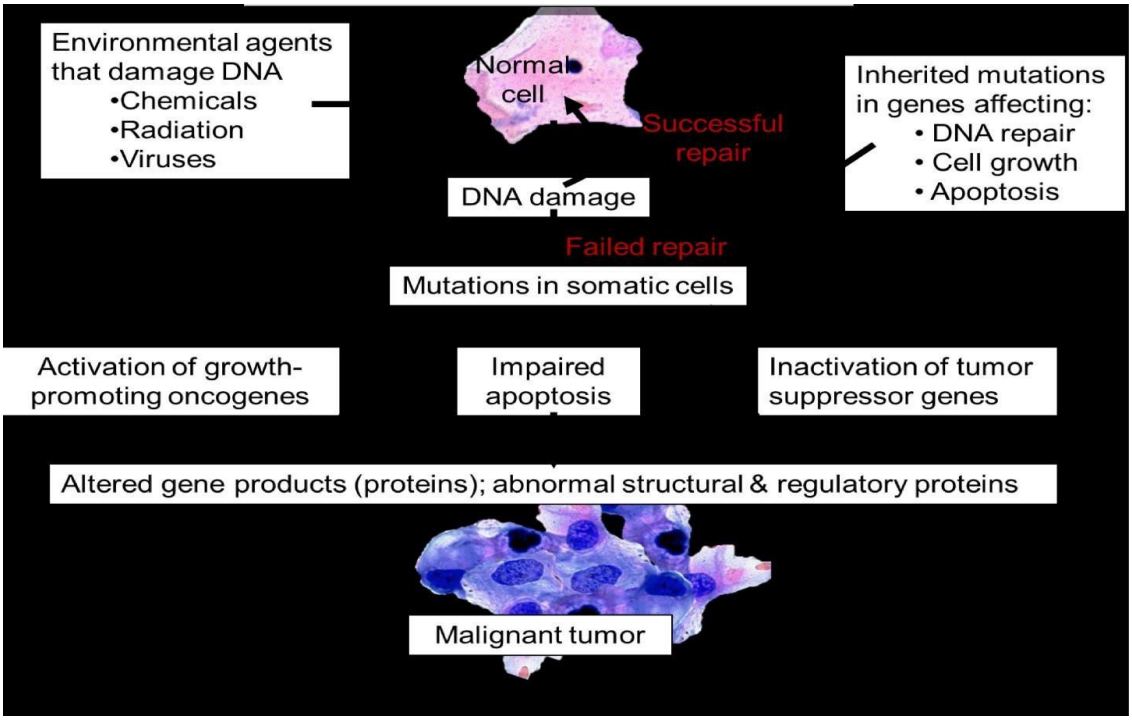


Fig. 3 Overview of Cancer

1.3 Mechanism of Cancer (Kamal N etal;2022)

Cancer is a complicated genetic illness that results from the interaction of genetic predisposition and environmental variables. Cancer progresses from the cellular to the organismal level by somatic DNA changes, which are followed by exposure to carcinogens. These mutations can cause gene translocation and amplification, resulting in abnormal gene expression patterns known as oncogenes. Because these mutations occur often during cell reproduction, the genetic harm they produce is frequently permanent.

Genotoxic ants are compounds that cause mutations or DNA damage. They can act directly or indirectly. Direct-acting genotoxins include ethylene imine and its chloromethyl ether. Indirect-acting genotoxins, such as the hepatitis B virus and aflatoxin, can cause hepatocellular cancer. Lifestyle variables such as alcohol and tobacco use are also linked to an increased risk of mouth cancer. To cause cancer, indirect-acting genotoxins must first be activated metabolically. Polycyclic aromatic hydrocarbons and aromatic amines are two types of carcinogens associated to lung and bladder cancer.

Dietary and lifestyle variables play an important influence in cancer development. Various dietary components can cause cancer or genetic abnormalities. Pesticides in agricultural goods may constitute a health concern. Even natural items, such as herbal teas, may include naturally occurring carcinogens, such as pyrrolizidine alkaloids. Hydrazine in edible mushrooms, as well as safrole and alkenylbenzene in spices and flavorings, are carcinogenic. Mycotoxins, such as aflatoxin, which can be present in moldy food, are also carcinogenic and immunosuppressive.

Oxidative stress, generated by the production of free radicals, can damage DNA and contribute to tumor development. Unrepaired DNA damage can cause base mutations, DNA crosslinking, strand breakage, and chromosomal abnormalities. Our diet's phytochemicals, with their antioxidant characteristics, have the ability to control cancer genesis and tumor growth by scavenging free radicals and altering cell signaling, oxidative stress response, and inflammation. Flavonoids, carotenoids, phenolic acids, and organosulfurs have all been proven in studies to block certain carcinogenic pathways.

Fig. 4 Plant: As an anticancer agent(Allium sativum)

The plant species that is often referred to as allium sativum is a member of the family that is known as the Alliaceae. It is native to the region that extends from the Mediterranean to China. There are a number of different substances that may be found in it, including ajoene, allicin, alliin, allixin, -glutamyl-S-2-propenylcysteine, diallyl disulfide, methyl allyl disulfide, S-allyl-cysteine, and 1,2-vinyldiithin. Antitumor activity was discovered in mice that carried the L5178Y lymphoma strain. Allicin was reported to have this effect. The anticancer efficacy of the methanolic extract of A. sativum (MEAS) has been demonstrated against MCF7, A549, and DU145 cell carcinomas, in addition to cell carcinoma of the bladder (Thomson M etal; 2003).

Fig. 5 Annona muricata

Graviola (*Annona muricata*) is a tropical fruit tree found in Central and South America, Africa, and Asia. It is distinguished by its unusual chemical makeup, which includes acetogenins, sterols, phenols, alkaloids, and other antioxidants. *Acetogenins, graviola's* principal bioactive components, have been intensively researched for their possible anti-cancer capabilities. These chemicals prevent cancer cells from producing adenosine triphosphate (ATP), an essential energy molecule. *Acetogenins,* which impair ATP synthesis, can efficiently target and destroy cancer cells.Acetogenins have been shown in studies to have anticancer properties against a range of cancer cell lines, including pancreatic cancer, breast cancer, colonic adenocarcinoma, liver cancer, and lymphoma (Prakash Om et al., 2013).While graviola has shown promise as an anti-cancer treatment, further study is needed to completely understand its mechanisms of action and to assess its safety and efficacy in clinical studies.

Fig. 6 Astragalus membranaceus

Astragalus membranaceus, also known as Mongolian milkvetch, is a member of the family Fabaceae and is commonly known by that name. It includes compounds such as astragloside, astraglan, calycosin, soyasapogenoside, quercein, and kaempferol, among others. The majority of the time, you can discover this plant in China. Chinese medical practitioners make use of it in the treatment of more advanced cases of liver cancer. According to the findings of one study, patients with advanced stages of liver cancer who were given this plant in combination with conventional treatment had a significantly higher chance of survival when compared to patients who had received conventional treatment alone. The liver is shielded from the damaging effects of chemotherapy by the astragalus membranaceus plant. Swainsonine, a significant component of this plant's extract, has been shown to inhibit the development of metastases (Wang J etal;1991).Astragalus membranaceus, often referred to as Mongolian milkvetch, is a member of the family Fabaceae and is widely recognized by that name.

Azadirachta indica

Azadirachta indica belongs to the Meliaceae plant family and can be found throughout India andthe Indian subcontinent. It is made up of things like nimbin, nimbolide, ascorbic acid, n- hexacosanol, amino acid, and nimbiol,nimbanene, nimbandiol, among other things. This medicine has previously been used successfully to treat skin cancer, buccal cancer, breast cancer, prostate cancer, and stomach cancer. Azadirachta indica's ethanolic extract kills prostate cancer cells by inducing programmed cell death (apoptosis). The dose-dependent method of action increases DNA fragmentation (Kumar S et al., 2006).

Fig. 7 Berberis aristata

Berberis aristata is an invasive species of the Berberidaceae family that grows in temperate and subtropical regions of Asia, Europe, and the Americas. It is more widely known as Daruhaldi, and its roots include berberine, berbamine, oxyxanthine, jatrorhizine, and epiberberine, among other active components. The methanolic extract of B. aristata showed potential anticancer activity against a human colon cancer cell line and inhibited HT29 cells in a concentration-dependent manner. This chemical inhibits carcinogenesis caused by 20-methylcholantherene in a dependent upon dosage and substantial way (Saied S et al; 2007).

Fig. 8 Camellia sinensis

Camellia sinensis, formerly referred to as green tea, is a member of the Theaceae family. It contains caffeine, theobromine, gallic acid, catechin, ampelopsin, epicatechin, and variousother polyphenolic chemicals. This plant's natural habitats are in East Asia, Southeast Asia, and the Indian Subcontinent; however, it is now grown not only in tropical but also in subtropical regions around the world. It includes polyphenolics, which have anti-cancer and anti-mutagenic properties. Some data suggests that this plant may protect against colon and stomach cancers. According to one study, green tea extract dramatically

inhibited DNA production in liver and leukaemia tumor cells. Furthermore, it fights the development of cancer by eliminating damaging free radicals from the body. (Dreosti IE;1996).

Fig. 9 *Cannabis sativa*

Cannabinaceae *i*s the family to which Cannabis sativa belongs, and South Africa is the plant's natural habitat. Cannabinoids, cannabinol, anandamide, pinene, and myrcene are all components of this compound. Based on the results of experiments conducted using animal models of cancer as well as cultured cell models, cannabinoids have been shown to have the potential to inhibit cancer. Cannabinoids offer intriguing medical applications, including antiemetics, analgesics, hunger stimulants, and treatment for debilitating disorders such multiple sclerosis, spinal Tourette's syndrome, cord injuries, glaucoma, and epilepsy. Cannabinoids possess qualities that demonstrate the actions of N-acylethanolamines. These features include anti-cancer and pro-apoptotic activity. Cannabinoids kill cancer cells through a process known as apoptosis and prevent their development in the body.

Fig. 10 Catharanthus roseus

Catharanthus roseus is a plant that is utilised in traditional medicine and is a member of the Apocynaceae family. In common parlance, it is referred to as the Madagascar

periwinkle. Actineoplastidemeric, vinblastin, vincrestine, vindesine, vindeline, tabersonine, and a few other things can be found in it. Catharanthus is a plant native to Madagascar that is now grown in several other countries, including Tanzania, Kenya, and Kisi. This plant is used as medicine for a variety of conditions, including cancer, diabetes, fever, and high blood pressure. It is packed with many different types of bioactive compounds, some of which are ajmalicine, vinblastine, vincristine, and serpentine. Both vinblastine and vincristin are frequently applied therapeutically in the fight against leukaemia and lymphoma (Don G;2003).

Fig. 11 Curcuma longa

Haldi in Hindi, Harida in Sanskrit, and turmeric in English are all common names for the same plant species, Curcuma longa. The family Zingiberaceae is the one to which it belongs. This plant is native to Southern Asia, and in addition to its other applications, it is used as a colouring agent in the cuisine of Bangladesh and India, as well as for a variety of other purposes. Curcumin, curcuminoids, essential oil, turmerone, monoterpenes, diarylpentanoids, diterpenes, sesquiterpenes, triterpenoids, sterols, alkaloids, and a variety of other compounds are found in it. This plant is used for both the prevention and treatment of cancer. The active component of this plant is called curcumin, and it is a polyphenol that is derived from the rhizome of this plant. The protective effect of curcumin is demonstrated by its ability to inhibit the growth of several genes associated with tumour development and angiogenesis. Curcumin has an anti-proliferative effect because it inhibits the expression of a wide variety of genes. These genes include activator protein 1, NF-kappa B, cycloxygenase 2, epidermal growth receptor 1, nitric oxidase synthase, and tumour necrosis factor. Curcumin also inhibits the expression of nitric oxide synthase (Pushkarev VM etal;2008).

Fig. 12 Glycine max

Glycine max is a member of the Fabaceae family and can be found naturally occurring in East Asia. It is abundant in a variety of nutrients, including selenium, zinc, vitamins, isoflavones, amino acids, phytosterols, and saponons, and is more commonly referred to as soy bean. According to the findings of one study, the presence of soybean agglutinin in rats prevented the development of tumours. Isoflavones induce cell differentiation, which results in cancer cells being transformed into normal cells. Apoptosis is induced in cancer cells by the compound genistein (Liener IE;1994).

Fig. 13 Linum usitatissimum

Linum usitatissimum is a member of the family Linaceae and contains a significant amount of lignans. The western and southern regions of Europe, as well as western Asia, are home to this short-lived perennial plant. Through bacterial fermentation in the colon, these plant lignans are transformed into mammalian lignans, which are then capable of acting as estrogens. These mammalian lignans are enterodiol and enterolactone. It would appear that mammalian lignans have anti-carcinogenic properties. Lignan metabolites have

a similar structural makeup to estrogens and have the ability to bind to oestrogen receptors, which in turn inhibits the growth of estrogen-driven breast cancer. In the human breast cell line MCF7, root extract of Linum usitatissimum induced a significant amount of inhibition of cell vitality and proliferation without performing the strong cytotoxicity (Szewczyk M etal; 2014)

1.4 Role of Phytopharmaceuticals in cancer therapy:

At this point in time, cancer poses a significant threat. Despite the presence of various treatments, cancer was responsible for the deaths of a significant number of patients each year. In the field of health care pharmaceuticals, the predominant research direction is currently toward the development of side effects and side effects without the necessity of treating anti-cancer. In this regard, the chemical entities found in factories have proven to have a lot of potential. They believe that cancer cells change without affecting normal cells, making the phytochemical biological activity the more desirable option. The process of oncogenesis is a complicated one that includes many different signalling events. Because phytochemicals have functions that go in multiple directions and can target these events in multiple ways, they are the best candidates for the development of anti-cancer drugs. The development of key candidates derived from phytochemicals that are capable of halting or delaying the progression of cancer without causing any adverse effects is currently under way.

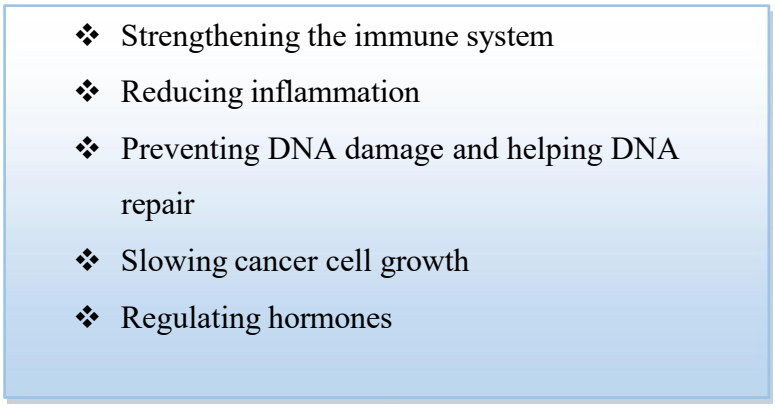


Fig. 14 Prospective benefits of Phytochemicals include:

Several phytochemicals have been shown to inhibit the growth of tumours both in vitro and in vivo. The precise mechanism by which phytochemicals exert their anticancer functions is still a topic of investigation in the field of research. They carry out a diverse array of operations on the nuclear and cytostatic factors that are present in cancer cells. In a cell that has been transfected, they are capable of either directly absorbing reactive oxygen species (ROS) or promoting the activity of antioxidant enzymes (such as superoxide dismutase, glutathione, and catalase). Either the beginning of a precancerous cell's transformation into a cancerous cell or the metabolic transformation of a carcinogen can be stopped by the molecule found in plants. In addition, they have the ability to regulate cellular events and signalling that are involved in the growth, invasion, and metastasis of cancer cells (Singh Sukhdev etal; 2016).

Phytopharmaceuticals	**Mechanism of action**
Tannins	Ellagic acid from pomegranate induces apoptosis in prostate and breast cancer cells and prevents the spread of several cancer types. Epigallocatechin gallate (EGCG) inhibits the activity of ornithine decarboxylase, an enzyme that causes cells to proliferate faster and avoid apoptosis. Luteolin prevents epithelial mesenchymal transition.
Flavonoids	Ellagic acid, contained in pomegranates, induces apoptosis in prostate and breast cancer cells and prevents the spread of a range of malignancies. The chemical epigallocatechin gallate (EGCG) inhibits the action of an enzyme called ornithine decarboxylase. This enzyme instructs the cell to increase its rate of proliferation while avoiding the death process known as apoptosis. Luteolin inhibits the epithelial-to-mesenchymal transition. Flavanones, isoflavones, and lignans are three types of chemicals that prevent the binding of oestrogen to cancer cells, hence slowing their growth. Another method is that the nuclear factor-kappa B (NF-kB) family of transcription factors inhibits inflammatory processes, resulting in reduced inflammation. Apigenin, a flavone found in parsley, celery, and chamomile, inhibits the leptin/leptin receptor pathway, causing lung cancer cells to commit death via apoptosis. It induces extrinsic caspase-dependent apoptosis in HER-2 overexpressing BT-474 breast cancer cells by inhibiting signal transducer and activator of transcription 3 (STAT3) signalling. Genistein, an isoflavone found in soybeans, has anticancer effects because it inhibits the NF-kB and Akt signalling pathways. Resveratrol inhibits carcinogenesis by a number of methods, including the overexpression of p53 and BAX proteins and the downregulation of NF-B, AP-1, hypoxia induced factor 1 (HIF-1), MMPs, Bcl-2, cytokines, cyclins, cyclin-dependent kinases (CDKs), and COX-2 proteins.

Polyphenol & Anthocyanins	Quercetin, curcumin, anthocyanins obtained from bilberries, EGCG, caffeic acid, and derivatives all function via the NF-B pathway. Curcumin, a polyphenol found in *Curcuma longa,* can suppress the growth of human glioblastoma cells by regulating a variety of molecular components. It accomplishes this by increasing the expressions of p21, p16, p53, early growth response protein 1 (Egr-1), extracellular signal regulated kinase (Erk), c-Jun-N-terminal kinase (JNK), ElK-1 (a member of the ETS oncogenic family), Bcl-2 associated X protein (Bax), and Caspase-3, 8, 9 proteins, and by decreasing the levels of In response to UVB-induced DNA damage, the main bioactive component of S. marianum, silibinin, significantly activated the DNA-PK-p53 pathway for apoptosis..
Carotenoid	Crocetin, a carotenoid found in Crocus sativus and Gardenia jasminoides, inhibits GATA binding protein 4 and the MEK-ERK1/2 pathway, protecting against cardiac hypertrophy.Crocetin, a carotenoid present in Crocus sativus and Gardenia jasminoides, inhibits GATA binding protein 4 and the MEK-ERK1/2 pathway, providing protection against coronary hypertrophy.
Glycosides	Red berries contain cyanidin glycosides, which are responsible for their antioxidant and anticancer properties. They accomplish this by blocking mitogen-induced metabolic pathways in colon cancer cells and downregulating the expression of genes encoding inducible nitric oxide synthase (iNOS) and cyclooxygenase-2.
Alkoloids	Podophyllotoxin induces ER stress, autophagy, and cell cycle arrest in non-small cell lung cancer cells, all of which promote apoptosis. Vinblastine and taxol are medicines that inhibit signalling pathways involving activator protein 1 (AP-1).
Sulforaphane	Sulforaphane, also known as 1-isothiocyanato-4-methylsulfinylbutane, is a significant byproduct of the glucoraphanin hydrolysis reaction. Sulfuraphane is an

	aliphatic hydrocarbon. Furthermore, studies have demonstrated that sulforaphane protects the central nervous system. It achieves this by activating nuclear factor (erythroid derivative)-like 2 (Nrf2), which reduces oxidative stress and inflammation in nerve cells. In a mouse model, sulforaphane reduced the generation of iNOS in macrophages activated with lipopolysaccharide. Activation of the Nrf2 pathway resulted in a reduction in cytokine production that was proportional to the concentration of sulforaphane supplied to the mice in the study undertaken to further show the compound's anti-inflammatory capabilities. In addition, T cell proliferation was significantly reduced.

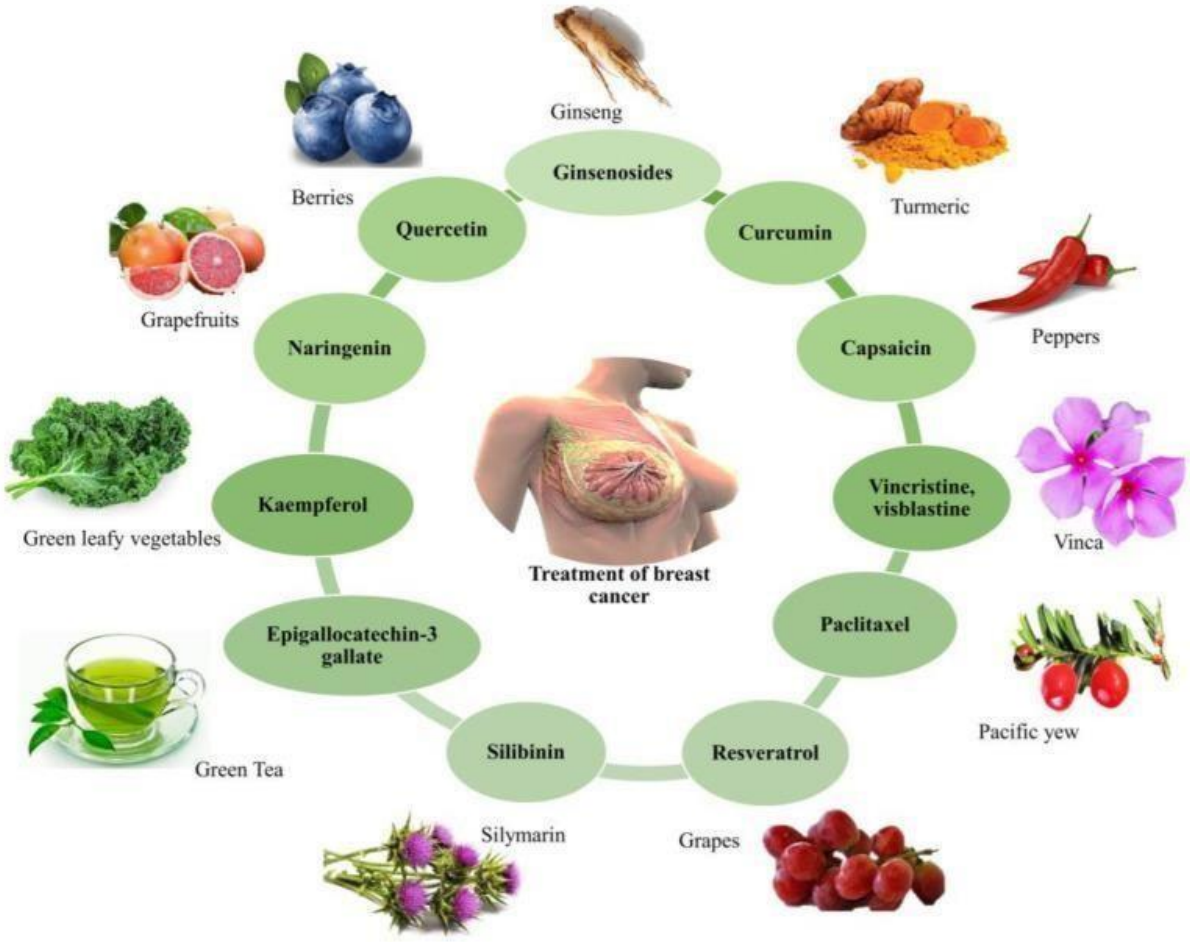


Fig. 15 Role of botanical in treatment of Breast cancer

Novel Drug Delivery System

Microencapsulation technology was initially conceived of as an alternative method of administering medication sometime between the 1940s and the 1960s. In the 1980s, the technology involving polymers and membranes came to be recognised as being at the forefront of the ongoing search for a more sophisticated system. Attaching bioactive compounds to liposomes, biopolymers, implants, monoclonal antibodies, and particle carriers enables precise site-specific targeting and distribution. This can be accomplished by using site-specific targeting and delivery methods (such as nanoparticles and microspheres).

Because it is so simple to use, the oral route of drug administration is universally regarded as the one that offers the greatest number of advantages. However, if the drug is one that is degraded significantly in the first pass through the liver, oral administration is not the best route to take for many medications. Therefore, because the gastrointestinal tract does not allow for systemic absorption, researchers have been looking into alternative methods of administering drugs. These methods include parenteral, intramuscular, subcutaneous, intranasal, and transdermal administration. The conventional method of taking a drug by mouth does not typically result in a noticeably elevated blood concentration of the drug. This frequently reaches levels that are toxic, and after a relatively brief period of time at levels that are therapeutic, the drug levels eventually drop until they are re-administered. To get the maximum therapeutic impact, the medicine must be administered to the target tissue in the appropriate amount during the required time period, with as minimal toxicity and side effects as feasible. Targeted drug delivery, also known as smart drug delivery, is a way of giving medication to a patient that involves raising the concentration of the medication in one region of the body over others. This strategy is sometimes known as "smart drug delivery." The goals of a targeted drug delivery system are to stretch, localise, and target lesion tissue, as well as to guard against medication interactions. The usual technique of medication delivery involves absorption through the biological membrane. In contrast, the targeted release approach reduces the frequency of doses required by the patient. As a result, the drug's action is more consistent, and the unwanted effects are reduced. And a reduction in the variability of medication levels in the blood. The downsides of this system

are its expensive cost, which makes increasing production difficult and limits its capacity to alter dosages, as well as its limited dosage adjustment capability. Targeted drug delivery systems can use a wide range of vehicles, including the following: The ideal medication delivery vehicle would be non-toxic, compatible with living organisms, non-immunogenic, and breakdown spontaneously. Microspheres are spherical particles with micron-sized diameters, typically ranging from one micrometre to one thousand micrometres (1 mm). Microspheres are also known as tiny particles in some circumstances. Polymers, waxes, and other protective compounds are employed in their manufacture. H. Chemically changed natural compounds like starch, gum, proteins, lipids, and waxes, as well as biodegradable manmade polymers. Albumin and gelatin are two examples of natural macromolecules. Examples of synthetic polymers are polylactic acid and polyglycolic acid. Microspheres are differentiated by their small size and high surface-to-volume ratio. They have colloidal qualities when they are at the smaller end of their size range. Interfacial characteristics are extremely important in determining the activities of microspheres. (M.Alagusundaram etal; 2009).

Microspheres (MS), also known as emulsion cells or solids distributed in a continuous phase, are used in a wide range of sectors, including food, cosmetics, and pharmaceuticals, among others. Emulsions (or MS) are often created in a way that leads in substantial polydispersion across a large range when traditional emulsion production methods are applied. It is widely held that surfactants are responsible for a significant portion of the emulsification process's success. The interfacial tension is lowered by surfactants, which also contribute to the formation of emulsions. It is generally accepted that the surfactant contributes to the stabilisation of the emulsion by causing the droplets to repel one another (Najam ul Hassan etal; 2021).

Microspheres are spherical particles that have a diameter in the micron range, typically ranging from one micrometre to one thousand micrometres (1 mm). Microspheres are also referred to as fine particles in some contexts. Polymers, waxes, and various other protective materials are used in their construction. H. Biodegradable synthetic polymers and naturally occurring substances that have been chemically altered, such as starch, gum, proteins, fats, and waxes. Albumin and gelatin are both examples of natural macromolecules. The

synthetic polymers polylactic acid and polyglycolic acid are examples of these. Microspheres are distinguished by their diminutive size and high ratio of surface area to volume. They exhibit colloidal properties when they are at the smaller end of their size range. Microspheres' activities are frequently determined by their interfacial properties, which are of the utmost significance (Patel B etal; 2012).

In its most basic form, each particle is a drug dispersed in a polymer, with the pattern of drug release governed by a first order mechanism. The amount of medicine released is determined by the rate at which the substrate dissolves or degrades. Because of its size and shape, microspheres have a comparable effect to ball bearings. Microspheres can differ significantly in terms of quality, sphericity, particle homogeneity, and particle size distribution. It is critical that the proper microsphere be chosen for each application. There are numerous methods for creating microspheres, which can then be employed to modulate drug delivery.

Protects unstable compounds before, during, and after administration, as well as before they reach the site of action, allowing for more precise delivery of even small doses of strong medications. Reduces medication concentrations at places other than the intended site of action. We can alter the activities of medications in live beings by attaching them to carrier molecules. The activity of carrier molecules can have an effect on the kinetics of clearance, as well as on tissue metabolism and the drug interactions that occur within cells. It is possible that the application of these pharmacodynamic modifications will result in an increase in the efficacy of treatment (Fu X etal; 2005).

This controlled drug delivery system's goal is to immediately ensure that the therapeutic amount is immediately delivered, and that it has reached the treatment level, while also maintaining the desired drug concentration in the area of action (Ghalop SB etal; 2010).

The oral route is a practical option that is typically chosen for drug administration. Certain medications that have a short t1 / 2 and are readily absorbed in the gastrointestinal tract are quickly removed from the blood circulation. Managed drug delivery systems are able to circumvent issues that are present in conventional drug delivery systems and gradually release drugs into the G.I.T. Keep the concentration of the serum stable over a longer period of time.

1.5 Various advantages and disadvantage of Microsphere are a follows:

(Lachman L;1987& Asija R *etal*; 2014)

ADVANTAGES	DISADVANTAGES
Reliable means delivering the medicine to the target site with the desired level of specificity, should this be changed, andmaintaining the desired concentration at the place of interest while generating noundesired side effects. Solid biodegradable microspheres may allow for regulated medication release throughout the particle matrix. Microspheres have received a lot of interest not just for their potential to restrict the release of anticancer medications, but also fortheir ability to target the tumour. It has been discovered that the size of microspheres, as well as the surface charge and hydrophilicity of the surface, play important roles in determining the destiny of particles in vivo. Research on the uptake of microspheres by macrophages has shown that these particles have the potential to direct drugs directly to pathogens that are located inside of cells.	After being injected, the drug becomes more difficult to remove. During the process of preparation, non- uniformity of the drug's content may occasionally result.

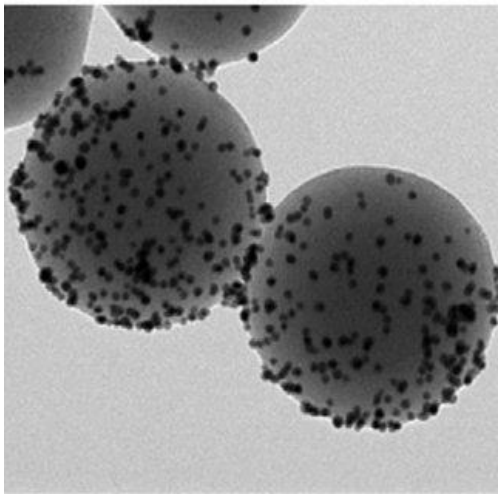
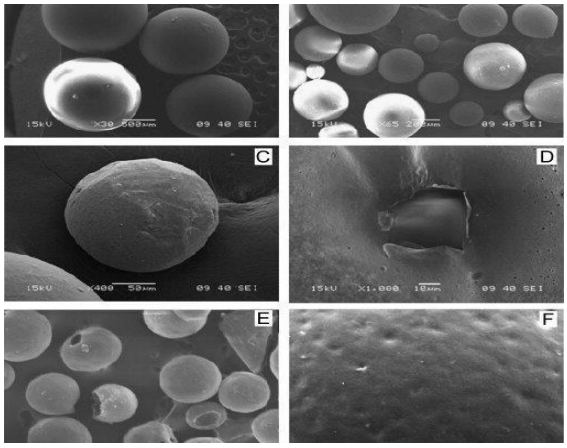
Blood flow measurement and analysis. Microspheres eliminate the possibility of incompatibilities between the drug and the recipients, particularly with the buffer. Dose dumping is mitigated by the use of microspheres. Microspheres offer drugs some degree of protection from the surrounding environment. The taste and odour are both concealed by the microspheres. The metabolism of microspheres is skipped in the first pass. Because of their diminutive size and spherical shape, microspheres can be effortlessly injected into the body. Both the biological half-life and the bioavailability are significantly improved thanks to the use of microspheres. Additionally, the risk of gastrointestinal irritation is decreased when using microspheres. Drug discharge in the stomach is inhibited, which is the reason why local adverse effects are decreased. In the case of microspheres, it is possible to achieve a better therapeutic effect for drugs that have a short half-life.	

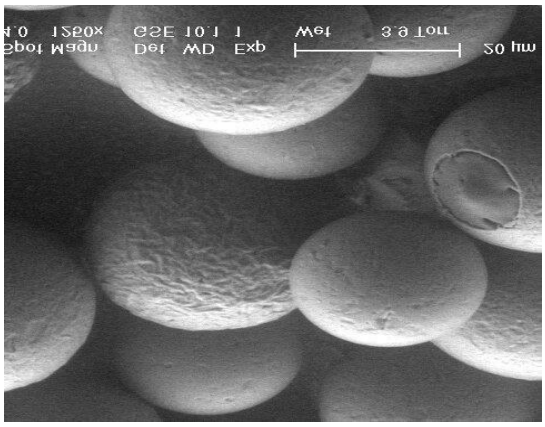
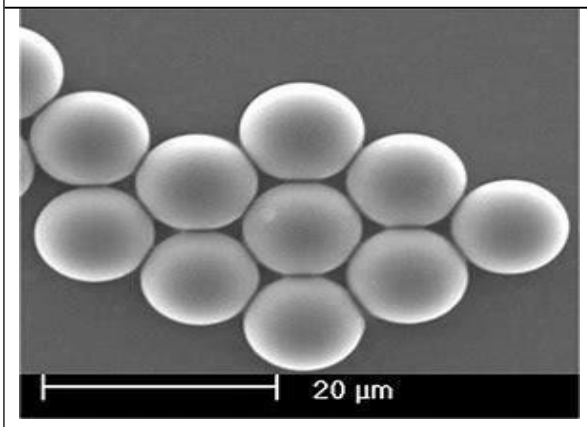
Limitations of microspheres (Kunchu K *etal*; 2014)

- ❖ The rate of regulated release of microspheres varies depending on a variety of circumstances, including diet, intestinal transit rate, mucin turnover rate, and so on.
- ❖ The amount of medication released varies according to dosing form.
- ❖ If extra-gastrointestinal microspheres are present, limit your medicine intake.
- ❖ When microspheres are administered intravenously, it is difficult to completely removethe carrier from the body. This is especially true for parenteral delivery.
- ❖ When injected parenterally, microspheres may interact with or form complexes with numerous blood components. There is some flexibility in the release formula.
- ❖ It is possible for the release sample to become toxic if its integrity is compromised inany way.

1.6 Types of Microspheres

Type	**Description**
Bio-adhesive microspheres **(Pawan Chaware etal;19991)** 	A good definition of adhesion would be "adherence to the membrane through the utilisation of the sticking properties of water-soluble polymer molecules." A bio-adhesive drug delivery system is a delivery system that makes use of the bioadhesion property of some of the polymers. These polymers become adhering on hydration and can be used for extended periods of time to direct medication to a particular area of the body. Because of this, the drug's absorption is improved, and as a result, its bioavailability is as well. This is because the patient is more compliant as a result of the decreased dosing frequency.

Magnetic microspheres **(Farah Hamad Farah, et. al;2016)** 	Magnetic microspheres are molecular particles that are small enough to move across capillaries without causing an esophageal occlusion (4 micrometres), but they are also extremely sensitive (ferromagnetic) enough to become trapped in micro-vessels and drawn by a magnetic field of 0.5-0.8 tesla through neighbouring tissues. Magnetic microspheres are essential necessary since they deliver medication straight to the damaged area of the body.
Floating microspheres **(Jagtap Yogesh Mukund, et. al;2012)** 	Microspheres that float in water and have a non-effervescent composition are the basis for the gastroretentive drug delivery method. Hollow microspheres, microballoons, and floating microparticles are all terms that are considered to be synonymous with the concept of floating microspheres. Floating microspheres are, to put it in layman's terms, small hollow objects that do not have a centre. These are cells that are free to move around and range in size from 1 to 1000 micrometres.

Radioactive microspheres **(Urs Häfeli, et. al;2016)** 	The subgroup of microspheres that exhibits radioactive interaction is typically handled in a manner that is analogous to that of microspheres that do not exhibit radioactive interaction. In spite of this, a radioactive microsphere will always contain at least one radionuclide and frequently more than one, in addition to the matrix material that both describes the microsphere and provides it with the targeting properties it needs to be effective in a specific organ or tissue. Even in very small quantities, radioactive microspheres are able to transport significant doses of radiation to a particular location without having any impact on the natural tissue that is located nearby.
Polymeric microspheres **(Lachman LA;1991)**	The following is a classification of the various kinds of polymeric microspheres that are available. A. Polymeric microspheres that degrade naturally B. Polymeric microspheres manufactured in a lab.
	

Materials used in formulation of Microspheres:

(P.M. Dandagi *etal* ;2017 & Chinna Gangadhar B *etal* ;2010)

Polymers are typically utilised to make microspheres. They can be divided into two distinct categories.

1. Synthetic Polymers

2. Natural polymers

Synthetic Polymers	**Natural polymers**
Polymers that do not degrade naturally: Epoxy polymers, acrolein, glycidyl methacrylate, and poly methyl methacrylate (PMMA), also known as poly methyl methacrylate (PMMA). Polymers that break down naturally: Lactides, glycolides, and the co-polymers of both of these Acrylates of polyalkyl cyanoalcohols and polyanhydrides.	Albumin, gelatin, and collagen are all types of proteins. Agarose, Carrageenan, Chitosan, and Starch are all examples of carbohydrates. Carbohydrates that have been chemically modified, including poly dextran and poly starch.

1.7 METHOD OF PREPARATION

In order to successfully prepare microspheres, certain criteria need to be met:

1. The ability to incorporate acceptable amounts of the medication at relatively highdoses.
2. The preparation's post-synthesis stability, as well as its therapeutically acceptable shelflife.
3. Particle size and dispersibility in aqueous vehicles for injection can be accurately regulated.
4. The precise and regulated release of the active reagent over a large time period.
5. Biocompatibility along with a biodegradability that can be regulated. susceptibility to alterations caused by chemical processes

Single Emulsion Technique

The single emulsion technique is used to create microparticulate carriers of natural polymers, such as proteins and carbohydrates. These natural polymers are referred to as natural polymers. After being dissolved or dispersed in an aqueous media, naturally occurring polymers are disseminated in a non-aqueous liquid, such as oil. The next step is to cross-link the dispersed globule. The molecules can be cross-linked using either heat or

chemical crosslinking agents. Cross-linking agents utilised in the chemical process include glutaraldehyde, formaldehyde, di acid chloride, and others. For temperature-sensitive compounds, heat denaturation is not an option. If the active component is introduced during preparation and then centrifuged, washed, and separated, it will be overexposed to the chemicals employed in the chemical cross-linking process, which is a drawback of this approach.

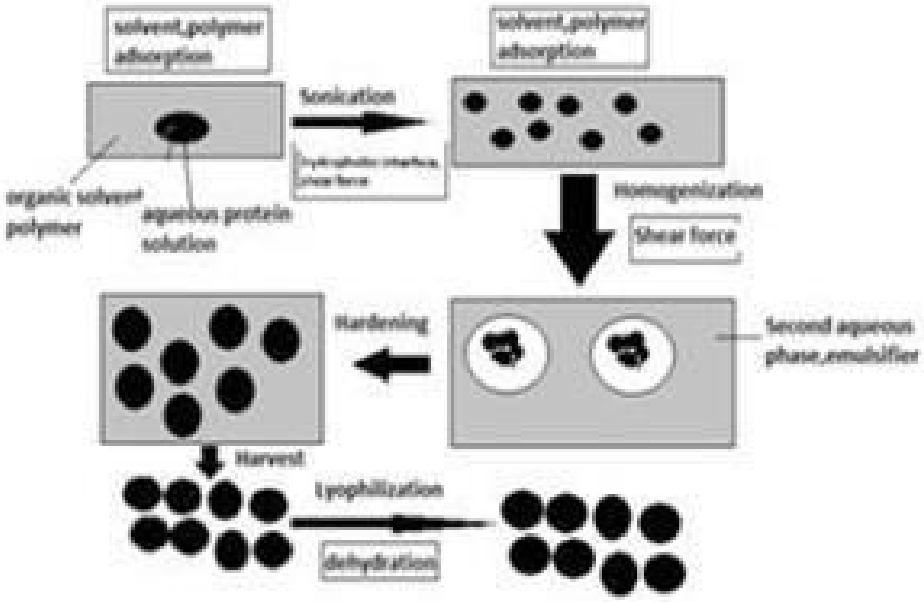


Fig. 16 Single Emulsion Technique by Chemical Cross-Linking

Double Emulsion Technique

The microspheres can be created using a technique known as the double emulsion method. This process involves the production of several emulsions or a double emulsion of the w/o/w variety. This approach is particularly effective when applied to water-soluble medicines, peptides, proteins, and vaccinations. This approach can be used with natural or synthetic polymers, depending on the project's needs. The lipophilic organic continuous phase contains an aqueous protein solution that is spread throughout. It is probable that the active components exist in this protein solution. The continuous phase is typically made up of the polymer solution that eventually encapsulates the protein from the scattered aqueous phase. After that, the primary emulsion is homogenised or sonicated before being added to

the polyvinyl alcohol in water solution. This results in the formation of a double emulsion. Following that, the emulsion's solvent is removed using either solvent evaporation or solvent extraction, depending on preference. Several hydrophilic medicines, including leutinizing hormone releasing hormone (LH-RH) agonists, vaccines, proteins/peptides, and conventional compounds, have been successfully integrated into microspheres using the double emulsion solvent evaporation/extraction approach.

1.8 Polymerization Techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization
II. Interfacial polymerization.

Both of these processes occur in the liquid phase. Normal polymerisation is achieved using a range of strategies, including bulk, suspension, precipitation, emulsion, and micellar polymerisation. When beginning a polymerisation process in bulk, it is usual practice to add heat to a monomer or a combination of monomers, as well as the initiator or catalyst. The resulting polymer can be formed into microspheres if required. During the polymerisation process, the drug may be loaded. Suspension polymerisation is sometimes known as pearl or bead polymerisation. In this case, it is performed by heating the monomer or mixture of monomers in droplets spread in a continuous phase of aqueous solution. It is possible that the droplets include an initiator in addition to other ingredients. The presence of an initiator in the aqueous phase of emulsion polymerisation, which diffuses to the surface of micelles, distinguishes it from suspension polymerisation. One advantage of bulk polymerisation is the ability to produce pure polymers. Interfacial polymerisation is the interaction of different monomers at the interface of two immiscible liquid phases to generate a film of polymer that essentially envelops the dispersed phase. This process is known as "interfacial polymerisation."

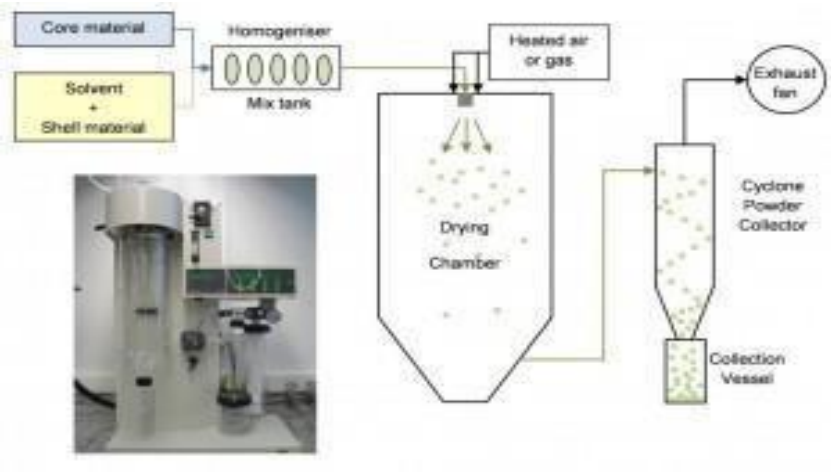


Fig. 17 Spray Drying Technique

Phase Separation Coacervation Technique

This procedure is based on the premise of limiting the solubility of the polymer in the organic phase. This is done in order to influence the formation of a polymer-rich phase that is referred to as the coacervates. First, the drug particles are distributed in a solution of the polymer, and then an incompatible polymer is introduced to the system. This is the technique that is being used. This results in the first polymer undergoing phase separation, which is followed by the drug particles being absorbed by it. The solidification of the polymer that takes place as a consequence of the incorporation of a non-solvent into the mixture. The utilisation of butadiene as the incompatible polymer has enabled the production of microspheres composed of polylactic acid (PLA) through the utilisation of this technical approach. As a result of the fact that it controls the dispersion of the polymer film, the particle size, and the agglomeration of the produced particles, the rate at which the coacervates are achieved is a very essential variable in the process. The factors of the procedure are of utmost significance. Agglomeration must be avoided at all costs by stirring the suspension with a sufficient speed stirrer. This is done in order to prevent the polymerise globules that have been created from adhering to one another and producing agglomerates. This is due to the fact that the origin of the microsphere creation process begins with the polymerise globules that have been created beginning to adhere to one

another. The process variables are of the utmost importance because it is via them that the kinetic of the produced particles is managed. This is due to the fact that there is no predetermined state of equilibrium that can be attained.

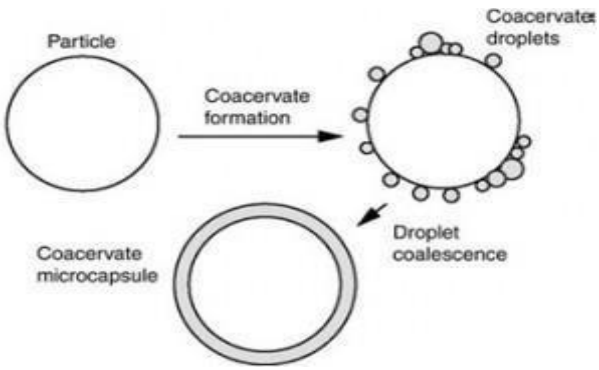


Fig. 18 Formation of Coacervates Around the Core Material

Spray Drying and Spray Congealing

The drying of a mist that contains both the medication and the polymer in the air around it is the central focus of these approaches. If the solution is cooled before or after the removal of the solvent, the two processes are referred to as spray drying and spray congealing, respectively. The nomenclature of these processes are decided by the order in which the solvent is removed. In the initial stage of the procedure, the polymer is dissolved in a suitable volatile organic solvent, such as dichloromethane, acetone, or another chemical that is analogous to it. The medication, in its solid form, is then homogenised at a high speed before being disseminated in the polymer solution. This stage comes after the previous one. A jet of hot air is then used to atomise the dispersion after this stage has been completed. Microspheres with diameters ranging from one to one hundred micrometres are formed as a result of the atomisation process, which results in the development of small droplets or a fine mist. The effect of this process is that the solvent evaporates instantaneously, resulting in the formation of microspheres. While the cyclone separator is used to remove the microparticles from the hot air, the vacuum drying process is used to remove any remnants of the solvent. Both phases of the process are carried out simultaneously. One of the most important benefits of the process is that it may be

successfully carried out under aseptic circumstances. This is one of the most significant advantages. We use the spray drying procedure in order to encapsulate the various forms of penicillin that are available. Thiamine mononitrate 14 and sulpha-ethylthiadizole 15 are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid by the process of spray congealing through the use of spray congealing. On the other hand, the creation of porous microparticles is brought about by the extremely quick evaporation of the solvent.

Solvent Evaporation

The removal of the organic phase through extraction of the organic solvent is a necessary step in the solvent evaporation method, which is utilised in the process of microparticle preparation. Isopropanol and other organic solvents that are miscible in water are utilised in this method. The organic phase is eliminated through the use of water extraction. The amount of time required for the microspheres to become hard is cut down by this process. Directly adding the drug or protein to the polymer organic solution is one of the variants of the process. The rate at which solvent is removed by the extraction method is contingent upon the temperature of the water, the ratio of emulsion volume to the volume of the water, and the solubility profile of the polymer (Kadam N.R and Suvarna V;2015).

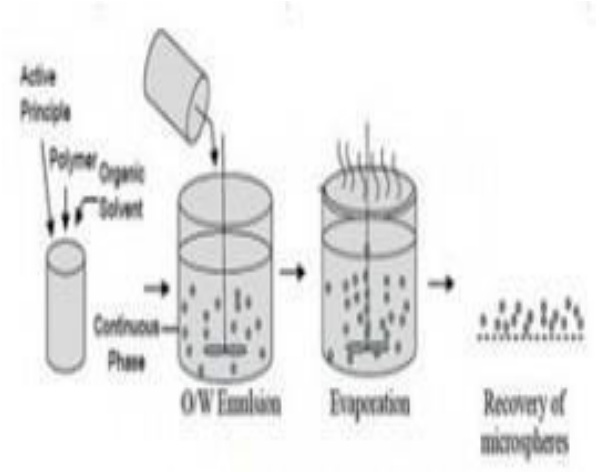
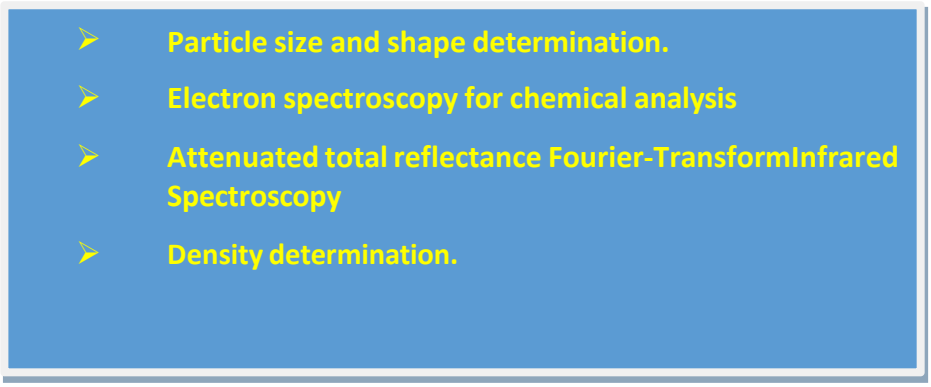


Fig. 19 Solvent Evaporation Technique Characterization

The characterization of the micro particle carrier is a significant phenomenon that is helpful in the design of appropriate carriers for the delivery of antigens, proteins, or drugs. The microstructures of these microspheres are distinct from one another. The release and stability of the carrier are both determined by these microstructures (Bansal H etal; 2011).



Applications of Microspheres

Every day, brand new applications for microspheres are found, and here are a few of those applications:**(Kadam N.R and Suvarna V; 2015):**

- **Ophthalmic Drug Delivery:** Microspheres made with polymers have favourablebiological properties such as bio-adhesion and permeation enhancing properties. Theyalso have interesting physicochemical properties, which distinguish them from other materials that can be used to make ophthalmic drug delivery vehicles like chitosan, alginate, and gelatin. These properties make microspheres one of a kind as a material for the production of ophthalmic drug delivery vehicles.
- **Oral Drug Delivery:** Because microspheres containing polymer have the ability to form films, they can be used in the formulation of film dosage forms, which is an alternative to the use of pharmaceutical tablets. Microspheres are better suited for oral drug delivery applications due to their pH sensitivity and the high reactivity of their primary amine groups. e.g. Chitosan, Gelatin.

- **Gene Delivery:** Because of their ability to adhere to and move through the gastrointestinal tract, microspheres have the potential to serve as effective oral gene carriers. Chitosan, gelatin, viral vectors, cationic liposomes, and polycationic complexes are a few examples of such substances.
- **Nasal Drug Delivery:** By having good bioadhesive properties and easily swelling upon contact with the nasal mucosa, polymer-based drug delivery systems like microspheres, liposomes, and gels have been shown to increase the bioavailability and residence time of drugs that are administered via the nasal route. Examples of such systems include starch, dextran, albumin, chitosan, and gelatin.
- **Intratumoral and Local Drug Delivery:** For the purpose of delivering paclitaxel to the site of the tumour at a concentration that is suitable for treatment, a polymeric film is fabricated. There is reason to be optimistic about the mixture of medications' application in the controlled delivery of to the oral cavity. Examples include gelatin and chitosan.
- **Buccal Drug Delivery:** The mucosal and bioadhesive properties of polymers make them ideal for buccal delivery because they can also serve as absorption enhancers. Polymers are excellent polymers (eg chitosan, sodium alginate).
- **Gastrointestinal Drug Delivery:** Internal cavities have been prepared in polymer through a de-acidification process that is then added to acidic and neutral media. These cavities have been found to be buoyant and have provided a controlled release of the drug (for example, Eudragit and Gelatin).
- **Transdermal drug delivery:** A film-forming polymer with desirable physical properties. The thickness of the membrane as well as the cross-linking of the film make it difficult for the devices to release the drug. Chitosan and alginate are two examples.
- **Colonic drug delivery:** The accurate delivery of insulin into the colon has been accomplished through the use of polymer. Chitosan, for example.

Phyto-nano formulations for Cancer Therapy

Recent developments in nanotechnology have led to the identification of the most cutting-edge assist system for transporting therapeutic agents to their intended locations. The carrier prevents the encapsulated therapeutic agent from being damaged by the environment and contributes to the product's increased stability.

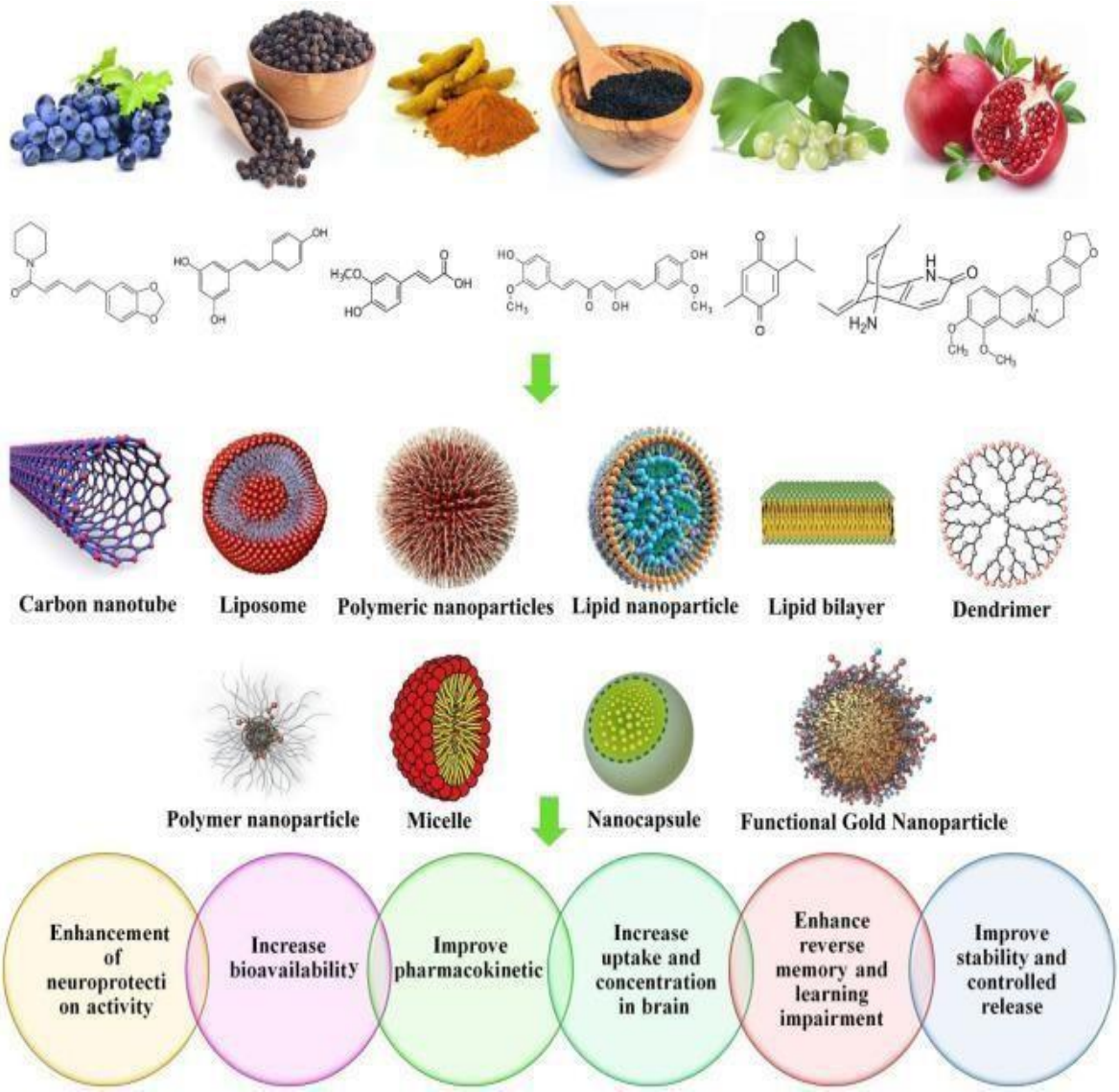


Fig. 20 Phyto-nano formulation

Different types of nano-formulations and shows potential activity against cancer cell type (P.More *etal;*2021)

Phytoconstituents	**Nano-formulation**	**Cancer type**
Silibinin	Nanomicelles	Liver cancer
Betulinic acid	Polymeric nanoparticles	Triple negative breast and laryngeal cancer
Epigallocatechin-3-gallate	Polymeric nanoparticle	Breast cancer
Trans-resveratrol and ferulic acid	Solid lipid nanoparticles	Colon cancer
Quercetin	Nanomicelles	Prostate cancer
Withanolide-A	Gold nanoparticles	Breast cancer
Berberine	Liquid crystalline nanoparticles	Breast cancer
Resveratrol	Liposomes	Hepatocellular carcinoma
Curcumin	Dendrimer	Hepatocellular carcinoma
Celastrol	Dendrimer	Colorectal cancer
Curcumin	Carbon nanotubes	Lung cancer
Silibinin	Magnetic nanoparticles	Lung cancer

Microsphere as Target drug delivery system

When it comes to the delivery of cargoes to specific locations in a manner that is both regulated and sustained, microspheres are one of the drug carriers that have been the subject of the most study and development. In addition to being biocompatible, they are also biodegradable, and the surfaces of these materials can be altered to suit specific needs.

Researchers have conducted experiments with a wide variety of polymers, each of which is capable of encapsulating pharmaceuticals that have morphological and chemical properties that are distinct from one another. They are a worthy candidate for targeting to numerous places in the body, such as the lungs, liver, bone, brain, colon, eye, and other malignant tissues situated in various regions of the body, with the combination of all of these qualities and methodologies making them a worthy choice. Additionally, they are able to target macrophages and other cellular organelles, such as mitochondria, with their attacks. This is a significant advantage.

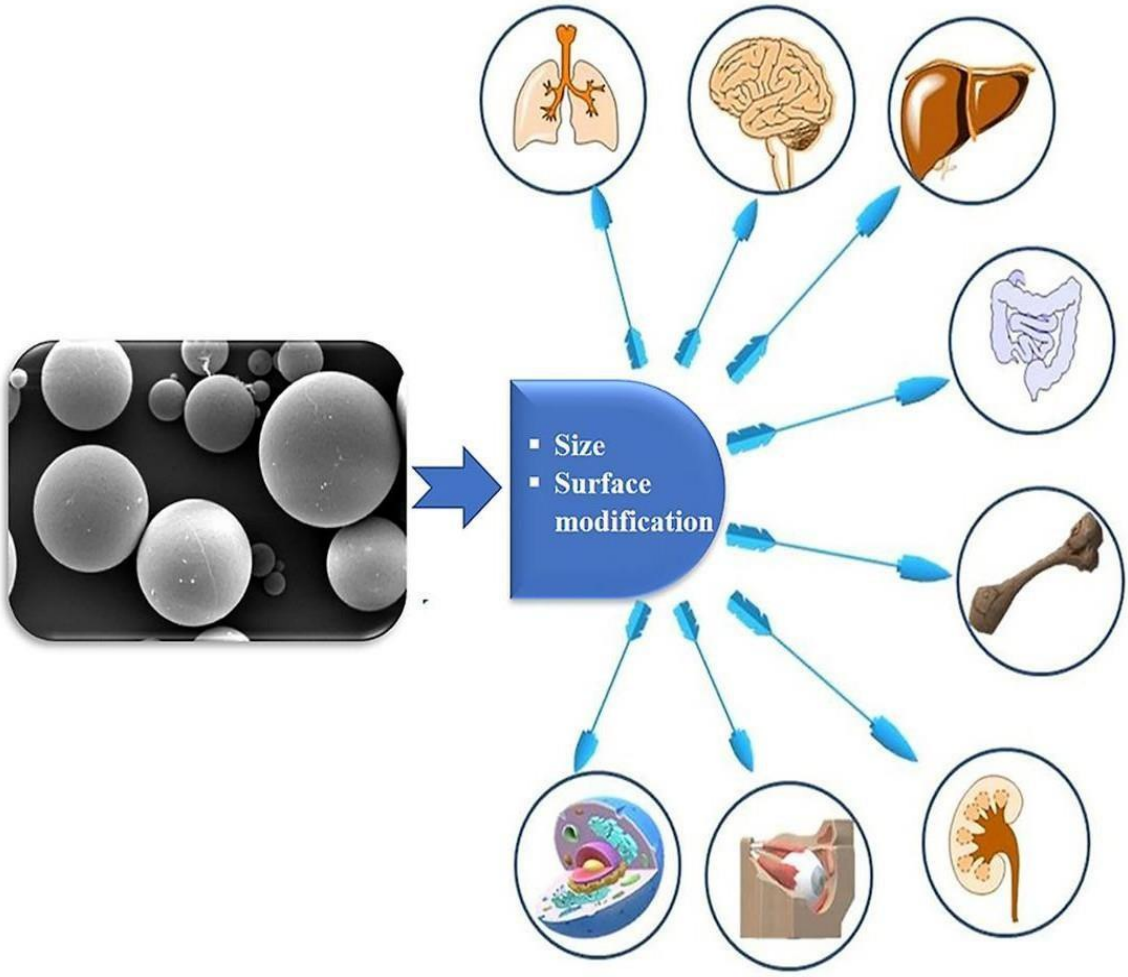


Fig. 21 Target Drug Delivery System

Application

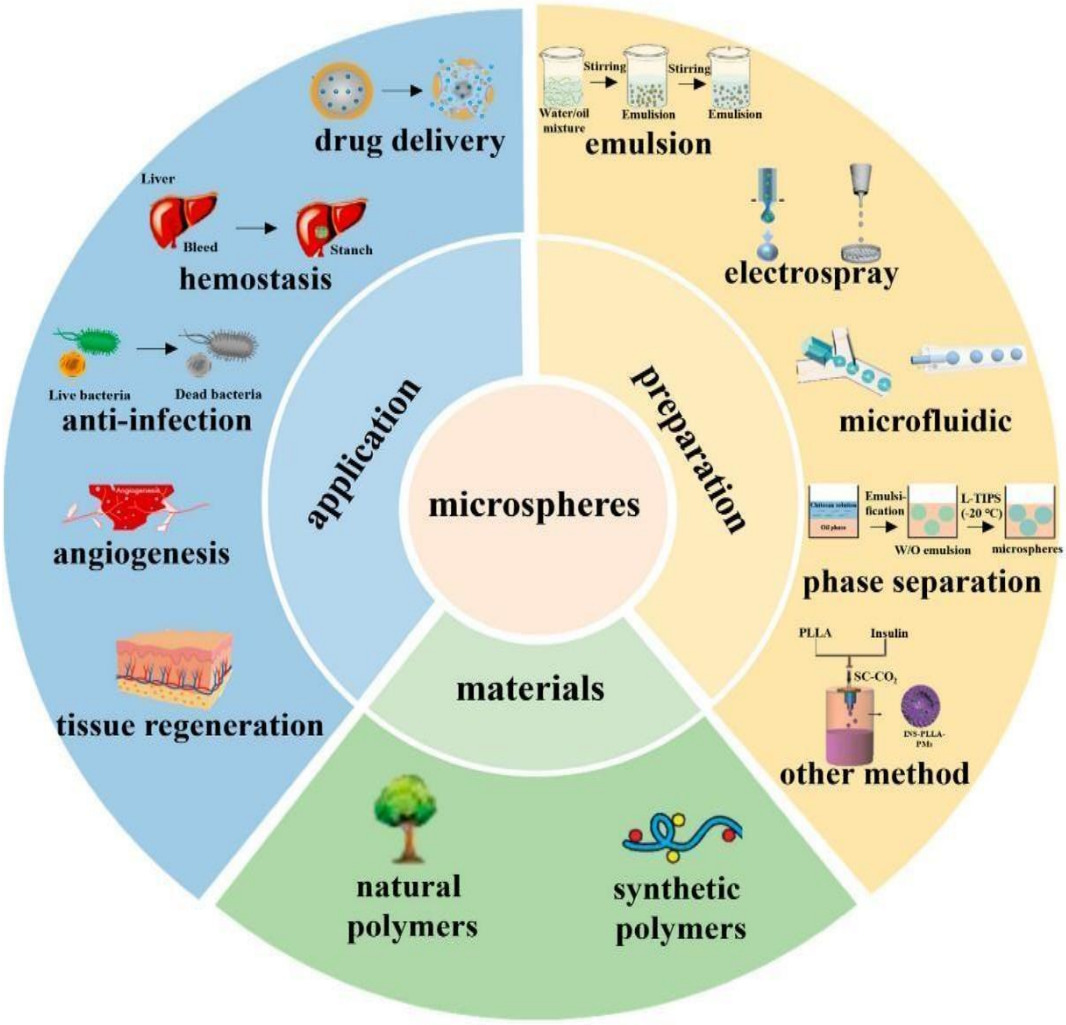


Fig. 22 Summarized Application of Microsphere

CHAPTER - 2
REVIEW OF LITERATURE

The points that follow are the primary goals of the literature review:

- Specifically, it explains how the proposed research is connected to previous research in the field of statistics.
- Both the originality and the significance of your study problem are demonstrated by this. Specifically, your study is distinct from that of other statisticians doing the same thing.
- Justifies the methods that you have proposed.
- The fact that you are prepared to finish the research is demonstrated by this.
- Prevent yourself from repeating the findings of other statisticians.
- Justify the significance of the research that you have proposed.

When conducting a literature review, it is common practice to include information that has been published in a certain area of research, and, on occasion, information that has been published within a particular period of time. In spite of the fact that a literature review could consist of nothing more than a list of the sources, it often adheres to an organisational framework and involves both summary and synthesis. In the context of a particular topic, the literature review is a written overview of key books and other sources on the subject. It is possible that the review will draw from a variety of sources, such as scholarly publications, journal papers, reports from the government, websites, and so on. Throughout the course of the literature review, each source is delineated, summarised, and appraised.

As per present investigation literature review was designed in following steps:

- ❖ Recent work done on Microspheres
- ❖ Recent advancement on Cancer research
- ❖ Previous work done on *Glycyrrhiza glabra*
- ❖ Work done Molecular modelling of cancer

Recent work done on Microspheres

The freeze-drying microfluidic technology, the integration of platelet-derived growth factor-BB (PDGF-BB), and the use of exogenous mesenchymal stem cells were utilised in order to create a live and injectable porous hydrogel microsphere that possessed long-term paracrine activity. The findings of the investigation carried out by Li X et al. 2023 revealed this. In order to stick to and grow on methacrylate gelatin (GelMA) hydrogel microspheres (GMs), exogenous stem cells are able to do so. This is made possible by the porous structure and exceptional mechanical property of GMs. In addition to enhancing the paracrine impact, this encourages connections between cells and the extracellular matrix (ECM) as well as between cells themselves. Moreover, the prolonged release of PDGF-BB has the capability of recruiting MSCs that are already present in the body, which contributes to the prolongation of the paracrine activity of the surviving GMs. Living GMs have been shown to have superior secretion properties and anti-inflammatory efficacy, and they have also been shown to be able to slow the progression of osteoarthritis (OA) by favouring the adherent microenvironment and making use of the synergistic effect of exogenous and endogenous mesenchymal stem cells (MSCs). These effects were validated through experiments conducted in vitro and in vivo. The fact that living GMs were able to slow down the course of osteoarthritis (OA) is evidence that this conclusion is correct. A living injectable porous hydrogel microsphere that is capable of boosting the paracrine activity of stem cells has been manufactured. This brings us to the conclusion that this microsphere has been created. The potential for future clinical translation of this microsphere in the treatment of osteoarthritis (OA) and other disorders is something that is anticipated to be discovered in the near future.

Chi H et al. 2023 carried out an excellent assignment of providing a summary of the latest advancements that have been made in the preparation technique of hydrogel microspheres and their application in skin restoration. In this article, a number of distinct methods for the creation of hydrogel microspheres were analysed, compared, and contrasted in great detail. Additionally, a review was carried out on the state of research concerning the application of hydrogel microspheres for the purpose of skin restoration. The review focused on the possible applications of hydrogel microspheres as delivery platforms (hydrogel

microspheres as a microcarrier of medications, bioactive substances, or cells) in the field of skin restoration. These microspheres include haemostatic microspheres, antibacterial microspheres, bioactive microspheres, and haemostatic microspheres. Following the conclusion of the presentation, a conversation was held regarding the potential drawbacks of the development of hydrogel microspheres and their application in the field of skin restoration. Additionally, the session concluded with a discussion of the bright future that lies ahead for this field. In the future, it is intended that this study will serve as a helpful reference for the formulation of a strategy for the preparation of hydrogel microspheres, and that it will also encourage the utilisation of hydrogel microspheres in the process of skin restoration.

Effective antigen distribution is a critical component in determining whether or not antiviral vaccinations are successful. The success of antiviral vaccines is proportional to the degree to which they protect against virus exposures. In order to enhance the delivery of the antigen for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a polymeric microsphere system that is controllable, biodegradable, stable, biocompatible, and nontoxic was developed. This system is designed to include chemically inactivated viral proteins. By encapsulating SARS-CoV-2 proteins in polymeric microspheres, it was possible to induce the development of powerful antiviral immunity. The fact that the viral antigen-loaded microsphere system has the ability to eliminate the need for recurrent injections is a significant factor that highlights the system's potential value as a vaccine. In a short amount of time, the Food and Drug Administration (FDA) in the United States granted licenses to vaccines against SARS-CoV-2 that were not only efficacious but also safe. Nevertheless, each of the vaccinations needs to be updated in order to account for the emergence of new strains of the disease. Previous research provides the foundation for our hypothesis, which states that injectable biodegradable polymers have the potential to serve as a method for the persistent release of novel viral antigens. By making predictions about the genetic variability of viral strains, this concept offers a way that can be used to limit the number of times that vaccinations are administered. There is a possibility that this technique will result in antiviral protective immunity that is maintained for a longer total duration. These measurements have been accomplished by the proof-of-concept multipolymer investigation that is now being carried out for SARS-CoV-2.

A method to biomedical engineering that draws from a variety of disciplines, bone tissue engineering is characterised by the utilisation of scaffold materials, seed cells, and "growth factors" as its three primary constituents. The traditional way of construction, on the other hand, is not flexible and cannot be adapted to the specific shape of the flaw. Consequently, the cells that are trapped within the bone are unable to obtain the necessary nourishment, which can result in additional issues further down the road. As a consequence of this, a solution that is not only straightforward but also effective by utilising the "bottom-up" strategy is proposed. In the form of a scaffold or as a drug delivery system, microspheres are structures that can be utilised as supports for the growth of cells. Microspheres can operate in any of these capacities. Their diameters can range anywhere from one micrometre to one thousand micrometres, and each micrometre in between can be any size. In this article, Feng Z and colleagues 2023 address a number of different approaches to the manufacture of microspheres. These approaches include the categorisation of raw materials and the loading of microspheres with drugs. In addition to this, they study innovative approaches to the utilisation of microspheres in the field of bone tissue engineering. In addition to this, we consider things from fresh perspectives and investigate the various possible avenues that could lead to progress in the future.

A tissue injury, which is one of the most common types of traumatic injuries encountered in ordinary life, can quickly lead to the development of a secondary wound infection. This is because tissue injuries are easily transmitable. There are numerous types of wound dressings that have been designed for the purpose of facilitating wound healing and reducing the production of scar tissue. Some examples of these wound dressings include gauze, bandages, sponges, patches, and microspheres. Among these, microsphere-based tissue dressings have received an increasing degree of interest due to the fact that they are simple to create, demonstrate superior drug release capabilities, and have outstanding physicochemical performance. During the initial phase of this investigation, we started out by talking about the traditional methods that are used to prepare microspheres. Emulsification-solvent method, electrospray method, microfluidic technology, and phase separation methods are some of the approaches that fall under this category. Following that, we went over a concise synopsis of the many biomaterials that were utilised in the manufacturing of the microspheres during the process. There were both natural and

synthetic polymers incorporated in these biomaterials mixtures. Subsequently, Yang C et al.2023 demonstrated the utilisation of a variety of microspheres that were produced through a variety of processing techniques in the context of wound healing, in addition to other applications. In conclusion, a discussion of the potential future development directions for microspheres was offered, along with an examination of the restrictions that were present.

(Chang H et al.; 2022) It is absolutely necessary for the normal construction and function of the tissue to ensure that the metabolism of extracellular matrix (ECM) is kept in a healthy equilibrium. In spite of this, it can be difficult to maintain control of the metabolism of the extracellular matrix (ECM) over an extended period of time in an aberrant microenvironment. This is due of the complex interplay that exists between the ECM, the resident cells, and the tissue microenvironment. In this investigation, an injectable circRNA silencing-hydrogel microsphere, which was referred to as psh-circSTC2-lipo@MS, was produced by grafting circSTC2 silencing genes-loaded cationic liposomes onto methacrylated hyaluronic acid (HAMA) microspheres through the utilisation of amide bonds. The nucleus pulposus (NP) cells that have this construct have the ability to have pathogenic genes silenced in order to regulate the metabolism of the extracellular matrix (ECM). The preparation of HAMA microspheres was accomplished by the use of microfluidics, and the microspheres exhibited remarkable degradability, swellability, and injectability. It is possible to efficiently load lipoplexes by the utilisation of chemical grafting, and the contents of these lipoplexes can be released for a period of up to 27 days. In NP cells, the production of ECM catabolism-related proteases is inhibited by psh-circSTC2-lipo@MS when these cells are cocultured with these cells for a period of 72 hours under conditions that restrict the availability of nutrients. The amount of proteins related to the extracellular matrix (ECM) that are synthesised as a consequence of this is significantly increased. The local injection of psh-circSTC2-lipo@MS, administered after eight weeks, stimulates extracellular matrix (ECM) formation and recovers NP tissue in the rat intravenous diet (IVD) nutrient-restricted paradigm. This study's findings suggest that the psh-circSTC2-lipo@MS targeted gene delivery system, which is both safe and capable of being controlled, possesses a large degree of promise for the regulation of the ECM

metabolic balance in an aberrant microenvironment. In conclusion, the findings of this study demonstrate that its potential is significant.

A macrophage-modulated and injectable 'building block' drug delivery method was created by **Ji X and colleagues 2022** specifically for the purpose of this study. Hydrogel made of hydroxypropyl chitin (HPCH) and porous chitosan (CS) microspheres were the components that made up this substance. Encapsulation of the dimethyloxallyl glycine (DMOG) was performed in the thermosensitive HPCH hydrogel (HD), and the conjugation of the kartogenin (KGN) was performed using the CSK-PMS reaction. The HD/CSK-PMS composite scaffold that was constructed was successful in altering the microenvironment at the location of the defect, attaining local macrophage M2 polarisation, and encouraging cartilage regeneration. These three goals were accomplished. When compared to the extremely stable CSK-PMS, which supported the ossification of endochondral cartilage and promoted the regeneration of subchondral bone, the rapidly degradable HD was found to be more effective in promoting the regeneration of hyaline cartilage. Evaluations that were carried out in vitro and in vivo demonstrated that the recently developed HD/CSK-PMS, which is a controlled drug delivery system, was able to successfully produce an M2 macrophage milieu and orchestrate osteochondral (OC) regeneration. A comparison of the outcomes of the two different testing procedures led to the discovery of this. It is possible that the immunomodulation-based hydrogel/PMS composite system could be a promising candidate for OC regeneration as a result of these findings, which demonstrate that the immune milieu and subchondral bone play an essential role in the quality of cartilage repair.

Osteoarthritis is a degenerative joint illness that is associated to lubrication failure in the joint. Two of the hallmarks of osteoarthritis are the deterioration of the articular cartilage and the inflammation of the joint capsule. There is a consensus among medical professionals that enhancing joint lubrication in conjunction with anti-inflammatory medication is a successful technique for the treatment of osteoarthritis. Using the microfluidic technology, a monodisperse, size-uniform microsphere is fabricated, and then a spontaneously modified microsphere with DMA-SBMA copolymer is grafted onto it using a one-step biomimetic grafting method. This results in the development of a

superlubricated microsphere. The microsphere is based on the ball-bearing-inspired super lubricity and the mussel-inspired adhesion. It is referred to as the microsphere. It is because of the tenacious hydration layer that is produced around the charged headgroups (-$N^+(CH_3)_2$ - and -SO_3-) of the grafted poly sulfobetaine methacrylate (pSBMA) that the microspheres are provided with increased lubricating. In addition, because of their porous structure, the microspheres are able to concurrently offer the capability of effective drug loading as well as release capability. It is important to note that the grafting of pSBMA onto microspheres confers upon them features that are beneficial for the intraarticular therapy of osteoarthritis. A few examples of these qualities are improved lubrication, decreased degradation, and sustained drug release. Additionally, the superlubricated microspheres that have good biocompatibility have the ability to block the destruction of chondrocytes that is generated by tumour necrosis factor (TNF) in vitro. Furthermore, when loaded with diclofenac sodium, these microspheres have the ability to exert a therapeutic impact on osteoarthritis in vivo patients. It is possible to accomplish this because the microspheres have the capacity to keep an excellent level of lubrication.

Drug delivery systems, which are often referred to as DDSs, have been the driving force behind a substantial amount of advancements in the immunotherapeutic regimen and combined therapy of cancer. Differential drug delivery systems (DDSs) possess the capability to present tumour antigens, medicines, immunostimulatory agents, or adjuvants in a spatiotemporal manner. Because of this, it is now feasible to directly manipulate immune cells in vivo, such as dendritic cells (DCs) or T-cells, which ultimately results in powerful immune responses against tumours. It has been proven that cancer vaccines, immune checkpoint blockade, and adoptive cell transfer are all effective therapeutic interventions in the clinic. The addition of DDSs has the potential to significantly boost anticancer efficacy while concurrently minimising unfavourable side effects. The purpose of this article is to discuss the utilisation of nano-, micro-, and macroscale drug delivery systems (DDSs) for the purpose of co-delivering a variety of immunostimulatory agents in order to retrain the immune system to be able to combat cancer. When it comes to drug delivery systems that are based on nanoparticles, we place a strong emphasis on the modification of the immunological environment of tumours through the use of nanoparticles, either on their own or in conjunction with gene therapy, photodynamic

treatment, or photothermal therapy. When it comes to DDSs that are based on microparticles or capsules, an overview of the carrier type, fabrication process, and co-delivery of cancer vaccines and adjuvants is offered. Last but not least, macroscale drug delivery systems (DDSs) such hydrogels and scaffolds are also addressed, and a description of the role that these DDSs play in adoptive cell transfer therapy and tailored vaccine delivery is offered. The perspectives on DDS-based cancer immunotherapy and its application in clinical settings are also covered in this article. When it comes to developing cancer immunotherapy, both in terms of basic research and clinical translation, we believe that DDSs have a great amount of potential that has not yet been fully realised. One of the most promising new techniques to treating cancer is immunotherapy, which is rapidly becoming becoming more widespread. Drug delivery systems, which are often referred to as DDSs, have been the driving force behind a substantial amount of advancements in the immunotherapeutic regimen and combined therapy of cancer. The primary focus of this comprehensive analysis is on the application of DDSs at the nano-, micro-, and macroscales for the co-delivery of a range of immunostimulatory agents in an effort to retrain the immune system to fight cancer. This review is wide in its scope. According to According to the findings of the research conducted by Huang P et al. in 2019, a perspective was offered regarding the development of next-generation DDS-based cancer immunotherapy. The findings of this analysis indicate that drug delivery systems (DDSs) have the capacity to allow for the simultaneous administration of dual or multiple immunostimulatory medicines. Both the fundamental research and the clinical translation of cancer immunotherapy have the potential to be significantly advanced as a result of this. Additionally, this has the potential to improve the antitumor T-cell immunity.

In the year 2021**, Prajapati H and colleagues** were the ones who were responsible for the formulation of floating microspheres of baclofen as well as the examination of these microspheres. Evaporation of the solvent was the method that was employed in the process of preparing the microspheres. According to the results of the multiple regression analysis, increasing the concentration of Eudragit RL100 and Eudragit RS100 resulted in a decrease in the amount of drug that was released in vitro. Additionally, this led to an increase in the size of the particles, the percentage of drug entrapment efficiency, and the percentage of buoyancy. There is a percentage of drug entrapment that is 90.06 percent, and the buoyancy

of the optimised formulation is 90.76 percent. The particle size of the optimised formulation is 115.96 meters, and it has a buoyancy that is 90.76 percent. Baclofen floating microspheres were demonstrated to have a drug release that was maintained in vitro for a period of up to twenty-four hours during the experiment. In addition to being porous and fluid, the microspheres that were floating around possessed a form that was very close to being spherical. The Higuchi model was the one that was the one that was followed by the formulation, and the fickian diffusion mechanism was the one that was responsible for drug release, as indicated by the outcomes of the in-vitro drug release kinetics experiments.

Mishra Raghav;2021 carried out research with the intention of creating and studying amethopterin microspheres that float in the water. When these microspheres are administered orally, they have the potential to enhance the amount of time that the drug is present in the stomach, improve the bioavailability of the drug through prolonged release, decrease dose-dependent adverse effects, and improve patient compliance. Ethyl cellulose, polyvinyl alcohol, and polyvinyl pyrrolidone-K90 were the three components that were utilised in the production of floating microspheres by the application of the emulsification solvent evaporation procedure.

Ionic gelation was the procedure that was utilised in the research that **Shah Niyati and colleagues 2021** conducted in order to create microspheres of acebrophylline that had a prolonged release. Variable polymer ratios were present in these microspheres when examined. For the goal of conducting drug-excipient compatibility investigations, Fourier transform infrared spectroscopy was employed. The ideal formulation for microspheres was determined and produced by taking into consideration the production yield, the effectiveness of entrapment, and the in vitro release study among other factors. The FTIR, DSC, and SEM techniques were utilised in order to characterise a batch of microspheres that had been optimised and given the designation B2. A number of different release kinetic models were used to analyse the data that was collected regarding the quantity of medicine that was released from the batch that had been optimised. Over the course of one month, the batch of microspheres that had been optimised and given the designation B2 was subjected to a short-term stability test. The test was conducted against a temperature range of 40 2 degrees Celsius and a relative humidity of 75%. As a consequence of this, the

outcome indicated that the kinetics of drug release from the formulation of optimised microspheres (B2) adhere to a first order. Dissemination spectroscopy (DSC) was utilised in order to evaluate the melting behaviour of the medication that was present in the microspheres. The optimised microspheres were found to have a rough surface and a spherical shape with the help of a scanning electron microscope (SEM). During the course of the stability investigation, it was established that the improved formulation, B2, exhibited stability.

The formulation for the microspheres that were loaded with aceclofenac was developed by Sunil Datt Belwal and colleagues in the year 2020. Throughout the entirety of the process of producing the microspheres, the method of solvent evaporation was employed. Various amounts of ethyl cellulose and eudragit were used as polymers in conjunction with the medication, although the proportions of each were different. In the formulation (F), the first drug and ethyl cellulose were combined in a ratio of 1:3. On the other hand, in the formulations (F2, F3), the drug and both polymers were combined in ratios of 1:2:1 and 2:5:2, respectively. Particle size analysis, the amount of medicine that each formulation contained, and the rate at which it dissolved were all subjected to scrutiny during the evaluation process. As the concentration of these polymers grew, the amount of medication that could be contained within a microsphere also increased in proportion to the increasing concentration. Formulation F3 was shown to be the most effective formulation during the investigation. Both the solubility of the medicine that is poorly soluble and the rate at which it dissolves are improved by solid dispersion, as indicated by the findings of the overall study.

Through the utilisation of the natural polymer acacia nilotica gum, Abrar Anam and colleagues 2020 were able to successfully create the microspheres for the antiulcer medication. In order to produce famoidine microspheres, the ionotropic gelation method was utilised, and a cross-linking solution that was composed of calcium chloride, barium chloride, and aluminium chloride was utilised. As a consequence of the experiment, anumber of micrometric evaluations of natural polymer microspheres were presented. Better flow and better packaging properties are both contributed to by the micrometric parameters, which include bulk density, bulkiness, compressibility index Hausner's ratio, and angle of repose.

In 2018, **Ranu Biswas and Kalyan Kumar Sen** collaborated on the development and characterisation of a unique herbal formulation of Syzygium cumini seed extract. Polymeric microspheres were used as the delivery vehicle for this formulation throughout the process. In order to manufacture the extract-loaded microspheres that were made with ethyl cellulose (EC), a biological macromolecule, an o/w emulsion solvent evaporation approach was utilised. Polyvinyl alcohol (PVA) was used as an emulsifier. The utilisation of microsphere formulation as a novel carrier for the delivery of herbal medications appears to offer an abundance of potential, as indicated by the findings of this study.

A herbal mixture that had been adsorbed to PEG microspheres was researched by Ribeiro, Aliny A. L., et al. in 2018. The researchers looked at the effect that the mixture had on MCF-7 human breast cancer cells as well as these cells when they were co-cultured with blood MN cells. Furthermore, the MCF-7 cells and the blood mononuclear (MN) cells that were collected from the donors of blood were taken from the volunteers. The MN cells, the MCF-7 cells, and a co-culture consisting of MN cells and MCF-7 cells with or without herbal mixture (HM), polyethylene glycol (PEG) microspheres, or herbal mixture adsorbed in PEG microspheres (PEG-HM) were subjected to a pre-incubation period of twenty-four hours. The technique of flow cytometry was utilised in order to determine the levels of apoptosis, intracellular calcium, and cellular viability. As a consequence of the herbal mixture that has been adsorbed in PEG microspheres, the viability of MCF-7 cells has been reduced. The outcomes of the study suggested that the herbal mixture that was adsorbed by PEG microspheres exhibited apoptotic effects on human MCF-7 breast cancer cells. As a result, the herbal mixture was discovered to be a promising alternative form of treatment.

According to the findings of the research conducted by Alagusundaram et al. (2009), microspheres are distinguished by their capacity to flow freely as powders, their composition of biodegradable proteins or synthetic polymers, and their optimal particle size, which is less than 200 micrometres. A regulated drug delivery system that has been meticulously created has the potential to enhance the therapeutic efficacy of a particular medication. This is something that can be accomplished. Using this approach helps to get beyond some of the constraints that are associated with traditional therapy procedures. In order to achieve the objective of delivering a therapeutic chemical to the target site in a

manner that enables regulated and sustained release, there are a variety of alternative approaches that can be taken. At some point in the future, microspheres will play a pivotal role in the development of innovative drug delivery techniques by integrating a number of different methodologies.

Following the injection of the antibiotic through the pulmonary system, Ventura et al. (2007) recommended the preparation of chitosan microspheres that were loaded with moxifloxacin. Because of this, the medicine would be released over a longer period of time. The process of spray drying results in the production of spherical particles with sizes that are suitable for breathing because of their size. In addition to this, it has a high encapsulation efficiency for moxifloxacin, and the cross-linked microspheres allow the release of moxifloxacin to be prolonged for up to four days.

According to the findings of research that was carried out by Du Toit & colleagues (2006) on the subject of chemotherapy for tuberculosis and its current drug delivery techniques, new medications such as fluoroquinolones provide improved bioavailability with a sudden action and fewer adverse effects. Increasing the bioavailability of Rifampicin would be a step in the right direction towards solving the problem of treatment failure caused by non-compliance on the part of patients. This would be possible if Rifampicin and Isoniazide were administered to patients in a fixed dose combination with segregated delivery. There is a possibility that this would be possible.

The purpose of the research conducted by Riberio et al. (2005) was to synthesise alginate microspheres that were loaded with haemoglobin in order to examine the potential of microspheres as an oral protein delivery mechanism. Following coatings of microspheres with chitosan provide an efficient means of preserving protein in gastric medium and regulate the pace at which protein is released from microspheres into intestinal medium. Chitosan coatings are also an excellent protein preservation method. Almost every traditional medical practise include the use of medicines derived from plants as a significant component. In India, there are roughly 1250 different kinds of plants that are harvested for their medicinal properties. To produce medicinal preparations in accordance with Ayurveda and other traditional medical systems, these plants are employed in the production process.

Recent advancement on Cancer research

The availability of several medications for cancer treatment has advanced significantly; however, the continuous search for more effective treatments has led to the exploration of various new compounds. Among these, dihydropyrimidinones (DHPMs) have gained attention for their wide range of biological activities. Recent research has focused on the anticancer properties of unsaturated pyrimidine structures, including their synthesis methods and the mechanisms underlying their anticancer effects. This review provides an overview of the structure-activity relationship (SAR) of DHPMs as potential anticancer agents, summarizing their synthesis, action mechanisms, and extensive SAR studies (Prasad T et al., 2023).

Bhat AA et al. (2023) demonstrated that pyrrolidine compounds, both synthetic and naturally derived from plants, possess significant pharmacological properties. Various synthetic pyrrolidine derivatives, such as spirooxindole, thiazole, and coumarin, have shown strong anticancer potential. These molecules exhibit minimal adverse effects and efficiently target multiple cancer-related pathways, depending on their substitution patterns. This review covers recent advances in pyrrolidine synthesis and highlights their promising anticancer activity across various cancer cell lines, emphasizing the SAR behind these effects.

Flavonoids are known to inhibit cancer cell growth through several mechanisms, including the inhibition of topoisomerases, protein kinases, angiogenesis, and the induction of apoptosis. This review outlines the key structural features of flavonoids and their anticancer mechanisms. A detailed analysis of previously published data on flavonoids' anticancer properties is provided, focusing on the role of structural factors, such as the presence of a C2-C3 double bond, an oxo group at C4, and other key elements responsible for their anticancer activity. Additional research is required to improve the target specificity and selectivity of flavonoids as anticancer agents (Shah S et al., 2023).

Iron and heme have been identified as potential enhancers of anticancer therapies (ARTs). Zhang Y et al. (2023) demonstrated that zinc protoporphyrin-9 (ZnPPIX), a natural heme metabolite, significantly enhances the anticancer activity of dihydroartemisinin (DHA) in various cell lines. This combination increases intracellular free heme levels and reactive

oxygen species (ROS) production. In animal models, ZnPPIX boosted DHA's tumor-suppressing ability without causing toxicity, suggesting a promising synergy for improving ARTs in cancer treatment.

The unfolded protein response (UPR) helps cells cope with stress by preventing the accumulation of misfolded proteins in the endoplasmic reticulum (ER). However, cancer cells exploit this pathway to survive under chronic stress. Bartoszewska S et al. (2023) discussed the potential of targeting the inositol-requiring enzyme 1a (IRE1) pathway, a key component of UPR signaling, for cancer therapy, highlighting the development of IRE1 inhibitors as a promising therapeutic strategy.

Active peptides, particularly anticancer peptides (ACPs), show therapeutic potential through mechanisms like pore formation in cell membranes or targeting vital intracellular processes. This review discusses recent developments in ACPs and antimicrobial peptides (AMPs) with anticancer activity, focusing on their design, mechanism of action, and therapeutic applications. Despite challenges like high production costs and toxicity toward normal cells, computational methods have facilitated the design of novel ACPs, which could serve as promising cancer therapies (Kordi M et al., 2023).

Annona muricata, a plant from the Annonaceae family, has demonstrated potential anticancer properties due to its alkaloids, phenols, and acetogenins. Ilango S et al. (2022) reviewed the role of A. muricata in cancer treatment, focusing on its ability to modulate cellular proliferation, promote apoptosis, and reduce cancer cell invasion. The study highlighted the molecular mechanisms by which A. muricata affects cancer-related pathways, presenting its bioactive compounds as promising anticancer agents.

Sesquiterpenoids like drimanes and coloratanes, found in various marine organisms and plants, have shown cytotoxic activity against cancer cells. Despite their promising in vitro results, the structure-activity relationships (SAR) of these compounds have not been fully explored. Beckmann L et al. (2022) summarized the structures and derivatizations of these sesquiterpenoids, along with their anticancer activity, emphasizing the need for further research to fully understand their potential as novel anticancer agents.

Phycocyanobilin (PCB), a chromophore involved in various biological processes, has shown promise in combating cancer, inflammation, and oxidative stress. Li Y et al. (2022) reviewed the biological activities of PCB and its potential applications in disease treatment. PCB's antioxidant properties, along with its anti-inflammatory effects, make it a promising candidate for treating conditions like cancer, atherosclerosis, and COVID-19 complications, among others.

Jiang et al. 2016 provided evidence that GA possesses anticancer properties by testing it on two different human breast cancer cell lines (MCF-7 and MDA-MB-231) Research conducted using in vitro models demonstrated that GA caused significant cytotoxicity in both of the cancer cell lines. Other cancer cells, such as gastric cancer cell lines (MKN28, AGS, SGC7901, and MN45), were also affected by the GA treatment. Their growth cycle was arrested at the G2/M transition, which resulted in apoptosis of the cells.

Hasan, S.K. et al. 2016 found that GA inhibits the proliferation of HepG2 cells in liver cancer. In addition, GA causes an increase in the formation of ROS and NO production and causes a loss of the mitochondrial membrane potential.

In this sense, **Li, J.Y. et al. 2014** highlighted the in vitro and in vivo antioxidant activities of GA. Several other studies supported these findings. These methods have demonstrated antioxidant activity against reactive oxygen species, such as hydroxyl radicals, peroxyl radicals, and superoxide ions. These reactive oxygen species play an important role in the treatment of diseases involving reactive oxygen species (ROS) or in mechanisms related to photoaging. GA may impact cellular ROS levels through additional direct or indirect mechanisms, and it may activate the nuclear factor (erythroid-derived-2)-like 2 (Nrf2) pathway. This may occur because GA regulates the redox state of Keap1.

In the case of skin cancer, GA has been considered a natural antioxidant agent that protects mitochondrial functions in the presence of oxidative stress. This treatment is intended for tumours that are caused by exposure to UVB radiation. In other studies, GA was shown to have chemopreventive potential against DMH-induced colon carcinogenesis. This was accomplished by decreasing the immunostaining of Ki-67, NF-kB -p65, COX-2, iNOS, and VEGF in the colon of Wistar rats, while simultaneously increasing the immunostaining of

p53, connexin43, caspase-9, and cleaved caspase-3. Khan, R, et al., 2013 is the group that carried out this research.

The primary molecular antitumor mechanism of GA, as described by Ming, L.J.; et al. (2013) in the literature, involves the up-regulation of p53 and p21/Cip1 to protect DNA from damage and promote DNA repair while down-regulating proliferation, metastasis, cell-cycle arrest and apoptotic cell death, and transcription factor-NF-B.

According to Cherng, J.M. et al2011 .'s study, GA could prevent or postpone the development of UVB-induced skin cancer in a hairless mice skin model. GA's protective effects were mediated at the molecular level through NF-kB, COX-2, PGE2, and NO level inhibition. Because GA contains various isoflavonoids, including glabridine and its derivatives, these benefits were also linked to the substance's strong antioxidant properties.

The uterus and cervix carcinoma cell line SiHa were used in a 2008 study by Lee et al. to examine the harmful effects of GA. According to the findings, the GA activity may be linked to an increase in ROS production and GSH depletion via altering the permeability of the mitochondrial membrane, which releases cytochrome c and activates caspase-3.

In this field of study, Hoever et al. Studies have shown that GA has anti-cancer properties against many cancer cell types, including those of the mouth, stomach, breast, skin, cervix, and liver.

Horigome, H. et a(2001)'s in vivo investigations show that GA triggers programmed cell death, most likely through blocking the liver enzyme 11-hydroxysteroid dehydrogenase type I.

Previous work done on *Glycyrrhiza glabra*

In 2021**, Ilaria Marotti and colleagues** studied the impact of polyphenol extracts from the leaves, stems, and roots of 20-day-old licorice microgreen seedlings on the proliferation and viability of Caco-2 cells, which mimic the intestinal epithelium. This was done following the induction of inflammation using lipopolysaccharide (LPS). The results were then compared with the polyphenol, flavonoid, and anti-radical properties of the different tissue extracts.

In another study from 2021, Changchao Huan and colleagues explored recent findings related to the antiviral properties of glycyrrhizin, a key bioactive compound in licorice along with glycyrrhetinic acid. These triterpenoids have shown efficacy in inhibiting various viruses, including SARS-CoV-2. Their modes of action include inhibiting viral replication, directly inactivating viruses, reducing inflammation through HMGB1/TLR4 inhibition, and decreasing reactive oxygen species production.

Using in-silico techniques**, Saurabh K. Sinha et al., (2020)** aimed to identify the licorice compounds responsible for antiviral effects against COVID-19 protein targets. Through molecular docking simulations, glyasperin A exhibited strong affinity for Nsp15 endoribonuclease, while glycyrrhizic acid was effective in the binding pocket of spike glycoprotein, preventing viral entry into host cells. Molecular dynamics simulations were conducted to further assess the behavior of these docked molecules.

In 2019, Namavar Jahromi et al. studied the effects of Glycyrrhiza glabra (licorice), a cyclooxygenase-2 inhibitor (Celecoxib), and a gonadotropin-releasing hormone analogue (Diphereline) on endometrial implants in rats. The licorice group demonstrated significantly reduced implant area, volume, and HLM count compared to the control group.

Jun-Xian Zhou and colleagues (2019) investigated the phytochemical content and biological activities, including antioxidant, cytotoxic, and antimicrobial effects, of methanol extracts from Glycyrrhiza glabra, Paeonia lactiflora, and Eriobotrya japonica. They found that Paeonia lactiflora had strong radical scavenging and antibacterial properties, while Glycyrrhiza glabra exhibited moderate antibacterial activity. Further studies were recommended to assess their potential in antioxidant or antibacterial therapies.

Lateef M. and his team (2012) explored the antioxidant activity of Glycyrrhiza glabra root extracts using various solvents. They found that the crude methanolic extract had the highest antioxidant power, while the ethyl acetate fraction showed significant urease inhibition activity compared to thiourea.

Franceschelli S. et al. (2011) examined the anti-inflammatory properties of licochalcone C, a compound from licorice, in THP-1 cells under inflammatory conditions. The treatment reduced iNOS expression and activity, decreased extracellular oxygen production, and

modulated antioxidant enzyme activity, suggesting licochalcone C's potential as an antioxidant.

In 2011, Siracusa L. and colleagues evaluated the antioxidant, anti-genotoxic, and anti-inflammatory properties of Glycyrrhiza glabra leaf extracts. They identified various polyphenols, including flavonoids and dihydrostilbenes, as key active compounds, with the ethyl acetate extract showing the most significant bioactivity.

Li YJ et al. (2011) developed a novel method for screening antioxidants in licorice using DPPH spiking tests combined with HPLC-Q-TOF MS/MS. Their approach identified 21 antioxidant compounds in Glycyrrhiza species, 10 of which had known antioxidative activity, while 11 were newly identified as radical scavengers.

Mukherjee M et al. (2010) evaluated the anti-ulcer and antioxidant properties of Gut-Gard, a standardized extract of Glycyrrhiza glabra. Their study showed dose-dependent reductions in ulcer indices and increases in pH levels, along with strong antioxidant activity, supporting licorice's traditional use for treating ulcers.

D'Angelo S et al. (2009) investigated the antioxidant potential of methanolic licorice polyphenol extracts (LPEs) and found that LPEs offered significant protection against oxidative damage in human colon cancer cells, likely due to their high polyphenolic content.

Visavadiya NP et al. (2009) assessed the antioxidant activity of Glycyrrhiza glabra in vitro. Both aqueous and ethanolic extracts showed strong scavenging activity against various radicals and reduced lipid peroxidation, suggesting their potential in preventing oxidative stress-related diseases.

Yadav AS et al. (2007) evaluated the antioxidant effects of spices, including licorice, in rat liver homogenate. Licorice displayed significant metal chelating activity and DPPH radical scavenging, highlighting its potent antioxidant properties.

In a study on hypercholesterolemic rats, Visavadiya NP et al. (2006) found that Glycyrrhiza glabra root powder significantly lowered cholesterol levels and enhanced antioxidant enzyme activity, indicating its hypocholesterolemic and antioxidant effects.

Di Mambro VM et al. (2005) studied the antioxidant properties of Glycyrrhiza glabra and

Ginkgo biloba extracts in skin formulations. Both extracts demonstrated strong free radical scavenging activity, suggesting their potential for use in skincare products to protect against oxidative damage.

Lastly, Morteza et al. (2003) compared the antioxidant effects of licorice root extract with synthetic antioxidants in hydroquinone cream and found that licorice exhibited comparable antioxidant activity at various concentrations.

Fukai T et al. (2003) researched the anti-arthritis activity of glabridin, a compound derived from Glycyrrhiza glabra. Glabridin significantly reduced protein excretion in a nephritis model without affecting radical scavenging, indicating its potential as an anti-nephritis agent.

Work done Molecular modelling of cancer

In 2023, Guerfi M et al. examined a group of newly synthesized β-sulfamidophosphonate derivatives (3a-3g) for their anticancer properties against human cancer cell lines (PRI, K562, and JURKAT). When compared to the standard drug chlorambucil, the compounds showed moderate antitumor activity using the MTT assay. The IC50 values for compounds 3c and 3g were reported as 0.056-0.097 mM against PRI cells and 0.182-0.133 mM against K562 cells. A molecular docking study revealed that these compounds might inhibit glutamate carboxypeptidase II (GCPII), and further computational analysis using Density Functional Theory (DFT) supported these findings. Pharmacokinetics and toxicity assessments conducted using SwissADME and OSIRIS software indicated that all synthesized compounds exhibited acceptable bioavailability and non-toxic profiles.

In another study from 2023, Hadiyal SD et al. synthesized a series of benzofuran-thiazole hybrid compounds using a multi-step reaction. These compounds were screened by the National Cancer Institute for anticancer activity across 60 human cancer cell lines. Two of the compounds demonstrated significant lethality and were selected for further testing in five-dose assays. Compounds 8g and 8h exhibited GI50 values ranging from 0.295 to 4.15 μM and LC50 values between 4.43 and >100 μM, showing strong antiproliferative effects, with 8g outperforming the standard drug fluorouracil. Molecular docking studies into the

1HOV protein's binding regions provided insight into the binding interactions of these compounds.

Yousef TA et al. (2012) focused on developing new oxadiazole derivatives (8a-f) as inhibitors of tubulin polymerization. Their structural confirmation was carried out using element analysis, mass spectrometry, and NMR. Compounds 8e and 8f exhibited improved IC50 values of 3.19-8.21 μM against MCF-7, HCT116, and HepG2 cancer cell lines compared to colchicine. Molecular docking studies indicated key hydrogen bonding and hydrophobic interactions at the binding sites, and compounds 8e and 8f showed the highest tubulin inhibitory activity with IC50 values of 7.95 and 9.81 nM, respectively. These results suggest that the 1,3,4-oxadiazole scaffold has potential for the development of future anticancer drugs.

Erianin, a bisbenzyl compound found in Dendrobium chrysotoxum Lindl., has shown potential therapeutic effects in several cancers, including hepatoma, melanoma, non-small-cell lung carcinoma, leukemia, breast cancer, and osteosarcoma. Recent studies have demonstrated erianin's pharmacological activities, including its antioxidant and anticancer properties. Yan L. et al. conducted a systematic review of the anticancer mechanisms of erianin, compiling current data on its effects in specific cancers. The SwissTargetPrediction online tool was used to predict erianin's molecular targets, while the OMIM database identified potential treatment targets in various cancers. Kaplan-Meier and GEPIA tools were used to assess the prognostic value of these targets. Molecular docking was performed using Discovery Studio to confirm erianin's binding potential, and the KEGG pathway was used to map possible disrupted signaling pathways. The study also conducted an in silico prediction of erianin's ADMET properties. Overall, this research aimed to consolidate data on erianin's pharmacological effects, identify its therapeutic targets, and assess its ADMET characteristics for cancer treatment.

CHAPTER - 3
PROFILE OF SOME PROPOSED PLANTS FOR RESEARCH

GLYCYRRHIZA GLABRA

Fig.23: *GLYCYRRHIZA GLABRA*

Common/English Names	**Vernacular Names**
Black Sugar, Common Liquorice, Licorice, Licorice-Root, Liquorice, Liquorice Root, Persian Licorice, Rhizoma Glycyrrhizae, Russian Licorice, Russian Liquorice, Si-Pei Licorice, Sinkiang Licorice, Spanish Juice, Spanish Licorice, Spanish Liquorice Sweet Root, Sweet Wood, Sweet Wood Liquorice, True Licorice, True Liquorice	Afrikaans: Drop Albanian: Gliciriza E Shogët, Glicirizë Arabic: Irq As-Sus, Irqu As-Sus, Irqu Al-Sus, Sous, Sus Armenian: Madoodag, Matutak Azeri: Biyanlıq Basque: Erregaliz, Gotxerro, Makilgoxo Belarusian: Lakryčnik Brazil: Alcaçuz (Portuguese) Breton: Regalis Bulgarian: Sladnik, Sladuk Koren Burmese: NoekiyuCatalan: Regaléssia

Biological source: It is made up of the dried, peeled or unpeeled root and stolon of a plantthat is a member of the Leguminosae family.

Geographical Source: It has been reported that the drug is grown in Baramulla, and South India. Commercial cultivation of the drug takes place on a large scale in Spain, Sicily, England, and India.

Different Varieties of G.glabra:

1. G.glabra variety:Spanish liquorice:This plant has purplish blue coloured papilinaceous flowers. It gives out large no.of stolons.
2. G.glabra variety:Russian liquorice:It has a big root stock along with a number of elongated roots but does not bear stolons.
3. G.glabra variety:Violaceae:Persian liquorice:It has violet flowers.

Macroscopic Characters	**Microscopic Charaterstics**
This herbaceous perennial can grow up to one metre in height. Its pinnate leaves are 7 to 15 centimetres (3 to 6 inches) in length and have nine to seventeen leaflets each. The blossoms can be deep purple or pale white blue in colour, and they range in length from 0.8 to 1.2 millimetres (about 1/2 to 1/3 of an inch). The fruit is an oblong pod with several seeds that is 2 to 3 centimetres (about 1 inch) long. The main ingredient giving liquorice its sweet flavour is a substance called anethole, sometimes referred to as trans-1-methoxy-4-(prop-1-enyl)benzene. Anise, fennel, and several other herbs also contain anenethole, a fragrant, unsaturated ether molecule. A large portion of liquorice's sweetness comes from a substance called glycyrrhizin, which is sweeter than sugar. (Nadkarni K M;1989).	Secondary phloem is a broad band with cells of inner part cellulosic and outer lignified, radially arranged groups of about 10-50 fibres, surrounded by a sheath of pare. The cork of the stolon consists of 10-20 or more layers of tabular cells, with the outer layers containing reddish-brown amorphous contents, the inner 3 or 4 rows having thicker, colourless walls, the secondary cortex usually Root-T.S. resembling stolon with the exception that there is no medulla present, xylem tetrarch, usually four principal medullary rays at right angles to each other, in peeled drug cork shows phelloderm and sometimes without secondary phloem, all parenchymatous tissues containing abundant, simple, oval or rounded starch grains, 2-20 in length. Root (The Wealth of India;1982).

Chemical Constituents:

The main ingredient in liquorice is glycyrrhin, also known as glycyrrhic acid, a triterpenoid saponin that is the potassium and calcium salt of glycyrrhizinic acid. Another name for glycyrrhin is glycyrrhizinic acid. The hydrolysis of glycyrrhizinic acid, a glycoside, yields glycyrrhetinic acid, sometimes referred to as glycyrrhetic acid, which is structurally related to a triterpenoid. Antioxidant activity is significantly higher in isoflavones glabridin and hispaglabridins A and B, which are glabridin's isoflavones. Glycyrrhiza glabra Linn. contains triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and a host of other compounds. Glycyrrhizin, a kind of triterpenoid, is the chemical responsible for the sweet flavour of Glycyrrhiza glabra Linn root. The flavonoid content of Glycyrrhiza glabra Linn, which includes liquiritin, isoliquiritin (a chalcone), and other chemicals, is what gives the plant its yellow colour. Both glabrene and glabridin have estrogen-like characteristics and are more deeply implicated in the metabolism of corticosteroids, specifically glycyrrhizic acid.

There is a wide range of variability in the levels of glycyrrhizin found across the different varieties.

Spanish liquorice contains 5 to 10 %,

Russian variety contains about 10%, while

The proportion of glycyrrhizin in Persian liquorice varies from 7.5 to 13%. Other ingredients in liquorice include fat, asparagin (up to 4%), glucose (up to 4%), sucrose (2.5–6.5%), and resins containing the bitter substance glycyramarin.

Flavonoids, which are present in liquorice, are believed to have an antigastric action and may be useful in the management of peptic ulcers. This is just one more significant chemical element of liquorice. The flavonoids are isoliquiritin and liquidritin; they are both yellow in appearance.

Ethano-botanical uses (Ali Esmail Al-Snafi;2018):

Traditional uses	**Medicinal Uses**
➢ Expectorants and demulcents have historically been two of liquorice's most popular applications. In formulations containing quine, ammonium chloride, alkali iodides, quinine, cascara, and other like compounds, it is also employed as a flavouring ingredient. It is a component in cough mixes. Because it contains isoliquiritin, a flavonoid with antigastric properties, deglycyrrhized liquorice is used in the treatment of peptic ulcers (DGL). This form's decreasedminerocoeticoid activity makes it useful for treating peptic ulcers and encouraging healing.	In addition to its anti-inflammatory and antiviral characteristics, and it includes saponin, glycyrrhizin, and a variety of other vital substances required for optimal health. It is most commonly used to treat cervical cancer, kidney and bladder disorders, HIV, hepatitis B, herpes, mucous membrane inflammation, food poisoning, stomachaches, coughs, horse-voice, bronchitis, asthma, ulcers, arthritis, shingles, sunburns, infant fevers, and insect bites.
➢ Additionally, the drug hasantispasmodic qualities. Isoliquiritin, a flavonoid glycoside, is the cause of this. This glycoside's aglycone component is responsible for its antispasmodic properties. Because ofits minerocorticoid activity, which is brought on by glycyrrhetinic acid, itis used in place of corticosteroids to treat inflammations, rheumatoid arthritis, and Addison's disease. Nevertheless, the excessive dosages may result in salt retention, which may then lead to a serious electrolyte imbalance, hypertension, and water retention. The use of glycyrrhizin as an anti-inflammatory drug is well-established. ➢ The most popular use of liquorice is as a flavouring for stuff and chewing tobacco. The flavour of liquorice is sweet and licorice-like. Ammoniated glycyrrhiza is used as a flavouring additive in the	Glycyrrhizic acid from liquorice is about fifty times sweeter than sucrose. It is thought to support the adrenal glands, promote hormone excretion from the adrenal cortex, and increase hormone production, such as hydrocortisone. Liquorice possesses oestrogenic characteristics and can help regulate menstrual periods. It is also commonly accepted as an excellent treatment for a number of lung and spleen disorders. It is widely used in conjunction with other herbs to treat coughs, colds, sore throats, asthma, stomach and duodenal ulcers, hepatitis, hysteria, food poisoning, hypoglycemia, bronchitis, colitis, diverticulitis, gastritis, as well as some stress-related diseases, nausea, and inflammation. Liquorice root can aid in colon cleansing, adrenal gland stimulation, muscle spasm reduction, and increased mucus fluidity produced by the lungs and bronchial tubes.

beverage, confectionery, and pharmaceutical industries. ➢ Some stories state that the residue left over from the manufacture of the liquorice liquid extract was utilisedto stabilise the foam in a particular kind of foam fire extinguisher. According to some claims, the liquorice compound powder, whichincludes liquorice as a component, has a senna-like potentiating effect.	Licorice's medical characteristics include anodyne, antispasmodic, anti-inflammatory, demulcent, depurative, diuretic, cooling, expectorant, emollient, oestrogenic, expectorant, anti-arthritic, tonic, adrenal stimulant, anti-cholesterolemic, anti-gastritis, and anti-allergenic. Liquorice glycoside, which has been demonstrated to be useful in treating Addison's disease, is structurally similar to endogenous hormones produced by the body.

Pharmacological Activities (Dhingra D and Sharma A;2006 & Rodino S etal;2015):

- Antidepressant effect
- Antimicrobial effects
- Anticancer effect
- Antioxidant effect
- hepatoprotective potential
- Anti-inflammatory effect
- Effective against gastric duodenal ulcers:Carbenoxolone
- Hypolipidemic effect

EXCIPIENT PROFILE

Chitosan

Chitosan is a naturally occurring polysaccharide that is made up of glucosamine and n-acetylglucosamine in the form of a copolymer. This is achieved by removing a portion of the chitin from the crustacean shell and then partially deactivating it. Crustaceans, insects, and fungi are the three primary food sources for chitin. The term chitosan is used to describe various chitosan polymers with molecular weight (50 kDa 2000 kDa), viscosity (1% chitosan in 18-acetic acid <200 mPas), and degree of acetylation (4098%).

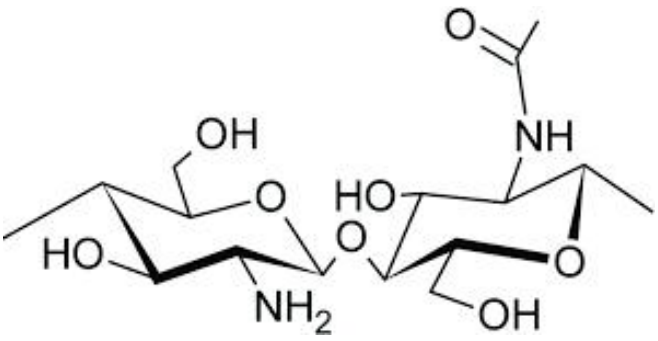


Fig. 24 Chemical Structure of Chitosan

Physicochemical Properties (Illum Lisbeth *etal*;1998)

- It is a linear polyamine that contains a number of amino groups that can participate in chemical reactions and can easily form salts.
- However, it is soluble in acids and forms salts with both inorganic and organic acids. It is insoluble at pH levels that are neutral and alkaline.
- Chitosan has a particle size of less than 30 micrometres, a density of 1.35-1.40 g/cc, and a pH ranging from 6.5 to 7.5.

Biological Properties

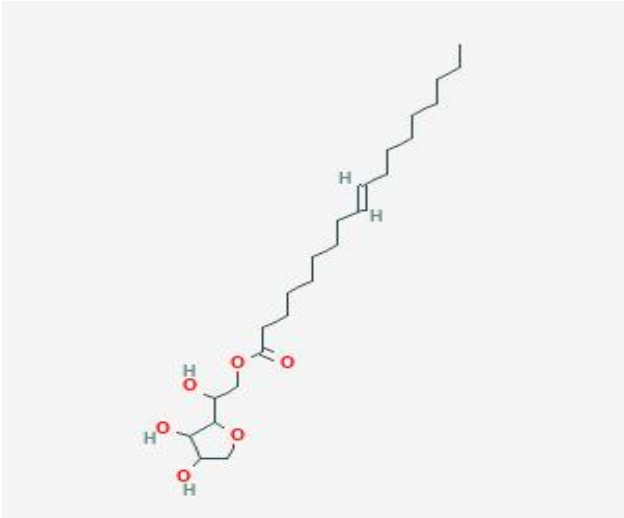
- This polymer is safe to use, it is compatible with living organisms, and it breaks down naturally.

Pharmacological Properties

It has been shown to be effective as a hypocholesterolemic agent, as well as a wound healer, an antacid, and against ulcers.

Pharmaceutical Applications

- Chitosan had excellent properties as an excipient for direct compression of tablets, which resulted in rapid disintegration when combined with a percentage of chitosan equal to or greater than 50 percent.
- Chitosan gel was put to use as a vehicle for the sustained release of the drugs that were not very soluble.

Properties	**Description**
Chemical Structure	
Molecular Formula	$C_{24}H_{44}O_6$
Synonyms	Sorbitan monooleate Sorbitan, mono-(9Z)-9-octadecenoate Arlacel 80 Span 80
Molecular Weight	428.6
Application	The United States of America and the European Union are two of the numerous countries that make use of polysorbate, which is a solid material. Numerous foods, including bread, cake mix, salad dressing, shortening oil, and chocolate, all make use of it as an emulsifier and a solubilizer through its application. The reaction between sorbitan fatty acid ester, which is a nonionic surfactant, and ethylene oxide results in the formation of polysorbate. Many countries outside of the United States make use of polysorbate.

	In order to create polysorbate 80, there are two different kinds of surfactants: hydrophilic and nonionic. We use it as a surfactant in soaps and cosmetics, and we use it as a lubricant in eye drops. A further application for it is in the cosmetics sector. In the context of food or medicinal items, it is conceivable that it could serve the purpose of an emulsifier. Polysorbate 80 is an excipient that is utilised for the purpose of stabilising aqueous formulations of certain drugs that are intended for parenteral administration or vaccines. Pharmaceuticals are the industry that makes the most frequent use of it. The utilisation of a solubilising agent, which also serves as a surfactant, is what makes one material more soluble in another substance. It is possible to bring a substance that would not ordinarily dissolve in a particular solution to a state where it is able to do so with the assistance of a solubilising agent. Once in this state, the substance will be able to dissolve. It is also known as an emulsifier, which is a substance that helps components mix together and prevents separation. Additionally, it is a component of water that contains small levels of salts, and it is included in a number of vaccines that have been granted official approval in the United States of America.

Fig. 25 Sorbitan monooleate(Drug bank)

CHAPTER - 4

AIM AND OBJECTIVES OF RESEARCH WORK

By incorporating the herbal drugs into more contemporary dosage forms, one is able to use them in a more conventional manner while simultaneously increasing their level of effectiveness. Developing original methods of drug delivery that are suitable for use with botanical components is one way to achieve this goal.

The current research is being conducted with the intention of establishing phytochemicals as a fruitful and productive area of research. There has been an increase in the incidence of cancer, and due to the high cost, various limitations of conventional treatments, including the high cost, and the high toxicity of current anticancer drugs, there are alternatives that are cost-effective, environmentally friendly, and biocompatible. Plant molecules, which are expected to revolutionise cancer treatment over the course of the next decade, are a strategy that is friendlier to the environment in this scenario. Together with their novel drug delivery system, the high biodegradability and biocompatibility of these plant molecules have contributed to their increased effectiveness in the treatment of cancer. Both the rate at which the drug is released and its capacity to pass through biological membranes are altered by the controlled release system. This approach to increased pharmacological activity while simultaneously reducing the number of adverse effects and the drug dose. The sustained release system is an effective and convenient tool for the delivery of drugs at specific times and locations.

As a result, the purpose of this project is to formulate microspheres that are loaded with Phytoconstituent of and evaluate their effectiveness as a sustained release drug delivery system. Microspheres are an essential component of this innovative system for the delivery of drugs. The significance of these microspheres, however, is constrained by the fact that they spend only a brief amount of time at the site of absorption. Further *in-vitro* and *in-silico* validation of microsphere was carried out to assess the antiproliferative potential.

CHAPTER - 5

PLAN OF WORK

Step1

- Literature Review.
- Collection and identification of plants.
- Pharmacognostic evaluation of plants.
- To prepare various extracts (Ethanolic, methanolic& petroleum ether) of selected plant material.
- Isolation of Glycyrrhizin
- Characterization of isolated moiety by various modern analytical techniques.

Step 2

- Preformulation studies
  - Melting point determination
  - Solubility studies
  - FT-IR study of drug
  - Determination of λ max (maximum wavelength)
  - Calibration curve of drug
  - Partition Coefficient
- Preparation and optimization of microspheres.
- *In- vitro* evaluation of capsules containing drug-loaded microspheres
- Percent Yield (%), Drug entrapment efficiency,
- Particle size distribution & FT-IR study of formulation

Step 3

- Proposed Mechanism studies by Molecular docking approach.
- Evaluation of Anti-proliferative activity

CHAPTER - 6

MATERIAL AND METHOD

All chemicals and solvent used in the preparation of Glycyrrhizin loaded microsphere purchased from different resources as shown in table1 were highly purified.

Table 1. List of chemical/solvents used in the preparation

S.No	Chemical/Solvents	Source
1.	Glycyrrhizin	Isolated from *G.glbra* and characterized.
2.	Hydrochloric acid	Rankem lab
3.	Potassium Hydroxide	Loba Chemie Pvt Ltd
4.	n-Octanol	Rankem lab
5.	Acetone	Loba chemie Pvt Ltd
6.	Ethanol	Loba chemie Pvt Ltd
7.	Chitosan	Rankem lab
8.	Glutaraldehyde	Rankem lab
9.	Sodium Chloride	Rankem lab
10.	Acetic acid	Rankem lab
11.	Light liquid paraffin	Loba chemie Pvt Ltd
12.	Heavy liquid Paraffin	Loba chemie Pvt Ltd
13.	Petroleum Ether	Rankem lab
14.	Water (HPLC grade)	Rankem lab

Table 2. List of Instruments used

S.No	Instruments	Makers
1.	Weighing Balance	Wensar^{TM}
2.	Sonicator	Khera instrument Pvt Ltd
3.	FT-IR (for preformulation study)	Bruker Germany
4.	FT-IR (for formulation study)	Shimadzu 8300
5.	UV spectrophotometer	1700 pharmaspec shimadzu
6.	Microscope	LEICA DM1000

6.1 Plant material collection

Evaluation of Physicochemical Parameters:

A. Physical Evaluation:

- **Determination of Foreign Matter (Harborne, 1984):**

  Macroscopic examination of medicinal plants is crucial for accurate identification and to detect any adulterants.

B. Determination of Solvent Extractive Value (Mukherjee, 2002):

a) Water-Soluble Extractive Value:

- 5 g of the powdered drug was macerated in 100 ml of water for 2 hours in a closed flask. It was then allowed to stand for 18 hours, with periodic shaking for the first 6 hours. After filtration, 25 ml of the filtrate was evaporated to dryness in a shallow flat-bottomed dish at 105°C and weighed. The percentage of water-soluble extractives was calculated based on the air-dried sample.

b) Alcohol-Soluble Extractive Value:

Alcohol is an ideal solvent for extracting components like tannins, alkaloids, and resins. To determine the alcohol-soluble extractive value, 5 g of powdered drug was macerated in 100 ml of 95% ethanol for 24 hours, with periodic shaking for 6 hours, and then allowed to stand for 18 hours before filtering. The filtrate was evaporated to dryness at 105°C in a shallow dish and weighed. The percentage of alcohol-soluble extractives was calculated using the air-dried drug.

c) Determination of Moisture Content (Mukherjee, 2002):

Moisture content is critical in controlling the quality of crude drugs, as excessive Moisture can lead to chemical degradation or microbial growth.

Procedure:

5 g of the powdered sample was placed in an infrared moisture balance. The weight loss due to moisture was expressed as a percentage based on the air-dried sample.

C. Determination of Ash Value (Mukherjee, 2002):

Ash value helps determine the presence of inorganic matter, which may either occur naturally or be added deliberately as adulterants. This includes components like sand, soil, or other mineral elements. The ash value is a measure of purity for crude drugs. Total ash and acid-insoluble ash were measured as follows:

a) Total Ash Determination:

2 g of the air-dried drug sample was weighed and placed in a pre-tared silica dish, then ignited at a temperature not exceeding 450°C until the residue was free of carbon. The ash content was calculated.

b) Acid-Insoluble Ash Determination:

The ash obtained from the total ash procedure was boiled with 25 ml of 2M HCl for 5 minutes. The insoluble residue was collected on an ash-free filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was calculated using the air-dried sample as the reference.

c. Determination of water soluble ash:

The inert substance was subsequently gathered on ash-free filter paper, boiled in hot water for 15 minutes, and ignited while the temperature remained below 450°C. The ash was initially heated in 25ml of water for 5 minutes. To determine the amount of water soluble ash, subtract the weight that contains the insoluble material from the weight of the ash. The proportion of water soluble ash was determined using the air-dried sample as a reference.

Table: 3 Physiochemical properties root of *G.glabra*

S.No	Ash values	Observation in (w/w)
1	Total ash	4.7%
2	Acid insoluble ash	0.55%
3	Water soluble ash	6.55%
4	LOD	5.5%

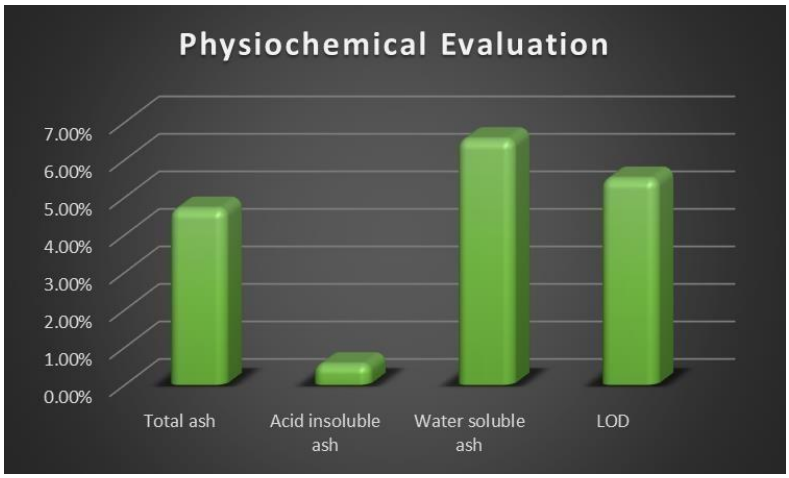


Fig. 26 Graph: 1 Physiochemical properties of root of *G.glabra*

Determination of alcohol soluble extractive value

In a closed flask, 100 millilitres of alcohol were macerated with five grammes of powdered, air-dried medication that had been carefully weighed. The maceration lasted for twenty-four hours, with regular shaking occurring during the first six hours and standing time lasting for eighteen hours. Following that, it was filtered as rapidly as possible to prevent any loss of solvent. Twenty-five millilitres of the filtrate were evaporated until it was completely dry, then dried at 105 degrees Celsius until it reached a constant weight, and finally, it was weighed. The dish had a flat bottom and had been covered with tar. We used

the air-dried medicine as a foundation in order to calculate the percentage of the extractive that is soluble in alcohol. For the purpose of carrying out the computation, the following formula was utilised.

To calculate the percentage of water-soluble extractive value, multiply the weight of the extract by 100 and then divide that number by 25 and then multiply the sample weight by the total weight of the extract.

Determination of water soluble extractive value

In a closed flask, 5 g of meticulously measured powdered, air-dried medicine was macerated for 24 hours with 100 ml of chloroform water, shaking frequently during the first six hours and standing for 18 hours. Following that, it was rapidly filtered to prevent solvent loss. In a shallow dish with a flat bottom coated in tar, 25 cc of the filtrate was evaporated to dryness, dried to a constant weight, and weighed. The percentage of water-soluble extractive was calculated using air-dried medicine as a reference.

Table 4: % Extractive value of root extract of *G.glabra*

Extractive value	**Inference**
Alcohol soluble	8.8%
Water soluble	18.6%

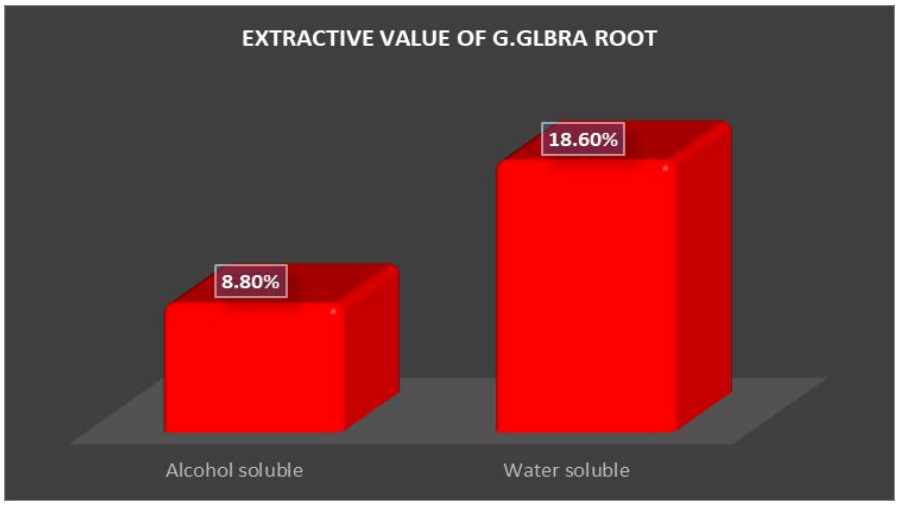


Fig. 27 Graph 2: % extractive value of root of G.glabra

Extraction

50 grammes of powdered root were extracted with a Soxhlet apparatus and 250 millilitres of hot water until the water was depleted. The extract was filtered and concentrated by vacuum evaporation.

Fig. 28: Extraction Process

6.2 Preliminary Phytochemical Screening of Plant Extracts

Physico-chemical parameters were determined as per standard procedures Soni H etal. 2011.

Preliminary qualitative test

The extract was subjected to preliminary qualitative phytochemical investigation Khandelwal; 2002.

Alkaloids

Prior to filtering, a small amount of alcohol extract was combined with a few drops of diluted hydrochloric acid. A variety of alkaloid reagents, including:, were then employed to extensively analyse the filtrate.

(a) Mayer's reagents

Before filtering, a small amount of alcohol extract was combined with a few drops of diluted hydrochloric acid. A variety of alkaloid reagents, including: were then employed to extensively analyse the filtrate.

(b) Dragendorff's reagent

Dragendorff's reagent causes an orange-brown precipitate to form when used with alkaloids. When 1 ml of extract was mixed with 1 ml of potassium bismuth iodide solution, an orange-red precipitate formed, suggesting the presence of alkaloids.

(c) Hager's reagent

Alkaloids yield a precipitate that is yellow when using Hager's reagent. A yellow precipitate produced after adding Hager's reagent, a saturated aqueous picric acid solution, to 1 ml of extract, indicating the presence of alkaloids.

(d) Wagner's reagent

When combined with alkaloids, Wagner's reagents cause a reddish brown precipitate. A reddish brown precipitate appears when 1 ml of extract is mixed with 2 ml of Wagner's reagent (iodine in potassium iodide), demonstrating the presence of alkaloids.

ii. Carbohydrates and glycosides:

Separately, distilled water was used to dilute each extract before it was filtered. The following carbohydrate test is run on the filtrate.

(a) Molisch's Test

Two milligrammes of extract, 1 ml of -naphthol solution, and 1 ml of concentrated sulphuric acid were mixed through the test tube's side. The emergence of a purple or reddish violet hue at the intersection of the two liquids indicated the presence of carbs.

(b) Fehling's solution

When Fehling's A and Fehling's B, both copper sulphate in distilled water and potassium tartrate and sodium hydroxide in distilled water, are heated with a few drops of extract solution, they produce a brick red cuprous oxide precipitate.

(c) Benedict's test

When Benedict's reagent (an alkaline solution containing cupric citrate complex) was added to a 1 ml extract solution, it produced a reddish brown precipitate, indicating the presence of reducing sugar when heated on a water bath.

iii. Proteins and free amino acids:

The Millon's, Biuret, and Ninhydrin techniques were used to test a little amount of alcohol extract dissolved in a few millilitres of water.

iv. Gums and mucilage:

For assessment of the swelling characteristics that 10 ml of the extract was gradually added to 25 ml of 100% alcohol while stirring, then filtered and air dried.

v. Terpenoids:

The red colour of Sudan III (an alcoholic solution) after the thin part of the material was added suggested the presence of terpenoids.

vi. Volatile oil:

By the hydro-distillation process.

vii. Tannins:

Aqueous bromine solutions, a 1 percent gelatin solution containing 10% sodium chloride, and a weak ferric chloride solution were used to test small amounts of alcohol extracts in water for tannins and phenolic compounds (5 percent).

viii. Flavonoid:

Shinoda Test (Magnesium Hydrochloride reduction test): Check the colour of the test solution after adding 0.5g of magnesium turnings and a few drops of strong hydrochloric acid.

Zinc Hydrochloride Reduction Test: Incorporate strong hydrochloric acid and zinc dust into the test solution. Examine the colour after heating the mixture.

Table No 5: Phytochemical analysis of aqueous & ethanolic root extract of *G.glabra*

S.No.	Test	Aqueous root extract	Ethanolic root extract
1	**Tests for sterols**		
	Salkowski's Test	+	-
	Libermann Burchard's Test	+	-
2	**Test for glycosides**		
	Baljet's Test	+	+
	Brontrager Test	-	+
3	**Tests for saponins**		
	Foam Test	+	+
4	**Test for carbohydrates**		
	Molish's Test	-	
	Barfoed's Test		
	Benedict's Test	+	
		-	
5	**Tests for alkaloids**	**-**	
	Mayer's Test.	-	
	Wagner's Test.		
	Dragendorff's Test	-	
6	**Tests for flavonoids**		
	Ferric chloride Test.	-	-
	Shinoda Test.		
	Alkaline Reagent Test.	+	+
	Lead Acetate Test.	-	-
7	**Tests for tannins**		-
	Ferric chloride Test.	-	
	Gelatin Test	-	
8	**Test for amino acid and protein**		
	Biurete test	+	**-**

6.3 Isolation of Glycyrrhizin

The Soxhlet equipment was used to extract 50 grammes of powdered root with 250 cc of water until the water ran out. The extract was filtered and concentrated by vacuum evaporation. Glycyrrhizin will precipitate after adding HCl (pH= 3-3.5) and dissolving the residue in water. Crude glycyrrhizin was produced by filtering, washing with water, and drying the precipitate (Crude GA). Oxalic acid was added to crude GA (50 mg) in 20 ml of a 1:1 MeOH:water solution, which was then heated to 60°C for 24 hours. The reaction mixture was extract with ethyl acetate (EtOAc) (2 x 250 ml) to get an aqueous fraction containing sugars and an EtOAc fraction containing the partly hydrolysed product. Glycyrrhizin was generated by concentrating the EtOAC fraction and purifying it using normal phase PTLC using the solvent system n-hexane/EtOAC (70:30).

Table 6: General Physical Properties of isolated Glycyrrhizin

S.No.	Physical properties	Inference
1.	Appearance	powder
2.	Color	Yellowish brown
3.	Solubility	Chloroform, methanol & slightly soluble in Pet.ether
4.	Melting point	221^0C

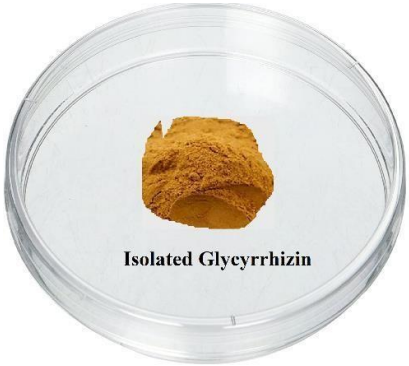


Fig 29: Isolated Glycyrrhizin

General and Physical Properties: The isolated compound appearance, colour, solubility, and melting point will be identified.

6.4 TLC chromatography

The subsequent mobile phases, as well as a pre-coated aluminium sheet with silica gel GF254, were used to compare isolated glycyrrhizin to conventional glycyrrhizin via TLC. Toluene, ethyl acetate, and glacial acetic acid are mixed (12.5:7.5:0.5) to make a spray reagent. (2010) Hemesh Soni et al.

Table 7: TLC chromatography Analysis of Glycyrrhizin

Solvent System	R_f(standard)	R_f(isolated sample)
Toluene: ethyl acetate: glacial acetic acid (12.5:7.5:0.5)	0.43 (Dark violet purple spot)	0.41 (Dark violet purple spot)

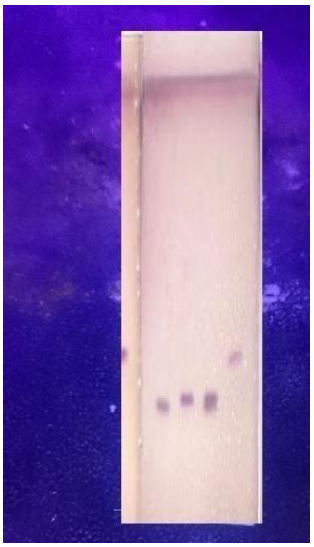


Fig 30: TLC chromatogram of isolated compound (Glycyrrhizin)

Spectrophotometric analysis

The isolated Glycyrrhizin was dissolved in methanol, and the UV absorption peaks were measured. The Shimadzu 1700 UV spectrophotometer was used in the spectrophotometer-based analysis. Rajpal, V. (2002).

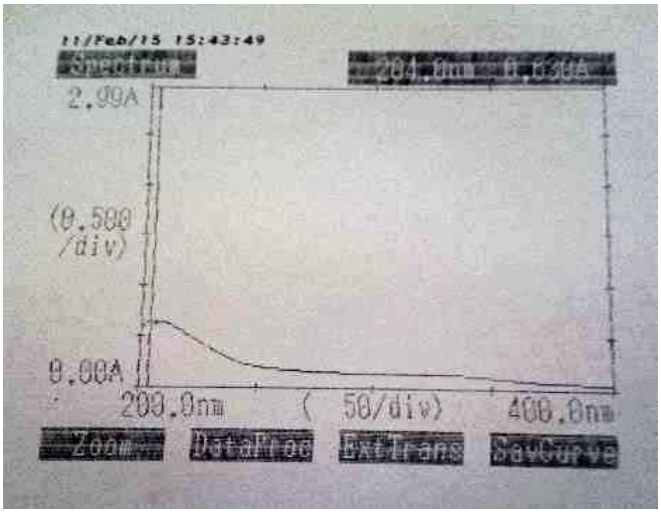


Fig 31: UV Scan of isolated compound (Glycyrrhizin)

6.5 HPLC analysis

The Salo Torrace LC-100 CyberlabTM with an LC-UV-100 UV detector was used for the HPLC investigation in Millburry, Massachusetts, USA. The chromatographic separations were performed using a C-18 (CAPCELL) HPLC-packed column with a size of 4.6 mm I.D. x 250 mm, type MG 5 m, and number AKAD/05245. One percent of the mobile phase was phosphoric acid: acetone to 60:40 ratio. The flow rate was 0.2 mL/min, and the temperature of the column was 25°C. The injection volume was 25 l, with a UV detection wavelength of 254 nm.

Table: 8 HPLC Analysis of Glycyrrhizin

S.No.	Sample	Height	Area	Conc.	RT	Inference
1.	Standard Glycyrrhizin	49442	1251681.2	96.7	8.00	Glycyrrhizin
2.	Isolated Glycyrrhizin	32582	417227.2	29.0598	7.58	Glycyrrhizin

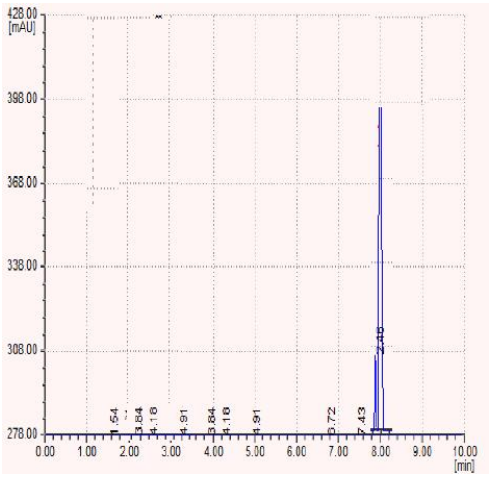


Fig 32:HPLC chromatogram of Std Glycyrrhizin

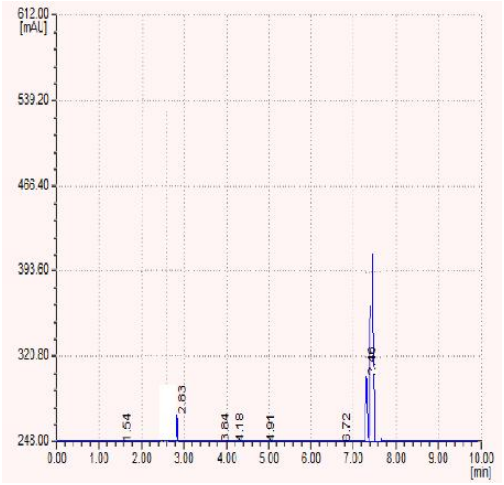


Fig 33:HPLCchromatogram of Glycyrrhizin (isolated)

IR analysis

IR spectral data was acquired using a Bruker (AT-IR).

Table: 9 IR Analysis of Glycyrrhizin (isolated)

cm^{-1}	**Functional Group**
3343	O-H (stretch)
3355	O-H (stretch)
3755	Aromatic
2874	CH stretch
1639	C=O stretch
1620	C=C
1364	C-O-C

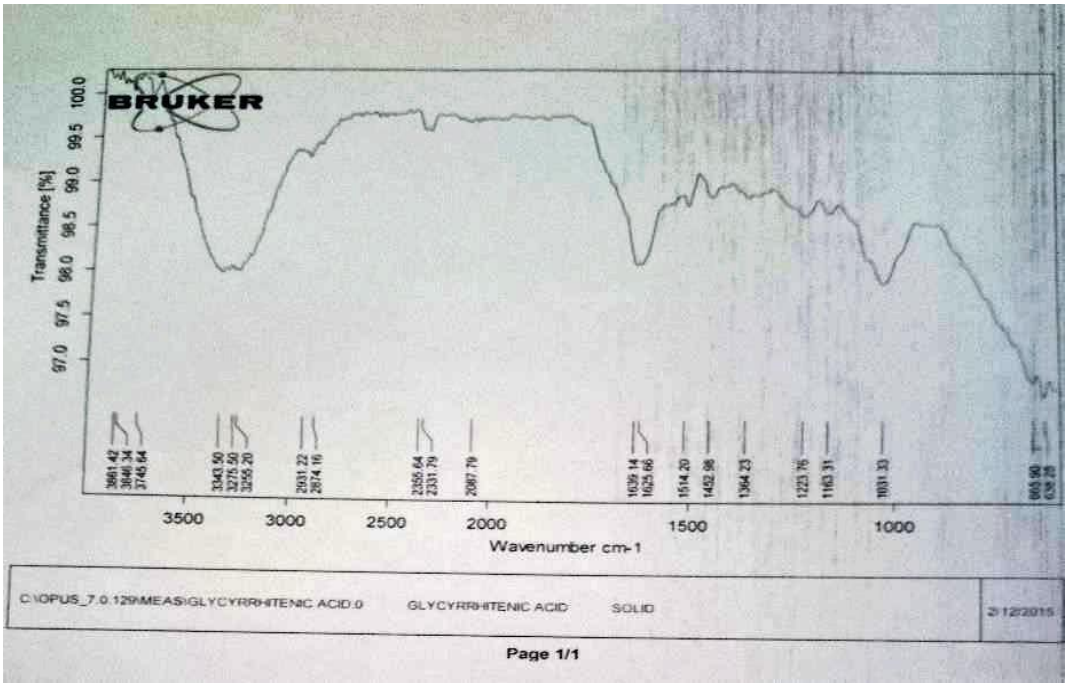


Fig 34: IR spectra of Glycyrrhizin (isolated)

6.6 Preformulation studies of Glycyrrhizin

Discovery of a new pharmaceutical substance is a huge scientific achievement, and if it successfully undergoes toxicity testing, where the potential benefits may surpass the dangers, it becomes much more noteworthy. The ultimate result of the new chemical entity

depends on its presence at the site of action after being effectively delivered by the appropriate route in the correct form. Consequently, following a successful pharmacological and toxicological screening, a new challenge arises: transforming a potential active new therapeutic entity into a pharmaceutical formulation. In general terms, it can be defined as the process of investigating the physical, chemical, analytical, pharmacokinetic, and pharmacodynamic properties of a new chemical entity and using the acquired findings to develop and create a very effective, stable, and safer dosage form. As part of the multidisciplinary approach, the preformulation study incorporates several components from pharmacological, toxicological, clinical, biochemical, medicinal, and analytical chemistry. The primary objective of the preformulation phase or study is to establish the foundation for transforming a new medicinal substance into a pharmaceutical formulation that can be accurately delivered, in the appropriate dosage, and, perhaps most crucially, to the intended target. The secondary objective of the preformulation study is to enhance the stability of the formulation and evaluate the efficacy of the developed formulation by appropriately designing and protecting pharmacological components from the environment.

Instruments

Researchers utilised a Brookfield Rotational Viscometer, a Franz diffusion cell type glass, a Shimadzu 1700 UV spectrophotometer from Japan, a Digital pH metre, and an ATR Bruker FTIR instrument from Germany.

Organoleptic Characteristics:

The drug's appearance, melting point, solubility tests, and partition coefficient were assessed (Soni H etal; 2020).

Melting Point determination

The melting point of glycyrrhizin was determined using capillary melting point apparatus. A little amount of drug sample was collected in a capillary tube, which was then placed into a melting point device and turned on. The drug in the capillary tube melted as a result of the

heat, and a digital thermometer displayed the temperature. The average reading was taken three times throughout the operation.

Solubility: The solubility of glycyrrhizin in ethanol, chloroform, water DMSO, and 7.4 phosphate buffer was assessed.

Partition Coefficient

Following the precise measurement of 10 milligrammes of glycyrrhizin, it was combined with 10 millilitres of phosphate buffer solution and 10 millilitres of n-octanol prior to being transferred into a separate funnel. After shaking this mixture for thirty seconds every ten minutes for a period of one hour, it was allowed to stand for a period of twenty-four hours. There is a separation between two phases that are incompatible with one another in a funnel that separates. It was possible to separate the aqueous phase, purify it, and then dilute it one hundred times with 0.1N potassium hydroxide with the use of filter paper. The maximum absorbance of the aqueous phase was determined to be 277 nm by the utilisation of a UV spectrophotometer. Through the utilisation of the drug standard curve, the concentration was determined, and the partition coefficient was determined through the utilisation of the following formula:

$Po/w = Coil/Caq$

Here, Po/w = Partition coefficient

Coil = Concentration of drug in organic phase. Caq = Concentration of drug in aqueous phase

The partition coefficient of Glycyrrhizin is 6.2.

Table.10: Physical Characterization of Glycyrrhizin

Appearance	Solid Powder
Colour	Yellowish
Taste	Sweat in taste
Odur	Characteristic
M.P.	221^0C
Solubility	Easily soluble in water and slightly soluble in ether
Partition coefficient	6.2

Preparation of standard curve for Glycyrrhizin:

Dissolving 10 mg of an isolated compound in phosphate buffer with a pH of 7.4 in a volumetric flask with a capacity of 10 ml was the first step in the process of creating the standard stock solution for the compound. Ethanol was combined with the same solvent in a ratio of 70:30, which resulted in the production of a solution that contained 1000 g/mL of glycyrrhizin. This enabled the final volume to be altered. It was necessary to make aliquots of working stock solutions of glycyrrhizin in the same solvent in order to attain concentrations of the acid that fell within the range of (initial to 28)g/ml.

The absorbance of the resulting solutions was measured at 254 nm, per Patil SK et al. (2012).

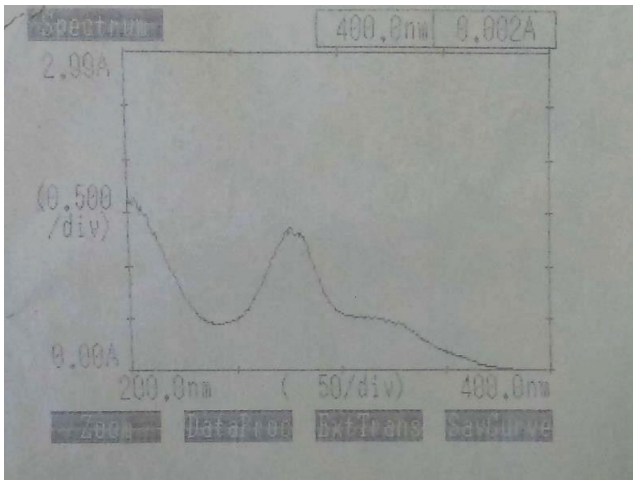


Fig. 35: Glycyrrhizin at λmax 254 nm

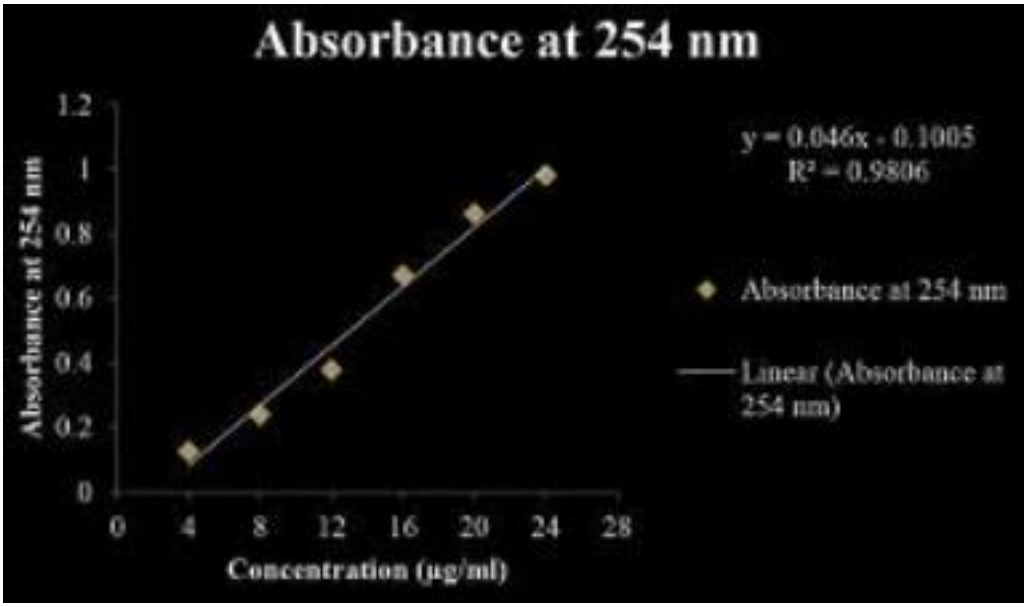


Fig. 36: Calibration curve of Glycyrrhizin

Table 11: UV absorbance of series of solution of Glycyrrhizin in phosphate buffer at254nm

S.No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	4	0.375
3.	8	0.676
4.	12	1.015
5.	16	1.287
6.	20	1.518
7.	28	2.012

Statistical Parameters

- Correlation coefficient $R^2 = 0.9806$
- Straight line equation: y= 0.046x + 0.10005

Glycyrrhizin's calibration curve, which was created in phosphate buffer with a pH of 7.4, was found to be linear. A regression value of 0.9806 was discovered.

Drug polymer Interaction:

Drug polymer interaction was studied by AT-IR.

A scan of the FT-IR spectrum of glycyrrhizin was performed, and the results are shown in figures 17(a) and 17.(b). The frequencies of the functional groups that were examined are presented in table 12, which indicates that the sample that was obtained did indeed contain glycyrrhizin and that it was unadulterated. It was discovered that the various peaks of the spectrum coincided with the typical spectrum of glycyrrhizin throughout the experiment.

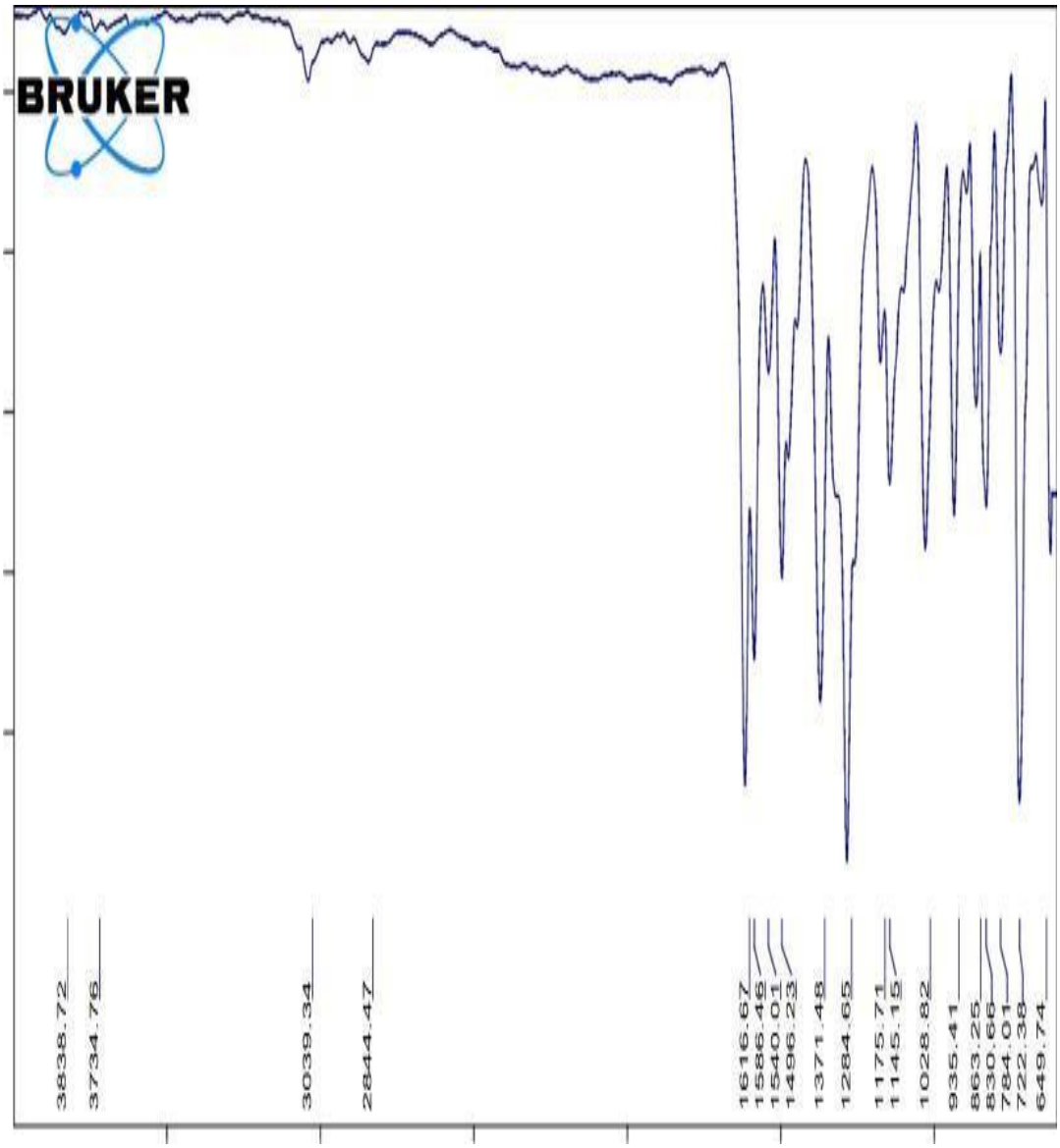


Fig.37 (a) FT-IR of Standard

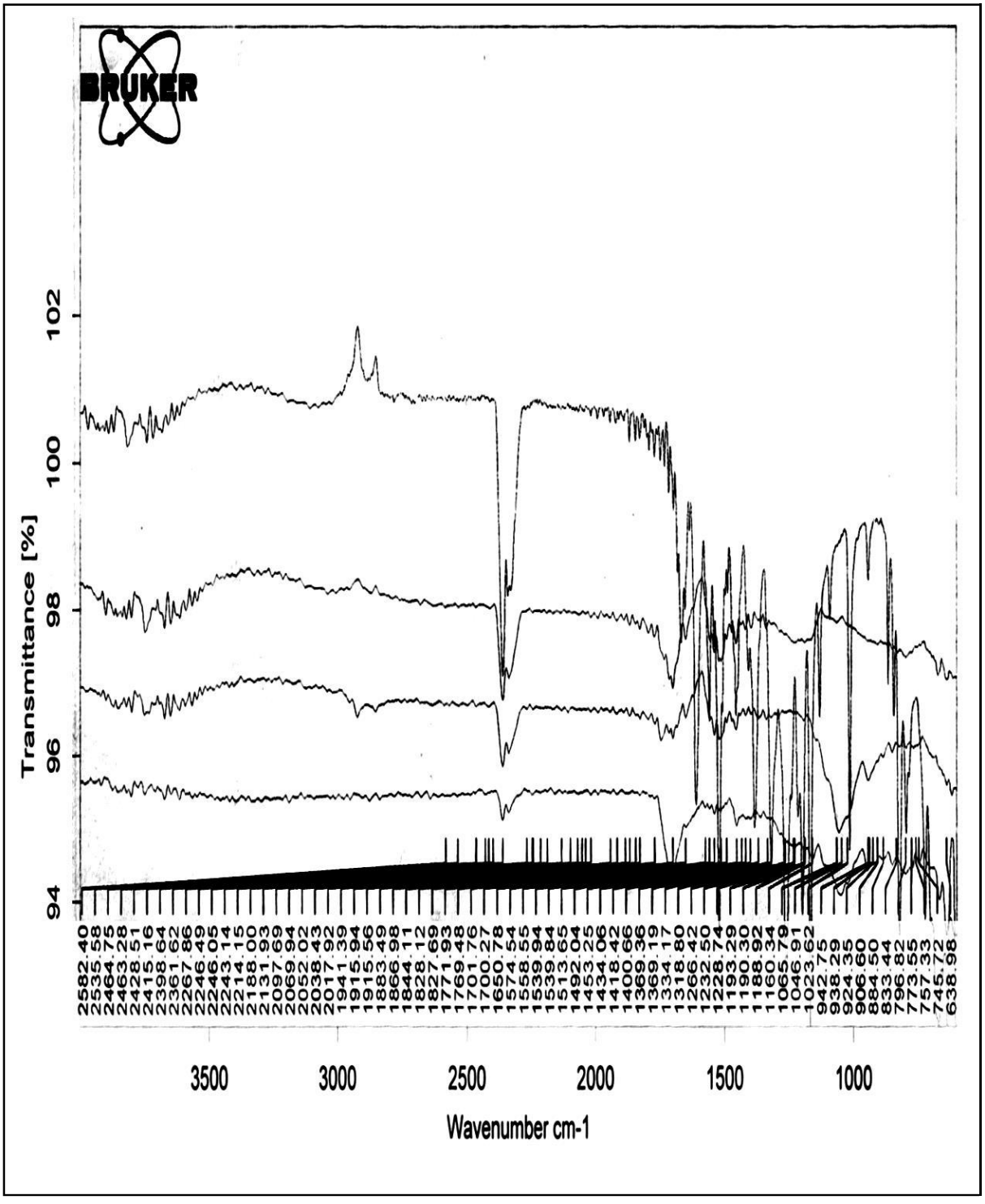


Fig.37 (b): Comparison of IR spectra of standard + Chitosan +sorbitan mono-oleate + Mixture

Table 12: IR interpretation of Glycyrrhizin

S.No.	Observation (cm^{-1})	Functional Group
1.	3343	O-H (stretch)
2.	3355	O-H (stretch)
3.	3755	Aromatic
4.	2874	CH stretch
5.	1639	C=O stretch

Inference: Drug and polymer interactions were investigated using FT-IR spectroscopy.

The drug-polymer combinations retain the peaks of the pure drug (table 12 & fig. 17(a & b), which are connected with specific functional groups of the drug.

6.7 Formulation & Evalaution

Preparation of Microspheres

Glutaraldehyde was utilised as a cross-linking agent during the emulsification cross-linking procedure to form the microspheres. A precisely weighed amount of chitosan was dissolved in aqueous acetic acid at 1% (v/v). The polymer solution and the drug (Glycyrrhizin) were mixed. A water-in-oil (w/o) emulsion was made by adding the dispersed phase drop-wise through a disposable syringe (10 ml) to the continuous phase, which was made up of light and heavy liquid paraffin in a 1:1 ratio with variable quantities of surfactant (Span 80). Stirring was carried out at different rates using a three-bladed stirrer. Following 20 minutes of stirring, a predetermined amount of aqueous glutaraldehyde (25 percent v/v) was added drop by drop. Following the final addition of glutaraldehyde, stirring continued for an additional hour. The microspheres that remained after centrifuging the preparation at 3000 rpm were washed four times with petroleum ether. Following the final wash, microspheres were collected, air dried at room temperature, and stored.

Table 13. Composition of Microspheres

S.No.	Code	Glcyrrhizin (mg)	Chitosan (mg)	Span80 (% v/v)	Gluter-Aldehyde(ml)
1	F_1	100	250	0.5	0.5
2	F_2	100	200	1.0	0.5
3	F_3	100	300	1.0	1.0
4	F_4	100	345	1.5	0.5
5	F_5	100	400	1.0	1.5

6.8 Evaluation of Microspheres

Percentage Yield

Weighing occurred on the prepared microsphere compositions F1-F5. The measured weight was divided by the sum of all non-volatile substances used to make the microspheres. The formula for calculating percent yield is as follows.

$$\% = \frac{\text{Wt.of microsphere}}{\text{recovered wt.(drug + Polymer)}} \times 100$$

DRUG ENTRAPMENT EFFICIENCY

To eliminate the drug from the microspheres, weighted microspheres were crushed and suspended in ethanol. The drug content of the filtrate was spectrophotometrically determined at 254 nm after 24 hours, with ethanol serving as a blank. Drug concentrations in the samples were calculated using a regression equation derived from the standard graph and the calibration plot. The equation for calculating drug entrapment efficiency is as follows.

$$\text{Entrapment efficiency} = \frac{\text{Acutual drug content}}{\text{Theoretical drug content}} \times 100$$

Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 18 shows a study of the IR spectra of glycyrrhizin-loaded microspheres, with distinct peaks for C-N at 1145.77 cm-1 and 1188.2 cm-1, C-F at 1338.66 cm-1, C-O at 1264.39 cm-1, and O-H at 2878.88 cm-1. The reported absorption peak values are equivalent to those from pure medicine.

As a result, the FT-IR examination shows that there was no polymer contact and that the drug was compatible with the polymer.

6.9 *IN-VITRO* DRUG RELEASE STUDY

In vitro drug release was tested using a USP dissolution device basket and 900 ml of phosphate buffer solution (pH 7.4) at 37 ± 0.5 °C and 100 rpm. Microsphere samples that had been properly weighed were added to the dissolution media. 5 ml of samples were obtained from the dissolution media at intervals of 0-30 min, 60-90 min, 120-150 min, 12-18 hr, and 24 hours. To maintain the washbasin state, an equal volume of new dissolving solution was put to it each time. These samples were spectrophotometrically evaluated using a standard curve equation and a UV-visible spectrophotometer set to 254nm.

PARTICLE SIZE DISTRIBUTION

The microspheres' size was measured using an optical microscope. Dry microspheres (10 mg) were manually shaken in a test tube containing phosphate buffer saline (pH 7.4) for 5 minutes. A drop of suspension was placed on a glass slide, and microspheres were counted at 100X magnification with a calibrated ocular micrometre.

Table 14: % Practical Yield of All Formulation

S.No.	Formulation Code	Theoretical Weight (mg)	Practical Yield (mg)	% Practical Yield (mg)
1.	F_1	350	268	76.5
2.	F_2	300	245	81.6
3.	F_3	400	297	74.2
4.	F_4	445	355	79.8
5.	F_5	500	385	77.0

Table 15: Drug entrapment efficiency of all formulations

S.No.	Formulation Code	Glycyrrhizin (mg)	Chitosan (mg)	% Drug Entrapment
1	F_1	100	250	92.1
2	F_2	100	200	93.5
3	F_3	100	300	86.5
4	F_4	100	345	90.1
5	F_5	100	400	82.2

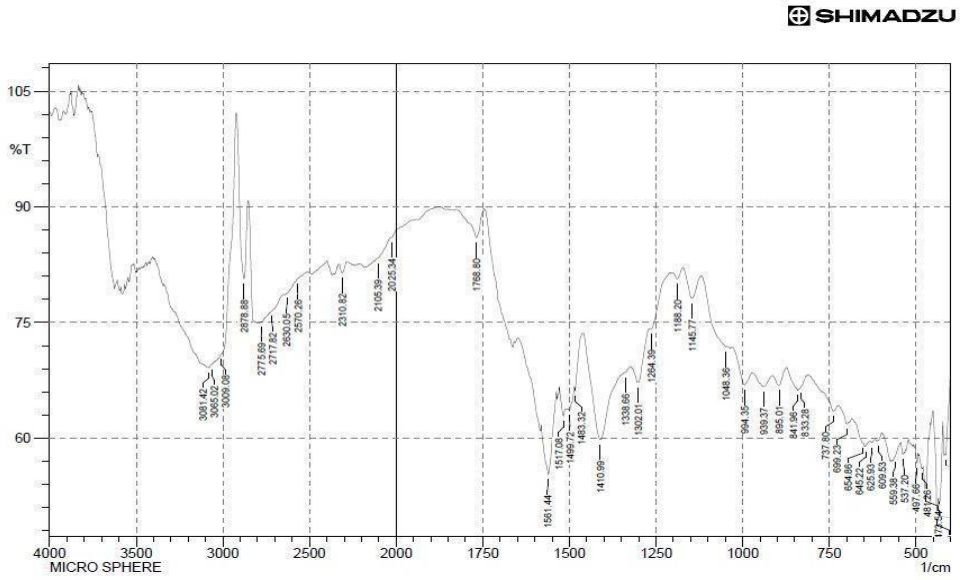


Figure.38: IR Spectra of Glycyrrhizin loaded microsphere

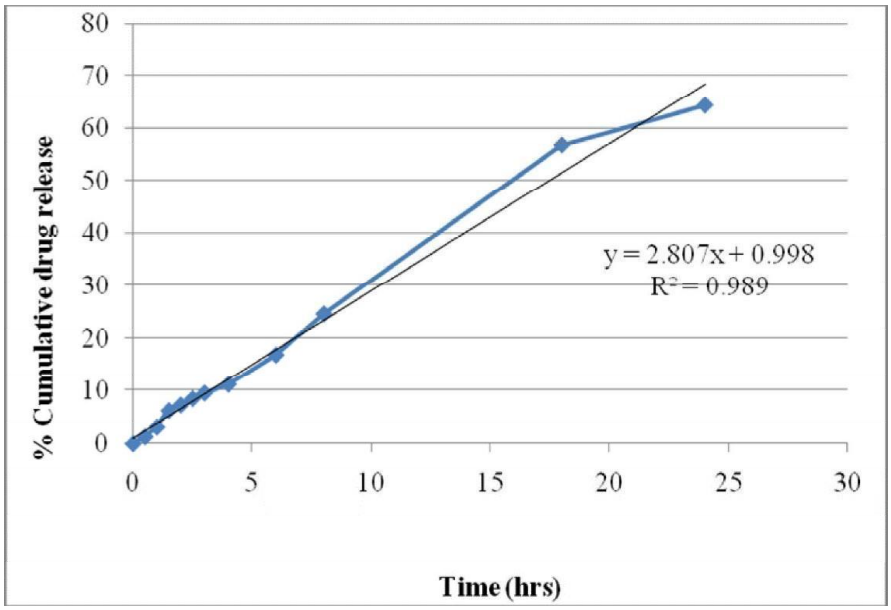
In Vitro Drug Release Study

With the help of a USP dissolve device type II, drug releases of formulation F2 were performed in vitro. Table 16 lists the F2 *in -vitro* drug release information.

Table 16: *In vitro* drug release study of formulation F_2

S.No.	Time (hrs)	Drug Release (%)
1.	0	0.00
2.	0.5	1.32
3.	1	3.12
4.	1.5	6.23
5.	2	7.32
6.	2.5	8.52
7.	3	9.56
8.	4	11.23
9.	6	16.87
10.	8	24.65
11.	18	56.78
12.	24	64.32

Zero order drug release of formulation (F_2)



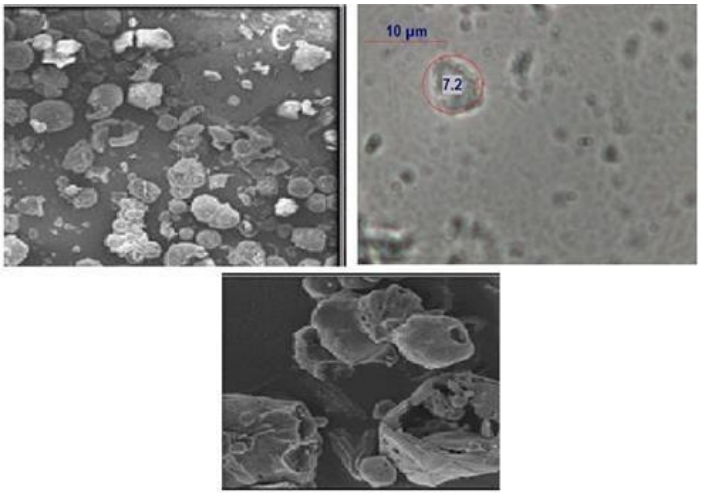


Fig.39: Photomicrograph of Particle Size Distribution

6.10 *In- silico* molecular docking for assessment of antiproliferative activity

Selection of Target Protein for docking studies

❖ **Caspase 9**

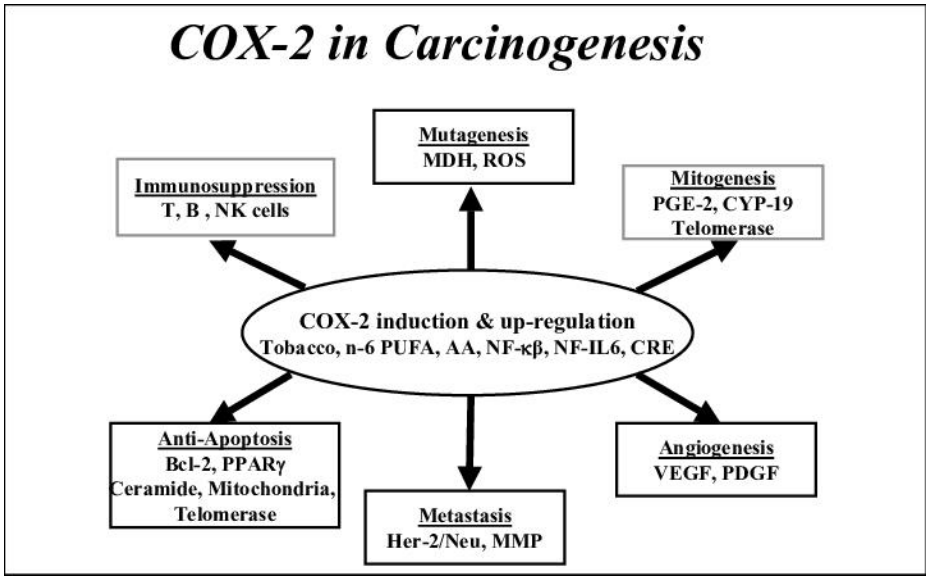
Despite recent improvements in our understanding of caspase-9's roles in the tumorigenic potential of human NSCLC and in CAR T therapy trials, its particular mode of activation/formation remains unknown. For example, we still don't fully understand how caspase-9 is activated for non-apoptotic reasons without resulting in cell death, nor do we understand the precise dynamics of caspase-9 activation and activity. Studies have tested our understanding of caspase-9 activity, revealing that it not only acts as a catalyst for cellular disassembly but also plays a role in controlling what happens to developing myoblasts. This discovery lends credence to the existence of a caspase-9 activation mechanism unrelated to cytochrome C, at least in certain cell types.

The exciting notion that caspase-9 may serve as a therapeutic is fuelled by fresh data that caspase-9 can be altered under a variety of clinical conditions. The requirement for DNA damage-induced neural precursor cell death requires caspase-9 but is not linked with a detectable loss of cytochrome c from mitochondria, implying that caspase-9 might operate directly as an apoptotic effector. Inhibiting caspase-9 activity may result in acquired chemotherapeutic resistance in certain types of human cancer cell lines. Cancer researchers

have long sought to selectively induce apoptosis in malignant cells. Further research into caspase-9 activation, activity, and regulation may potentially make this enzyme a promising therapeutic target for the treatment of degenerative and developmental issues as well as cancer (Olsson M etal;2011).

❖ **COX-2**

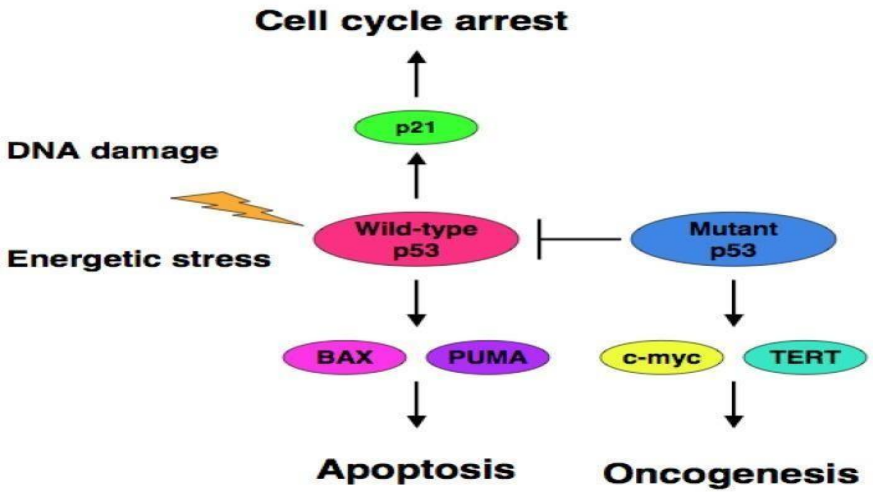
Cyclooxygenase-2 (COX-2) is highly expressed in a variety of cancers, where it plays a pleiotropic and multifunctional role in carcinogenesis initiation or promotion, as well as cancer cell resistance to chemotherapy and radiation. Cancer cells, macrophage type 2 (M2) cells, and cancer-associated fibroblasts (CAFs) all release COX-2 into the tumour microenvironment (TME). COX-2 promotes apoptotic resistance, cancer cell proliferation, angiogenesis, inflammation, invasion, and metastasis while also mimicking the behaviour of cancer stem cells. The presence of COX-2-mediated hypoxia within the TME, as well as its beneficial interactions with YAP1 and antiapoptotic mediators, makes cancer cells more resistant to chemotherapeutic treatments. (Hashemi Goradel N etal;2019).



❖ ***p53*** **protein**

Cyclooxygenase-2 (COX-2) has been identified in several different kinds of cancer, where it plays a pleiotropic and multifunctional role in the development or accelerated of carcinogenesis, as well as cancer cell resistance to chemotherapy and radiation. COX-2 is

produced by cancer cells, macrophage type 2 (M2) cells, and cancer-associated fibroblasts (CAFs) in the tumour microenvironment. COX-2 not only promotes apoptotic resistance, cancer cell proliferation, angiogenesis, inflammation, invasion, and metastasis, but it also mimics the behaviour of cancer stem cells. COX-2-mediated hypoxia within the TME, as well as its favourable interactions with YAP1 and antiapoptotic mediators, make cancer cells more resistant to chemotherapeutic treatments. (Hashemi Goradel N etal;2019).



❖ **Aurora kinases A**

The Aurora kinases, a family of serine/threonine kinases consisting of Aurora A (AURKA), Aurora B (AURKB), and Aurora C (AURKC), govern chromosomal segregation during mitosis. These kinases are critical for cell division. Aurora kinases may possibly influence meiosis in addition to mitosis. Eliminating Aurora kinases may inhibit cell division and hinder embryonic development. It has been determined that certain cancers contain Aurora kinase overexpression or gene amplification. Furthermore, an increasing number of studies have found that blocking Aurora kinases may improve the effects of chemotherapy. Over the last few decades, a variety of Aurora kinase inhibitors (AKIs) have successfully inhibited the growth and progression of numerous cancers, both in vivo and in vitro, indicating that Aurora kinases may offer a novel therapeutic target.

AURKA also mediates the oncogenic effects of Myc (N-Myc, c-Myc, and L-Myc) in cancers. Overexpression or activation of both Myc and AURKA is common in human cancers. AURKA can stimulate c-Myc transcription by binding to the CCCTCCCCA motif

in the NHE III1 region and acting as a Myc regulator. C-Myc, on the other hand, can up-regulate AURKA by binding to its promoter, resulting in a positive feedback loop. Furthermore, c-Myc activation induces the transcription of genes linked with the cell cycle, promoting cell proliferation and Myc-induced lymphomagenesis. (Lin X etal;2020).

6.11 Docking of Caspase 9 with Glycyrrhizin

Ligand Preparation:

ChemSketch [ACD/Structure Elucidator, version 2018.1] was used to create the two-dimensional structure of the ligand (glycyrrhizin), which was then translated into a three-dimensional structure and optimised using three-dimensional geometry. To ensure AutoDock compatibility, the optimised structure was saved in PDB format. The fundamental structure of the ligand, glycyrrhizin, is the following:

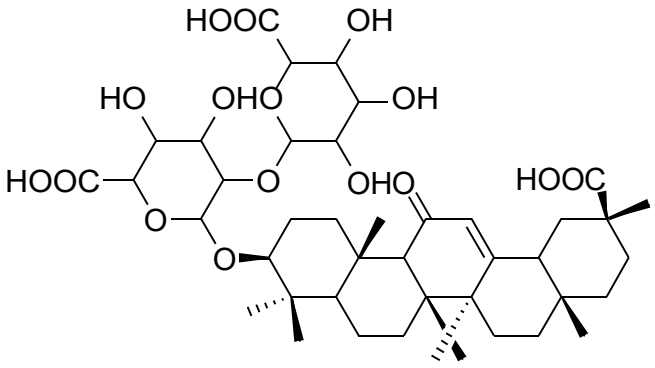


Fig. 40: 2D conformer of glycyrrhizin.

Preparation of the grid file

ChemSketch [ACD/Structure Elucidator, version 2018.1] was used to design the ligand's two-dimensional structure. The two-dimensional structure was then converted to a three-dimensional structure and optimised using 3D geometry. To ensure compatibility with AutoDock, the optimised structure was saved in PDB format. A description of the ligand's fundamental structure, glycyrrhizin, follows:

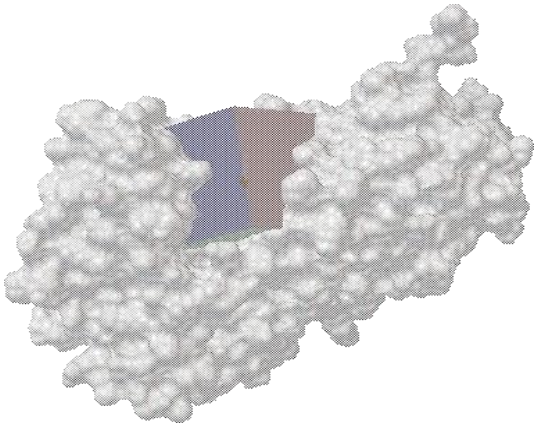


Fig 41: Grid box covering all active sites in receptor

Preparation of the docking file

The docking tool used for all the computations was Autodock4.2. Pymol, Chimera, DS visualizer, and MMP Plus were used to carry out the visualisation and other programmes required for docking experiments [Mujwar S etal; 2015].

Crystal structure

Determining the crystal structure of the receptor-containing protein was accomplished through the utilisation of the Protein Data Bank portal. It was determined that the 3v3k.pdb file from the Protein Data Bank, which includes all of the primary information regarding receptors and structures, needed to be utilised. [Berman HM et al., 2000].

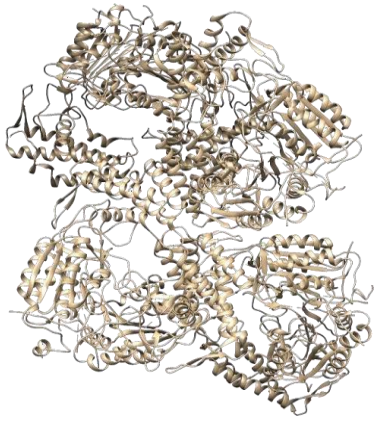


Fig 42: Crystal structure of Caspase 9 protein (PDB ID-3v3k)

Processing of Protein

Two chains, A and B, were selected for the experiment out of the sixteen chains that comprise the downloaded receptor protein. These chains are as follows: A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, and P. The molecule that was a macromolecular structure did not contain any ligands. [Shah K etal; 2019].

Molecular Docking Simulation Studies

Autodock was utilised to dock the glycyrrhizin ligand to the Caspase9 protein. Although no receptor residues were made flexible, all of the ligand's connections remained flexible. [Sharma KK etal; 2020].

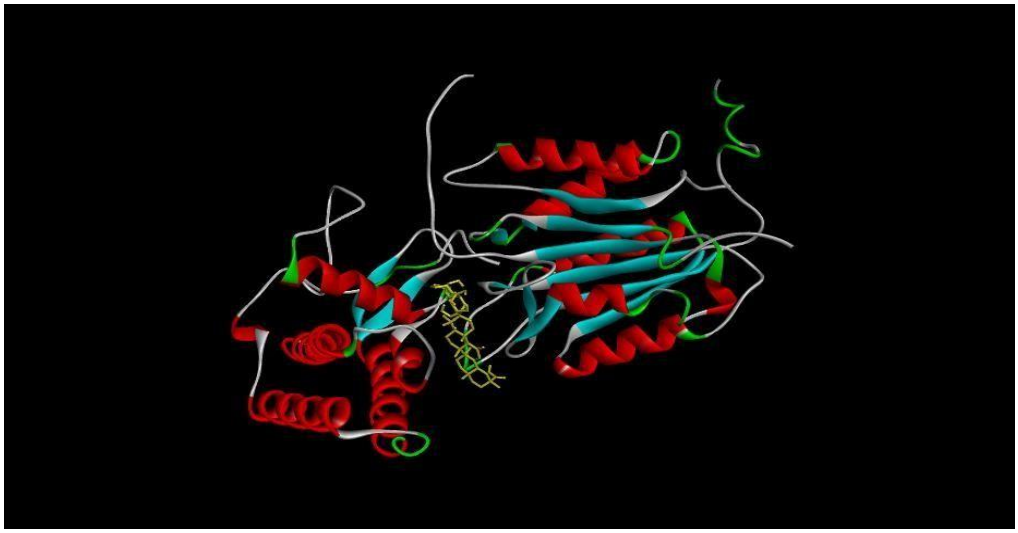
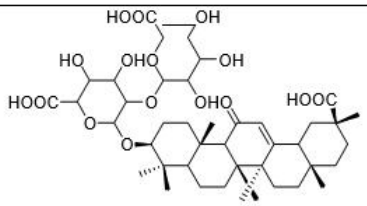


Fig 43: Binding mode of glycyrrhizin within the active site of Caspase9 protein receptor.

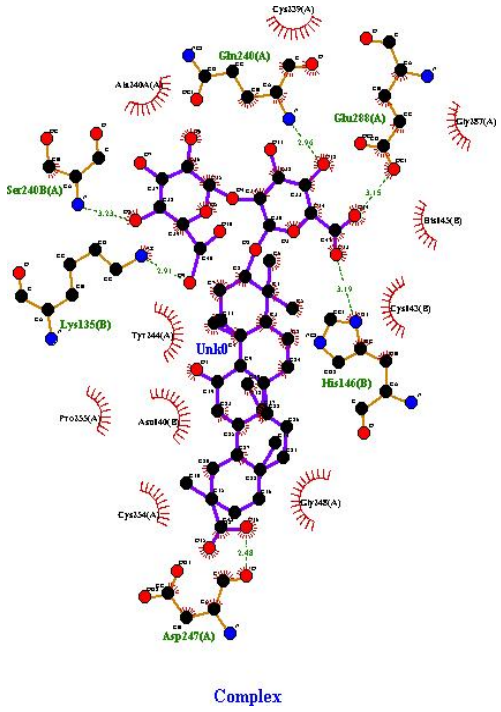
Toxicity & ADME-T Studies

According to Thomas Sander, the online program OSIRIS investigates modified lead compounds to anticipate the presence of hazardous groups, toxic groups, and ADME-T features.

Table 17: Results of docking of glycyrrhizin against Caspase 9 protein.

S. No	Compound Name	Structure	Binding Energy(Kcal/mole)	Ki (µM)
1	Glycyrrhizin		-1.41	6.82

Interactions



Complex

Fig 44: Binding interaction of glycyrrhizin with Caspase9 protein.

Glycyrrhizin interacts with Caspase9 protein residues Gln240A, Ser240B, Lys135B, Asp247A, His146B, and Glu288A to form a complex structure.

Toxicity & ADME-T Studies

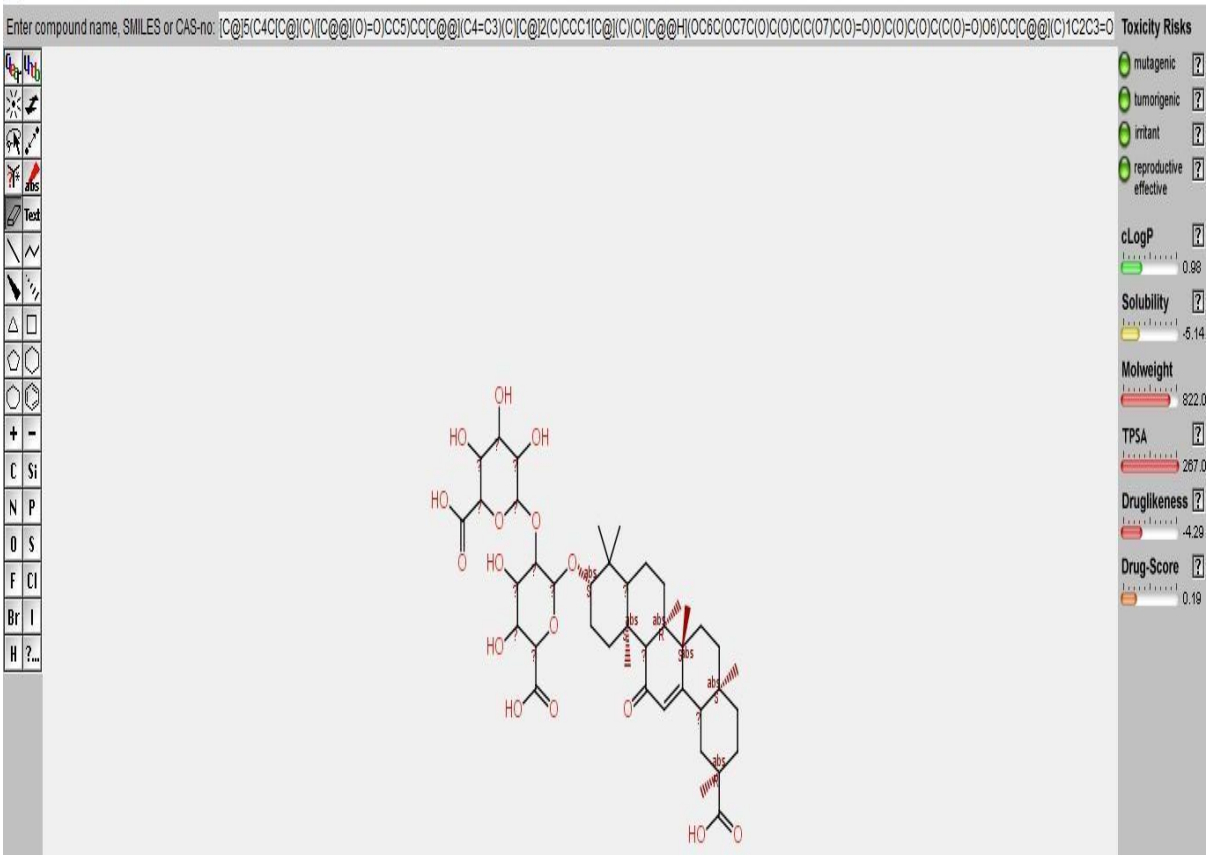


Fig 45: Pharmacokinetic and toxicity profiling of glycyrrhizin.

Molecular docking Glycyrrhizin with COX2

Ligand Preparation:

ChemSketch [ACD/Structure Elucidator; 2018] was used to create the two-dimensional structure of the ligand (glycyrrhizin), which was then translated into a three-dimensional structure and optimised using three-dimensional geometry. To ensure AutoDock compatibility, the optimised structure was saved in PDB format. The ligand's fundamental structure, glycyrrhizin, is as follows:

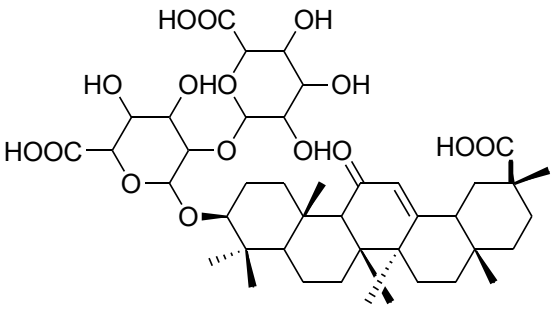


Fig 46: 2D conformer of glycyrrhizin.

Preparation of the grid file

Autodock's zones of interest have been identified by drawing a grid box around the active locations and factoring in grid area. Grid boxes are necessary for the docking process because they are designed to cover all amino acids other than those located in receptors, which are present in active sites and required for binding. The grid box contains three thumbwheel widgets that allow us to alter the number of points in the x, y, and z dimensions. Another thumbwheel can be used to adjust the distance between grid points; in this study, the value was 0.375. The number of points considered were 46, 44, and 46 points in the x, y, and z dimensions, as well as 38.042, 2.131, and 61.28 as the x, y, and z centres. [Mujwar S etal;2015].

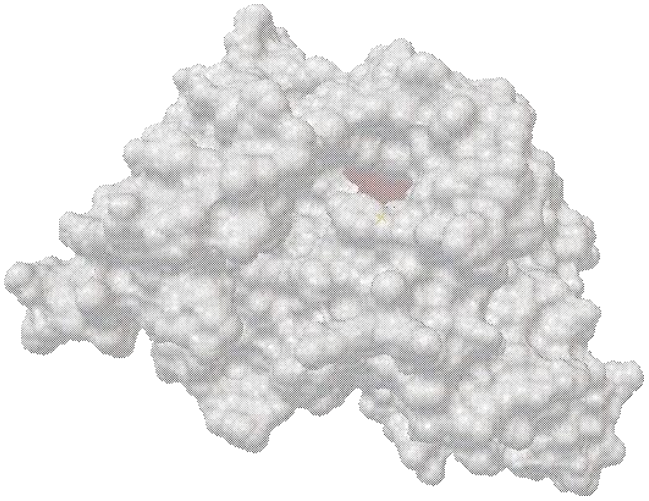


Fig 47: Grid box covering all active sites in receptor

Preparation of the docking file

Autodock4.2 served as the docking tool for all computations. The docking studies were carried out using Pymol, Chimaera, DS visualiser, and MMP Plus. [Mujwar S etal; 2015].

Docking of COX2 with Glycyrrhizin

Crystal structure

The crystal structure of the receptor-containing protein was downloaded via the Protein Data Bank portal. [Shah K et al; 2019]. All of the primary receptor and structural information (5ikr.pdb) stored in the Protein Data Bank was used.

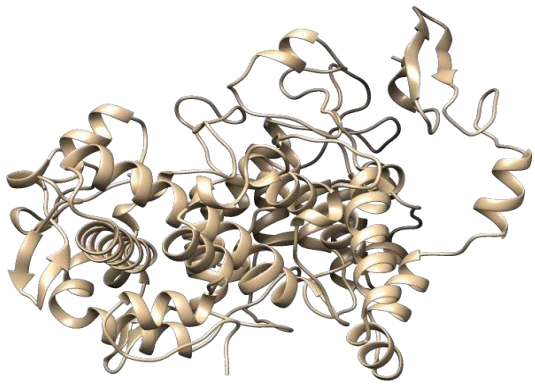


Fig 48: Crystal structure of COX2 protein (PDB ID-5ikr)

Processing of Protein

The downloaded receptor protein consists of two chains, A and B, with two chains A chosen for the experiment. The macromolecular molecule had a ligand for mefenamic acid. [Sharma KK et al., 2020].

Molecular Docking Simulation Studies

Autodock was utilised to dock the glycyrrhizin ligand to the COX2 protein. Although no receptor residues were made flexible, all of the ligand's connections remained flexible. [Berman HM etal; 2000].

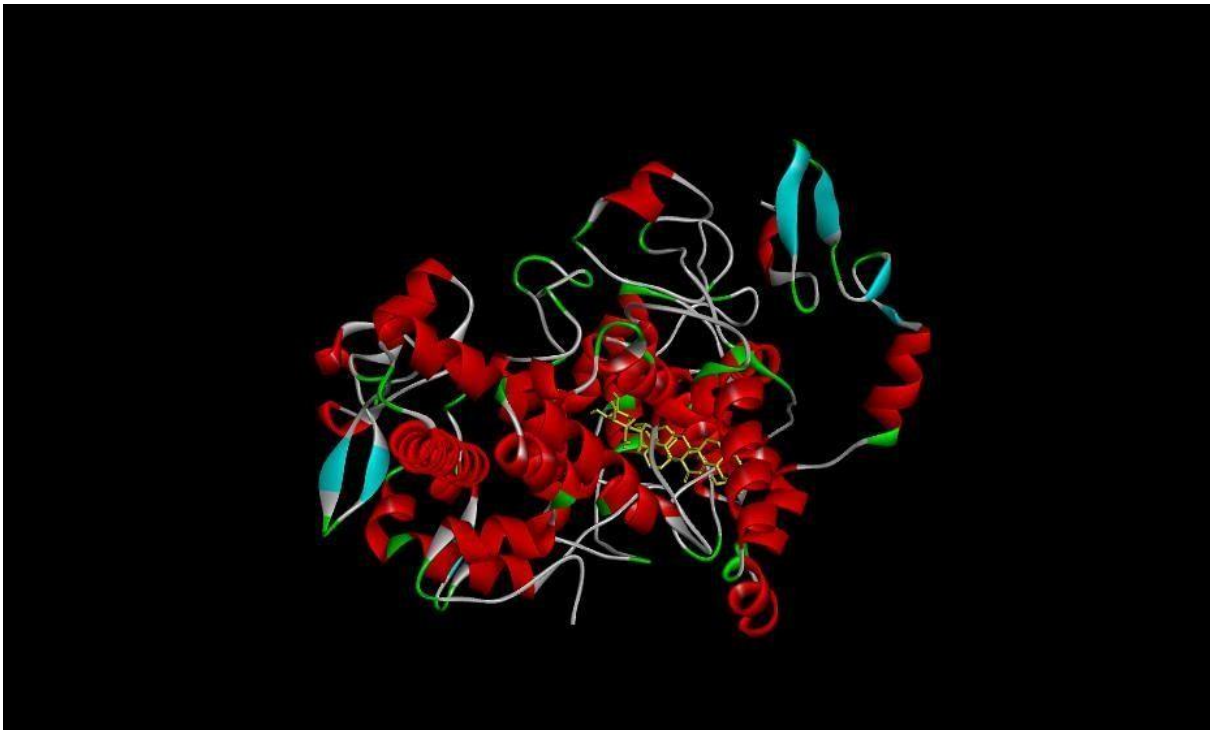
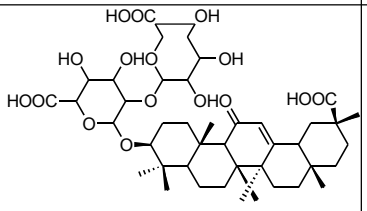


Fig 49: Binding mode of glycyrrhizin within the active site of COX2 protein receptor.

Table 18: Results of docking of glycyrrhizin against COX2 protein.

S.No	Compound Name	Structure	Binding Energy(Kcal/ mole)	Ki (µM)
1	Glycyrrhizin		7.7	2.28

Interactions

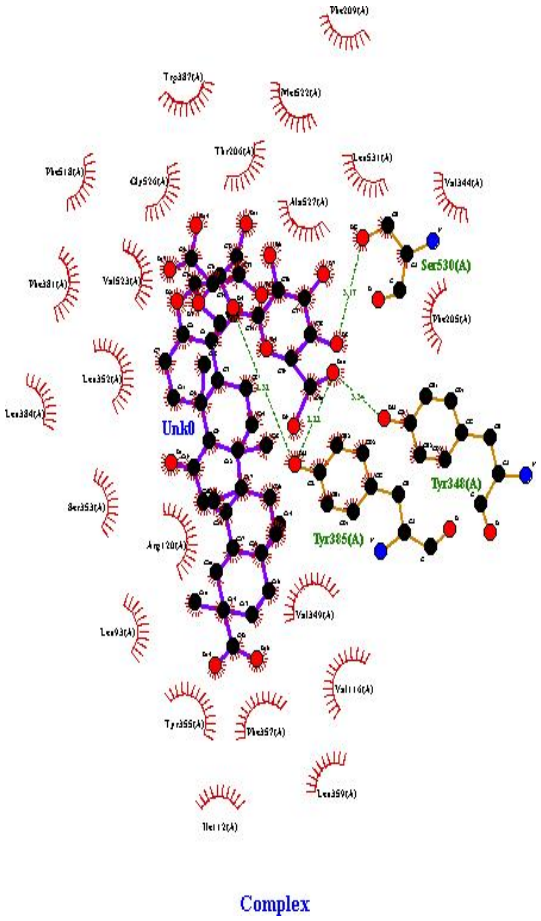


Fig 50: Binding interaction of glycyrrhizin with COX2 protein.

6.12 Molecular docking studies glycyrrhizin with mutant *p53* protein

Ligand Preparation:

ChemSketch [ACD/Structure Elucidator, version 2018] was used to sketch the ligand's two-dimensional structure (glycyrrhizin), which was then converted into a three-dimensional structure and optimised using three-dimensional geometry. To ensure AutoDock compatibility, the optimised structure was saved in PDB format.. The ligand's basic structure, glycyrrhizin, is as follows:

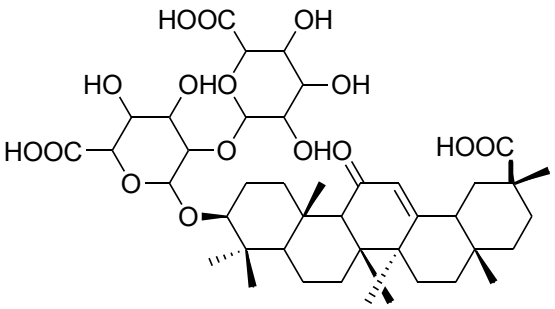


Fig 51: 2D conformer of glycyrrhizin.

Preparation of the grid file

Autodock's zones of interest were defined by drawing a grid box around the active locations and factoring in grid area. Grid boxes are necessary for the docking process because they are designed to cover all amino acids other than those located in receptors, which are present in active sites and required for binding. The grid box contains three thumbwheel widgets that allow us to alter the number of points in the x, y, and z dimensions. Another thumbwheel can be used to modify the distance between grid points; in this study, it was set to 0.497; the number of points considered were 52, 50, and 50 in the x, y, and z dimensions; and 8.505, -7.247, and 36.229 as the x, y, and z centres. [Morris GM et al; 2009].

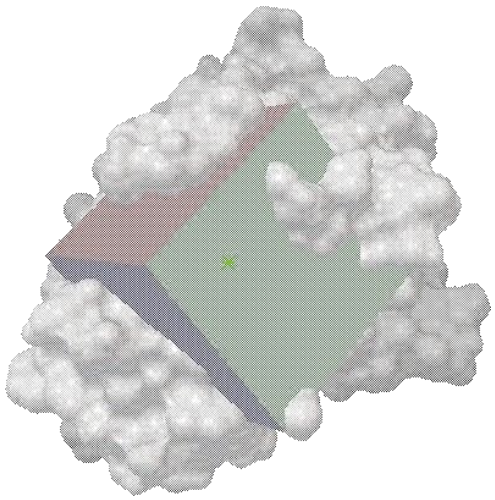


Fig 52: Grid box covering all active sites in receptor

Preparation of the docking file

The docking tool utilised for all computations was Autodock4.2. Pymol, Chimaera, DS visualiser, and MMP Plus were used to carry out the visualisation and other programs needed for docking investigations. [Mujwar S etal; 2015].

Crystal structure

The crystal structure of the receptor-containing protein was downloaded via the Protein Data Bank portal. [DeLano WLJCNopc, Pymol] All of the primary information about receptors and structures (4kvp.pdb) registered in the Protein Data Bank was used. The crystal structure of the receptor-containing protein was downloaded via the Protein Data Bank portal. [DeLano WLJCNopc, Pymol] All of the fundamental information on receptors and structures (4kvp.pdb) from the Protein Data Bank was utilised.

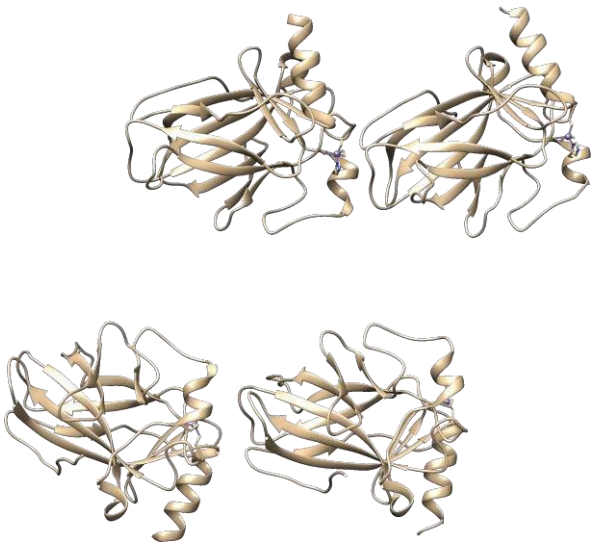


Fig 53: Crystal structure of p53protein (PDB ID-4kvp)

Processing of Protein

Chain A, one of the four chains that comprise the downloaded receptor protein (A, B, C, and D), has been selected for experimental use. The macromolecular molecule was free of any ligands. [Shah K etal; 2019].

Molecular Docking Simulation Studies Autodock was utilised to dock the glycyrrhizin ligand onto the p53 protein. Although no receptor residues were made flexible, all of the ligand's connections remained flexible. [Soni H etal; 2020].

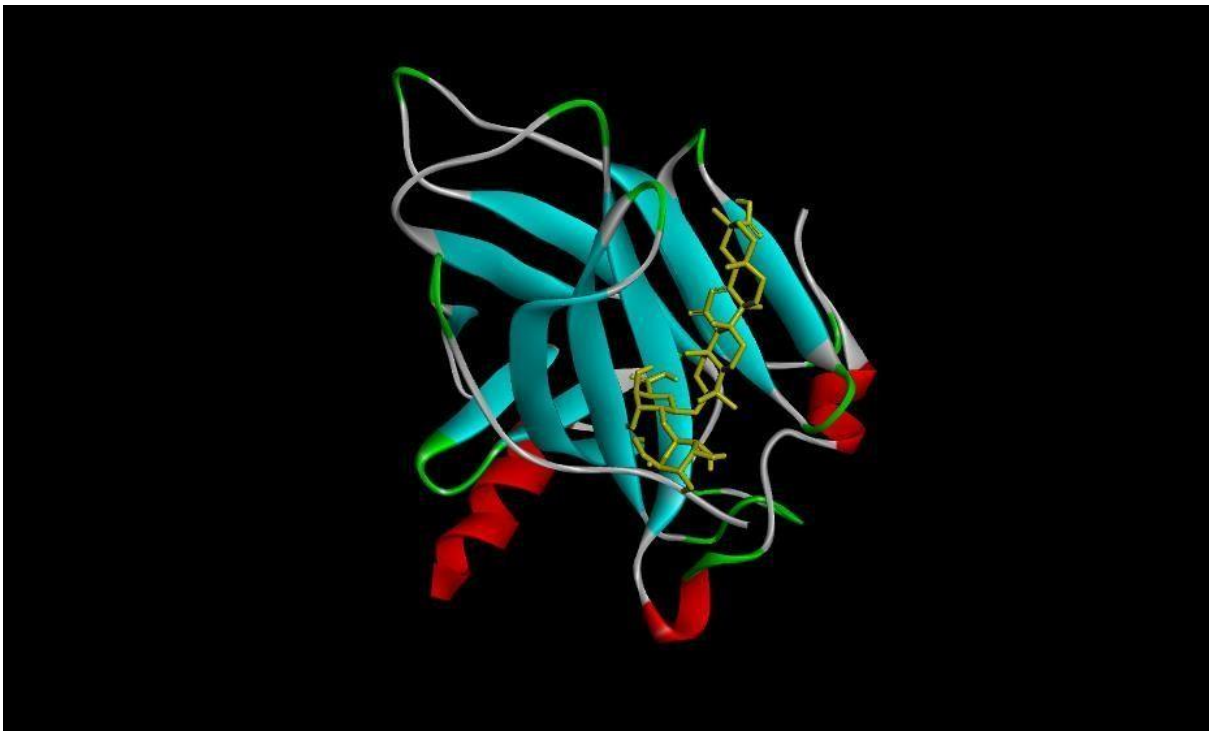
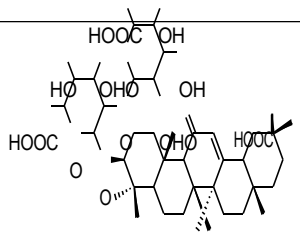


Fig 54: Binding mode of glycyrrhizin within the active site of p53 protein receptor.

Table 19: Results of docking of glycyrrhizin against p53 protein.

S. No	Compound Name	Structure	Binding Energy(Kcal/mole)	Ki (mM)
1	Glycyrrhizin		-3.54	2.55

Interactions

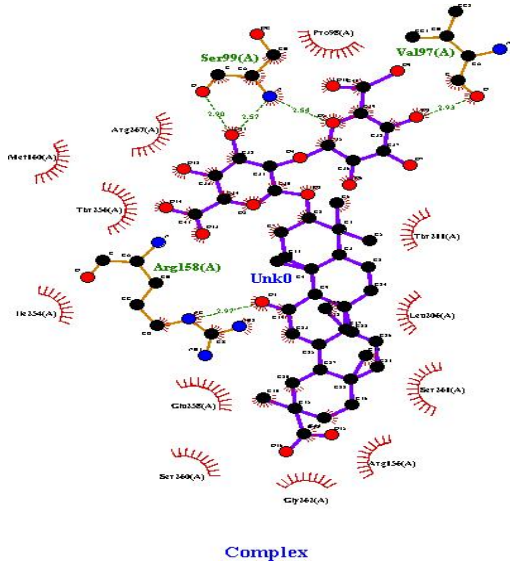


Fig 55: Binding interaction of glycyrrhizin with p53 protein.

6.13 Molecular docking of glycyrrhizin with AURKA Protein

Ligand Preparation:

ChemSketch [ACD/Structure Elucidator, version 2018.1] was used to create the two-dimensional structure of the ligand (glycyrrhizin), which was then translated into a three-dimensional structure and optimised using three-dimensional geometry. To ensure AutoDock compatibility, the optimised structure was saved in PDB format.. The ligand's basic structure, glycyrrhizin, is as follows:

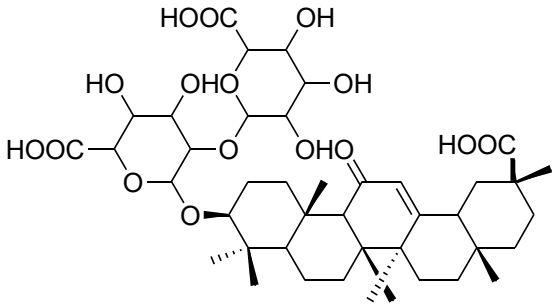


Fig 56: 2D conformer of glycyrrhizin.

Preparation of the grid file

Autodock's zones of interest were discovered by drawing a grid box around the active locations and taking the grid area into account. Grid boxes are critical to the docking process because they are designed to cover all amino acids other than those located in receptors, which are present in active sites and required for binding. Three thumbwheel widgets on the grid box allow us to change the number of points in the x, y, and z dimensions. Another thumbwheel can be used to adjust the distance between grid points; in this study, the value was 0.508; the number of points analysed were 40, 40, and 40 in the x, y, and z dimensions, with the x, y, and z centres being 32.888, 12.471, and -3.142, respectively.

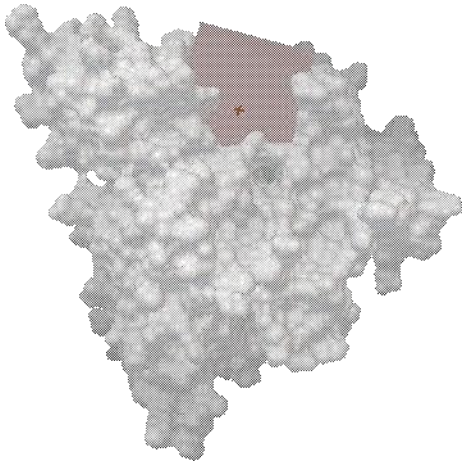


Fig 57: Grid box covering all active sites in receptor

Preparation of the docking file

Autodock4.2 served as the docking tool for all computations. The docking studies were carried out using Pymol, Chimaera, DS visualiser, and MMP Plus. [Mujwar S etal; 2015].

Crystal structure

It was possible to obtain the crystal structure of the receptor-containing protein by utilising the Protein Data Bank site. Utilised was the 3gcx.pdb file from the Protein Data Bank, which is a repository of all the fundamental information on receptors and their structures. [Berman HM; 2002].

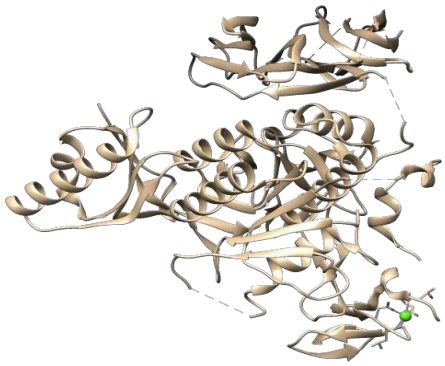


Fig 58: Crystal structure of AURKA protein (PDB ID-3gcx)

Processing of Protein

A, E, and P are the three chains that make up the receptor protein that was downloaded, and all of these chains were selected for the experiment itself. The molecule that was a macromolecular structure did not contain any ligands. [Shah K etal; 2019].

6.14 Molecular Docking Simulation Studies

Autodock was used to dock the glycyrrhizin ligand on the AURKA protein. All of the ligand's connections remained flexible, despite the fact that none of the receptor residues become flexible. [Sharma KK etal; 2020].

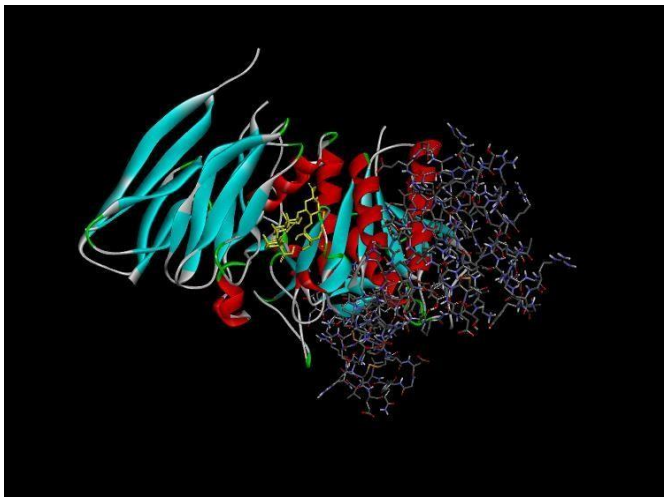
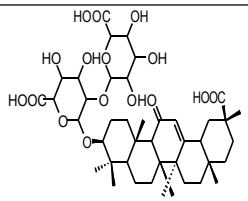


Fig 59: Binding mode of glycyrrhizin within the active site of AURKA proteinreceptor.

Table 20: Results of docking of glycyrrhizin against AURKA protein.

S. No	Compound Name	Structure	Binding Energy(Kcal/mole)	Ki (µM)
1	Glycyrrhizin		-7.11	972.11

Interactions

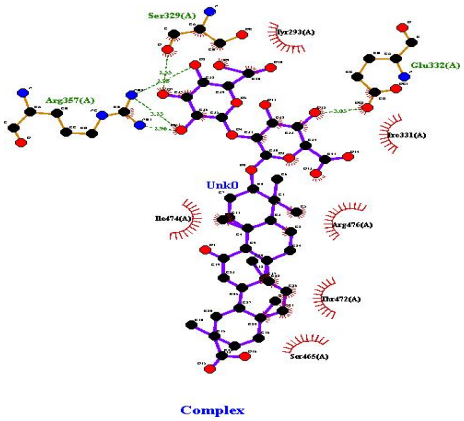


Fig 60: Binding interaction of glycyrrhizin with AURKA protein.

The glycyrrhizin interacts with the Arg357, Ser329, Glu332 and Arg476 residues of AURKA protein to form a complex structure.

6.15 *In -vitro* Assessment of Antiproliferative Potential of glycyrrhizin containing microsphere

Chemical, cell line and culture condition

During the course of this inquiry, the following illegal substances and chemical agents were utilised. The content of glycyrrhizinic acid was determined to be 98% pure through the use of high-performance liquid chromatography (HPLC). Himedia was the vendor for the

acquisition of the MTT kit, the RPMI 1640, and Dulbecco's Modified Eagle's Medium (DMEM).

The State Cancer Research Lab was kind enough to provide the MCF-7 human breast cancer cell line on donation. In order to maintain the cells' health, they were cultured in RPMI 1640 media that contained 10% FBS and antibiotics (100 U/ml penicillin G and 100 g/ml streptomycin).

MTT assay for cell proliferation

Principle: Through the use of cellular metabolic activity, the MTT test serves as a measurement tool for determining cell viability, proliferation, and cytotoxicity. For the purpose of this colorimetric assay, metabolically active cells are responsible for the transformation of purple formazan crystals into a yellow tetrazolium salt known as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT. The NAD(P)H-dependent oxidoreductase enzymes that are present in living cells are responsible for the transformation of the MTT into formazan. In order to dissolve the insoluble formazan crystals, a solubilisation solution is utilised. Following this, a multi-well spectrophotometer is utilised in order to measure the absorbance of the coloured solution that is produced at a wavelength range of 500–600 nanometres. The darker the solution is, the greater the number of living cells that are being metabolically active that are present in the solution. (Vistica VT etal;1991).

Procedure: The MTT assay, which is a colorimetric assay based on the breakdown of yellow-colored MTT by succinate dehydrogenase that is found in mitochondria, was utilised in order to evaluate the cytotoxic potential of glycyrrhizinic acid. In the process of entering living cells, MTT transforms into an insoluble formazan complex. The MCF-7 cells were seeded in a 96-well plate at a density of 2 x 105 cells per well, incubated for a period of 24 hours, and then subjected to different concentrations of glycyrrhizinic acid-containing microspheres (0. 5, 10, 25, 50, 100, or 200 M) for varying amounts of time. As the control group, the cells that had not been treated were maintained in their original position. After the cells had been cultivated for fifty minutes, they were put to one hundred litres of MTT solution and then washed twice with phosphate-buffered saline (PBS). At

last, an ELISA plate reader was utilised in order to determine the absorbance at a wavelength of 490 nm. (DR-5000, Diatek, China)

Colony Formation Assay

Principle: The survival of cells in vitro under test. It does this by determining whether or not a cell is capable of surviving and reproducing in order to produce healthy colonies. The initial implementation of this test occurred in the 1950s, and ever since then, it has been of critical importance to the field of radiobiology in terms of comprehending how radiation influences the proliferation and survival of cancer cells.

In order to determine the clonogenicity of cells, it is necessary to implant them at extremely low densities and then wait for them to have formed colonies for a period of one to three weeks. Staining with crystal violet makes the colonies visible, and then they are counted once they have been fixed. In order to do data analysis, plotting cell survival curves is necessary. (Rafehi H etal;2011).

Procedure: During the course of this experiment, MCF-7 cells were collected, and a hemocytometer was utilised to determine the total number of cells. Following the application of a treatment for a period of twenty-four hours, the cells were allowed to attach to one another and form a full monolayer. Each well contained two hundred cells when the cells were planted. One hundred milligrammes of the medicine known as glycyrrhizinic acid microsphere was introduced to the cell culture in a variety of different doses. After that, the cells were cultured for a period of seventy-two hours, rinsed with phosphate-buffered saline (PBS), and the colonies that had developed were fixed with methanol. Following twenty minutes of staining with crystal violet, the cells were counted using a light microscope.

Cell Invasion Assay

PrincipleAccording to Te Boekhorst et al. (2016), cell migration is an essential process that enables cells to adapt to their surroundings and move into the appropriate location in each environment so that they can perform their function. This phenomenon is essential for the processes of gastrulation, embryonic morphogenesis, the development of the nervous system, the maintenance of tissue homeostasis, and the trafficking of immune cells in

multicellular organisms. Moving cells, on the other hand, have the potential to become uncontrolled and contribute to a wide range of pathological events, including as inflammation and the spread of cancer. (Charras and Sahai ;2014).

Metastasis is the process by which cells from a tumour spread out from the original tumour and migrate via the lymphatic and circulatory systems, infiltrate through basement membranes and endothelial walls, and finally populate organs that are located in other parts of the body (Friedl and Wolf, 2003). It is crucial to understand and comprehend this process in order to combat disease because of the significant roles that cell migration, invasion, and adhesion play in this process. Furthermore, the spread of cancer cells to peripheral organs and the resultant damage to those organs is one of the primary causes of morbidity and mortality that are associated with cancer.

Procedure: In an experiment using a 24-well plate, we evaluated how the presence of glycyrrhizinic acid affected the capacity of MCF-7 cells to invade one another. Matrigel was applied to a polycarbonate filter that was manufactured without the use of polyvinyl pyrrolidone and had holes that were 6 millimetres in width. Following this, the upper chamber and coated filter were positioned on top of the bottom chamber, which had been filled with medium that contained 10% FBS in the past. Glycyrrhizinic acid was given to the MCF-7 cell culture at a number of different doses, including 0 M, 10 M, 50 M, and 100 M. The cell culture was maintained at a density of 2x106 cells per well. The treatment lasted for forty minutes on the cells. Following the seeding of the drug-containing cell culture into the upper chamber, the culture was allowed to continue growing there for an additional forty minutes. After that, the cells were frozen, stained with crystal violet concentrated at a concentration of 0.5 percent for twenty minutes, and examined with a phase contrast microscope.

Statistical analysis

We present the data as the mean plus or minus the standard deviation. In order to make a comparison between the untreated control group and the glycyrrhizinic acid-treated group, a Student's t-test performed. In order to determine whether differences were statistically significant, a p-value of 0.05 or less was required.

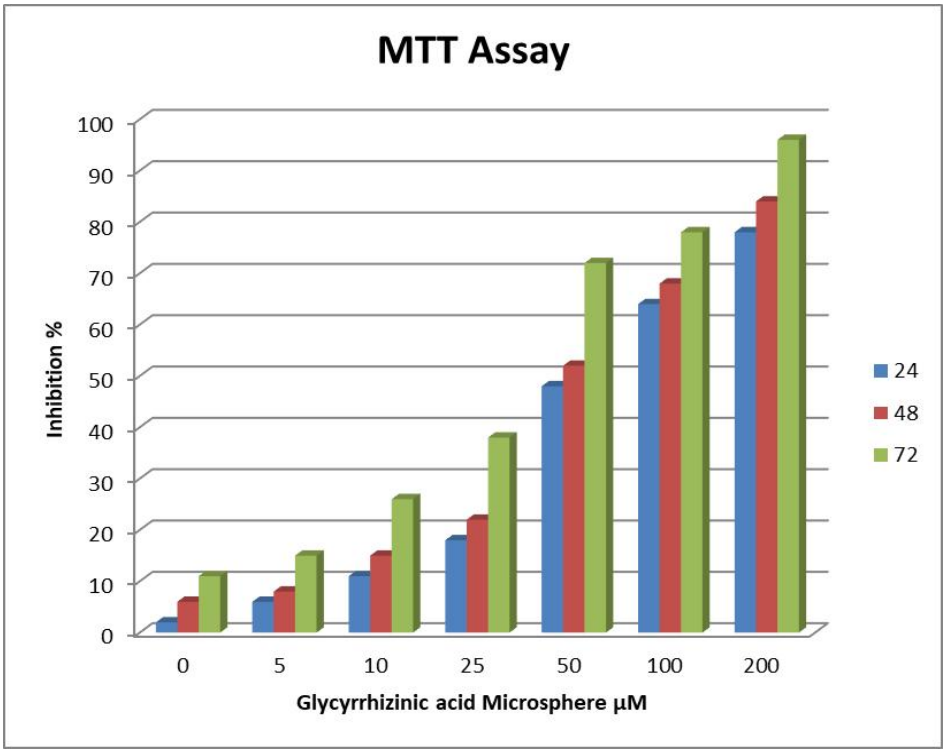


Fig 61: MTT Assay

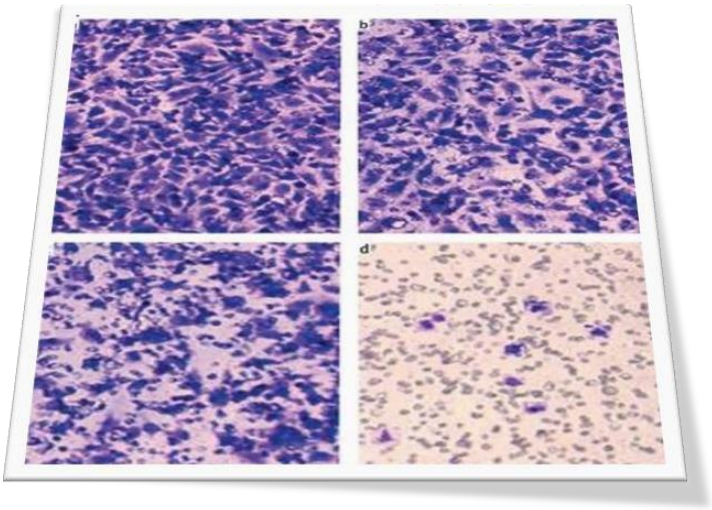


Fig 62: Glycyrrhizinic acid containing microsphere led to inhibition of cell invasion in MCF-7 human breast cancer cells. The cells were treated with 0 (a), 10 (b), 50 (c), and 100 µM (d) doses of Glycyrrhizinic acid for 48 hr.

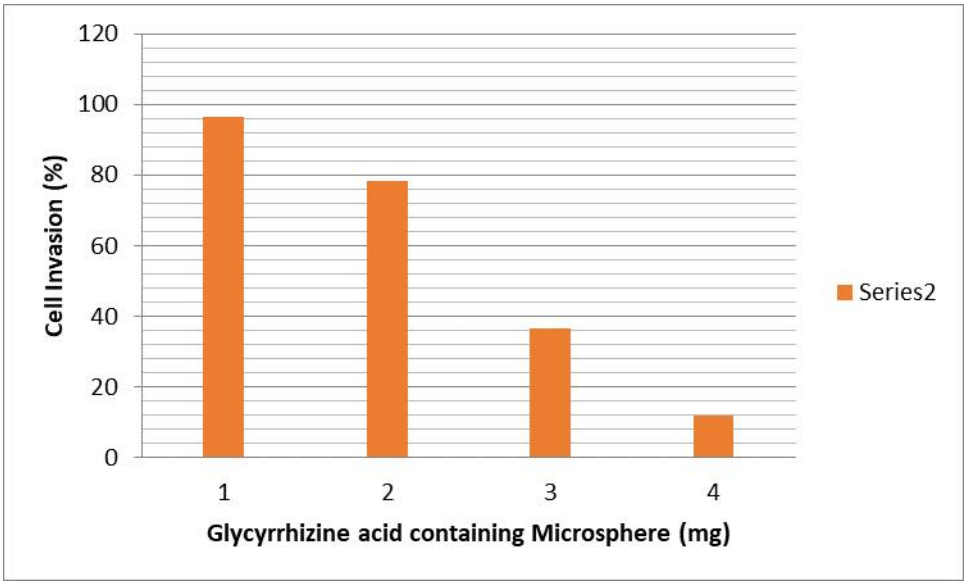


Fig 63: Bar graph showing the percentage of the invasive cells at indicated concentration of the drug

Data are shown as the mean ±SD of three independent experiments. *p*-value ≤ 0.05 vs 0 µM (Control)

CHAPTER – 7

RESULT AND DISCUSSION

The therapeutic approaches for cancer can also benefit greatly from lowering multi-drug Nature has always been a bizarre supply of medical ingredients, providing us with several medicinal plants that yield beneficial phytochemicals. Licorice, scientifically known as *Glycyrrhiza glabra*, belongs to the Leguminosae family and is widely used in Ayurvedic medicine. Extensive pharmacological studies have been conducted on various chemical constituents of *Glycyrrhiza glabra*, revealing its strong anticancer, antibacterial, anti-inflammatory, cardioprotective, hepatoprotective, and other beneficial properties.. Gastric and duodenal ulcers have traditionally been treated with liquorice as a prophylactic measure. It is used as an anti-inflammatory medication in dyspepsia during allergic responses. The stereoisomer forms of glycyrrhizin are 18α and 18 β. Due to its anti-inflammatory effects on neutrophil functions, especially the production of ROS, glycyrrhizin is regarded to be the most popular folk remedy (reactive oxygen species).

Most current cancer treatment strategies focus on the surgical removal of tumor masses, with chemotherapy and radiotherapy playing critical roles in slowing the spread of malignant cells. Often, these methods are combined to enhance treatment outcomes. However, it is well-documented that surgery, chemotherapy, and radiotherapy can also inhibit the growth of healthy cells, leading to significant side effects and a reduced quality of life due to their toxicity.

In recent years, innovative approaches have expanded the options available for cancer treatment beyond traditional methods. These include ligand or receptor-based targeting, triggered drug release, intracellular drug targeting, gene therapy, cancer stem cell therapy, magnetic drug targeting, and ultrasound-mediated drug delivery. These advanced techniques allow for the precise identification and elimination of cancerous cells while minimizing adverse effects. Additionally, overcoming drug resistance and enhancing the transport of targeted therapies into cancer cells remain important aspects of improving the efficacy of these novel treatment modalities.

Thus, the current examination investigated the different phytochemicals and physiochemical parameters of root was carried out. The normalization boundaries were misfortune on drying at 100-105^{0}C, ash value & LOD was tabulated in table1 & graph 1. Table 2 shows that the aqueous & ethanolic root extract contained a number of phytoceuticals. Glycyrrhizin was extracted from the aqueous root of *G.glabra* with a yield of 7.91 percent w/w. Additionally, extractive values can be used to assess the chemical components of a crude medication and estimate which components are soluble in which solvents. The identification of used-up or tampered pharmaceuticals is the main application of extractive values. The quality and purity of the medicine are determined by the extraction value of the crude substance. When evaluating crude pharmaceuticals, the extractive value that is water-soluble is very essential. A lower extractive value suggests the use of used materials, adulteration, or improper drying, storing, or formulation techniques.

The isolated compound glycyrrhizin was found to have a melting point of 221°C, consistent with previously reported values for glycyrrhizin. Table 3 presents the general properties of the isolated glycyrrhizin. Qualitative Thin Layer Chromatography (TLC) analysis revealed Rf values that closely matched the standard glycyrrhizin (Table 4, Fig. 4). The UV absorption spectrum of glycyrrhizin in methanol displayed two notable absorption peaks at 251 and 254 nm, indicating the presence of a triterpenoid structure (Fig. 5). Chromatographic fingerprints are essential for quality control in herbal medicine and should be considered in the global evaluation of herbal products. High-performance liquid chromatography (HPLC) was conducted using the LC-100 system with a UV detector. The retention times (RT) for the standard and isolated glycyrrhizin were 8.00 min and 7.58 min, respectively (Figs. 6 and 7). The infrared (IR) spectra of the isolated compound showed functional groups consistent with the reference compound, as detailed in Figure 8 and Table 6.

Further characterization of glycyrrhizin was carried out using modern analytical techniques. Preformulation studies of glycyrrhizin included organoleptic characteristics and UV spectral analysis. The isolated compound was found to be soluble in water and slightly soluble in ether, with a melting point of 221°C and a partition coefficient of 6.2, indicating its lipophilic nature and its ability to permeate biological membranes (Table 10).

Spectroscopic data were summarized in Table 11 and Figure 16, with a correlation coefficient (R2) of 0.9806. A standard curve for glycyrrhizin was established using UV spectroscopy at 254 nm. Drug-polymer interaction studies were conducted using FT-IR spectroscopy, and the resulting spectra showed that the characteristic peaks of glycyrrhizin were retained in the drug-polymer mixtures (Table 12, Fig. 17).

Microspheres containing 100 mg of glycyrrhizin were formulated using a cross-linking process with varying polymer concentrations (Table 13). The formulations (F1-F5) were evaluated for percent yield and drug entrapment efficiency. The F2 formulation exhibited optimal results, with a yield of 81.6% and a drug entrapment efficiency of 93.5% (Tables 14 and 15). The IR spectra of the glycyrrhizin-loaded microspheres were consistent with the standard compound (Fig. 18).

The F2 formulation was selected for further in vitro drug release studies. The drug release was investigated using a USP dissolution apparatus (basket type) with phosphate buffer (pH 7.4) as the dissolution medium at 37 ± 0.5°C and 100 rpm. Microspheres, with diameters ranging from 1 µm to 1000 µm, were produced from natural and synthetic materials. The particle size of the optimized formulation was measured using optical microscopy, revealing sizes between 7 and 10 µm (Fig. 20).

To investigate the mechanism of glycyrrhizin's antiproliferative activity, in-silico molecular docking studies were performed using Autodock. Molecular docking identifies the optimal orientation of a ligand to its target protein, minimizing free energy and forming a stable complex. This computational drug design method is more efficient and cost-effective compared to traditional cancer therapies. Docking analyses between glycyrrhizin and selected proteins—caspase 9, COX-2, p53, and AURKA—revealed binding energies that indicated high affinity and stability in the ligand-protein complexes.

Glycyrrhizin exhibited strong binding affinity with AURKA and COX-2, with binding energies of –7.11 kcal/mol and –7.7 kcal/mol, respectively. Glycyrrhizin was shown to inhibit the proliferation of cancer cells by inducing apoptosis at the G1 phase and cell cycle arrest at the G2/M phase. The docking interactions between glycyrrhizin and COX-2 revealed interactions with Tyr385 and Tyr348 residues, while binding with AURKA

involved Arg357, Ser329, Glu332, and Arg476 residues (Figs. 30 and 40). AURKA is known to be overexpressed in several cancers and is associated with poor survival outcomes. The docking and dynamic analysis confirmed glycyrrhizin as a potent AURKA inhibitor, while its binding to COX-2 suggests its potential use in developing new anti-COX-2 agents for cancer therapy.

Further in vitro studies on glycyrrhizin's antiproliferative effects were performed using models such as the MTT assay, colony formation assay, and cell invasion assay. Glycyrrhizin-containing microspheres demonstrated strong cytotoxicity and inhibition of colony formation. The MTT and clonogenic assays revealed that glycyrrhizin-loaded microspheres significantly inhibited the growth of MCF-7 breast cancer cells in a concentration- and time-dependent manner (Fig. 43).

The clonogenic assay also indicated that increasing doses of glycyrrhizin reduced the colony-forming ability of MCF-7 cells (Fig. 44), suggesting that glycyrrhizin exhibits a dual effect by inhibiting both cell viability and colony formation. This underscores glycyrrhizin’s potential as an effective therapeutic agent in cancer treatment.

CHAPTER – 8

SUMMARY AND CONCLUSION

The oral route is preferred and most practical for administering medications due to its simplicity. Due to the drug's first pass impact in the liver, oral administration is undesirable. Alternative medication delivery methods, including parenteral, intramuscular, subcutaneous, etc., are also employed to treat diseases.

The sustained and controlled drug delivery system, also known as smart drug delivery, is a technique for administering medication to a patient in a way that increases the concentration of the drug over a set length of time, hence reducing the frequency of administration. Micelles, liposomes, nanoparticles, microspheres, and other forms of vehicles are employed for sustained and regulated drug delivery systems.

Cancer is a disease that has a significant global impact on people. To treat and prevent this fatal condition, there is a continuing need for novel therapeutics. Natural substances are gaining attention from science and study because they are thought to have fewer hazardous side effects than existing treatments like chemotherapy. Natural secondary metabolites produced by the plant kingdom are being studied for their potential anticancer properties, which could lead to the creation of brand-new pharmaceuticals. New technologies are emerging to advance the field as a result of the success of these chemicals, which have been transformed into essential medications for the treatment of cancer. Nanoparticles for nanomedicines are a novel technology that aims to improve the anticancer effects of compounds produced from plants by managing the compound's release and researching alternative administration techniques.

Glycyrrhiza is derived from the ancient Greek term glykos, meaning sweet, and rhiza, meaning root. Glycyrrhiza glabra is known as mulaithi in north India. Glycyrrhiza glabra, also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. the plant is recommended as a common remedy for gastrointestinal problems, cough, bronchitis, and arthritis. In particular, it is still widely used to treat gastritis, peptic ulcers, respiratory infections, and tremors in folk medicine.

One of the goals that is being aggressively pursued in anticancer chemotherapy is improving the delivery of anticancer medicines in a targeted manner. Systemic anticancer medicines have a number of drawbacks, the most significant of which is that they are not selective for tumour tissue. This lack of selectivity results in severe side effects and low cure rates. A method that increases the therapeutic index of a cytotoxic agent by targeting it to the tumour is an example of an approach that can improve cancer therapy while simultaneously reducing the overall amount of toxicity that the treatment causes.

Microspheres are small spherical particles with diameter in the range typically 1µm to 1000 µm. They are sometimes referred as micro-particles. Microspheres can be manufactured from various natural and synthetic materials. The word glycyrrhiza is derived from the Greek words glykos, which means sweet, and rhiza, which means root. In north India, Glycyrrhiza glabra is referred to as mulaithi. The licorice and sweet wood plant, Glycyrrhiza glabra, is indigenous to the Mediterranean and some parts of Asia. The herb is suggested as a typical treatment for arthritis, bronchitis, coughs, and digestive issues. In particular, gastritis, peptic ulcers, respiratory infections, and tremors are still frequently treated with it in folk medicine.

The aim of this study was to isolated glycyrrhizin from *G.glabra* root followed by formulation, optimization and evaluation the glycyrrhizin loaded microspheres.

The preliminary physiochemical and phytochemical analysis of *G.glabra* root was carried out which revealed presence of glycoside, saponin & flavonoid. Then glycyrrhizin was isolated form aqueous root extract of *G.glabra* and characterized by modern analytical methods.

In order to formulate microsphere, glycyrrhizin firstly goes through preformulation studies, which includes determination of melting point, solubility, partition coefficient, Fourier Transform Infrared Spectroscopy (FT-IR), determination of λ max, and calibration curve of Glycyrrhizin.

By utilising gluteraldehyde as a cross-linking agent during the emulsification cross-linking process, the microspheres were created. The yield and drug loading effectiveness of the produced microspheres were further evaluated, as well as their particle size distribution and

in vitro drug release studies, using Fourier Transform Infrared Spectroscopy (FT-IR).

The prepared microspheres were spherical in shape and free flowing in nature. The drug loaded microspheres shows 74 to 81.5 % of yield and 82 to 93.5 % of entrapment. The infrared spectra showed stable character of glycyrrhizin with polymer and other excipients, and revealed the absence of drug excipient interaction. The distribution of particle size ranges from 2 µm to 200 µm and is spherical in shape. *In-vitro* studies were performed in Phosphate buffer saline (pH 7.4). Formulation (F2) was selected for in -vitro studies because of its high drug efficiency.

Formulation, optimization and evaluation of Glycyrrhizin loaded microspheres for controlled release was found to be potential and effective in terms of yield, encapsulation efficiency, particle size distribution and in *in-vitro* release characteristics. The outcome of study demonstrated that F2 formulation was found to be optimized formulation and F2 formulation attained zero order kinetic which have the potential to overcome the issues facing immediate-release.

The *in-silico* study conducted here compares the interaction of AURKA & COX2 with the selective inhibitor and the bioactive compound glycyrrhizin isolated from the roots of *G.glabra*. The study showed very encouraging results in terms of binding affinity and molecular dynamics to the validated inhibitor. From this study, we can determine that the glycyrrhizin can be potential therapeutic molecules for AURKA & COX2 protein. The findings in this study not only validate the ethnomedicinal use of *G.glabra* as an anticancer herb by predicting the possible modes of action by inhibiting the key residues of AURKA & COX2. Many AURKA protein have been found, and it has been shown that activation of AURKA plays a significant role in a variety of malignancies. AURKA protein, some of which are mitotic regulators, tumour suppressors, or oncogenes, can have their activities controlled by phosphorylation through the action of AURKA. Additionally, KEGG pathway enrichment and GO analysis have shown that AURKA-interacting proteins are involved in traditional carcinogenic pathways. All of these data support the notion that AURKA is a promising target for cancer treatment, and AURKA-targeting small molecules have been identified. Cancer-associated fibroblasts (CAFs), macrophage type 2 (M2) cells, and cancer cells produce the pro-inflammatory mediator COX-2 into the tumour

microenvironment (TME). In addition to encouraging apoptotic resistance, cancer cell proliferation, angiogenesis, inflammation, invasion, and metastasis, COX-2 also generates behaviour resembling that of cancer stem cells (CSCs). Cancer cells are more resistant to chemotherapy because of COX-2-mediated hypoxia within the TME, as well as its favourable interactions with YAP1 and antiapoptotic mediators. According to the results of molecular docking, glycyrrhizin has a strong inhibitory effect on the proteins COX2 and AURKA, acting as an antiproliferative agent. The *in-silico* validation was intended to be related to further in-vitro testing to determine the antiproliferative efficacy.

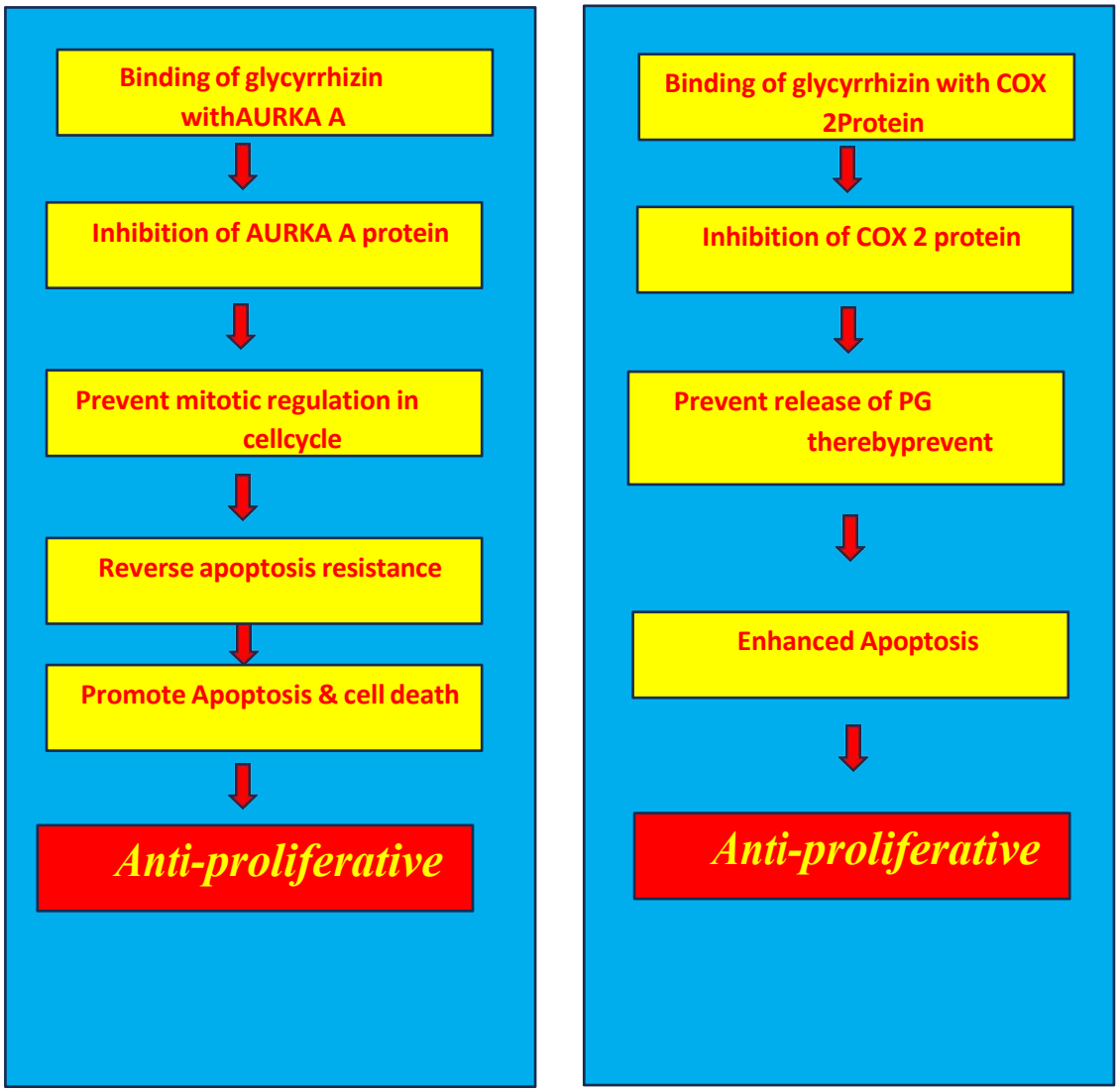
Initially, the MTT assay and clonogenic assays were used to evaluate the effects of glycyrrhizin containing Microsphere on cell viability and colony-forming tendency, which showed that this compound caused a strong cytotoxic effect and colony-forming efficiency of these cells. Our results are consistent with previous studies.

In addition, it has been shown that glycyrrhizin can inhibit the invasion of MCF-7 cancer cells in a concentration-dependent manner. These findings are promising because it is well established that breast cancer is one of the most invasive cancers and glycyrrhizin can prevent this behaviour.

In conclusion, glycyrrhizin containing Microsphere inhibited the growth of human MCF-7 breast cancer cells by inhibiting colony formation, inhibiting cell invasion. We strongly believe that glycyrrhizin may prove to be an important lead molecule. However, more in-depth mechanistic studies are needed. The glycyrrhizin-microsphere complex, in its entirety, was a multistep targeted drug delivery method that boosted drug accumulation in tumours, hence boosting anti-cancer efficacy and reducing adverse responses.

Divulgence of Investigation

There are several benefits to using microspheres as a medication delivery mechanism, including increased efficacy and decreased toxicity of the integrated drugs to non-targeted cells and tissues. Future systemic, oral, and localised cancer treatments using cytotoxic medicines and biological response modifiers are projected to benefit considerably from microspheres. The goal of the current inquiry was to develop and examine the potential of microspheres containing glycyrrhizin for the evaluation of anti-proliferative activity. The mechanism of glycyrrhizin against cancer was shown pictorially as follows:



CHAPTER - 9

SIGNIFICANCE OF INVESTIGATION

The rising global incidence of cancer has intensified the demand for alternative treatment options. Herbal therapy presents a practical alternative to traditional cancer treatments. Glycyrrhizin, a compound found in the root of the licorice plant (*Glycyrrhiza glabra*), is a triterpene glycoside with a wide range of pharmacological and biological properties. It is known for its anti-inflammatory, anti-ulcer, anti-allergic, antioxidant, anti-tumor, anti-diabetic, and hepatoprotective effects. These properties make it useful in treating conditions such as premenstrual syndrome, viral infections, hyperlipidemia, and hyperglycemia. Glycyrrhizin is also widely recognized for its efficacy in managing gastrointestinal disorders, including peptic ulcers.

Microspheres have emerged as a superior option compared to many traditional drug delivery systems. They hold significant potential for advanced drug delivery by integrating various techniques, especially in areas such as targeted drug delivery, diagnostics, gene therapy, and disease-specific cell sorting. Microspheres can mimic diseased organs and tissues for precise in vitro applications. They offer several advantages, including enhanced solubility for poorly soluble drugs, protection against enzymatic and photolytic degradation, reduced dosing frequency, improved bioavailability, and controlled release profiles, all of which reduce drug toxicity and improve therapeutic outcomes.

The present study focuses on the development, characterization, and evaluation of glycyrrhizin-loaded microspheres as an effective drug delivery system with anti-proliferative potential. This method shows promise for cancer treatment, offering targeted drug delivery with the potential to improve efficacy and reduce side effects compared to conventional treatments.

REFERENCE

1. Abdullaev FI. Plant derived agents against cancer. In: Gupta SK, editor. Pharmacology and therapeutics in the new millennium. Narosa publishing house: New Delhi, India 2001: 345-354.
2. ACD/Structure Elucidator, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
3. Vistica VT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd MR. 1991. Tetrazolium- based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer Res. 51(10):2515-20.
4. Rafehi H, Orlowski C, Georgiadis GT, Ververis K, El-Osta A, Karagiannis TC. Clonogenic assay: adherent cells. J Vis Exp. 2011 Mar 13;(49):2573.
5. Friedl, P., and Wolf, K. (2003). Tumour-cell invasion and migration: diversity and escapemechanisms. Nat. Rev. Cancer 3, 362–374.
6. Charras, G., and Sahai, E. (2014). Physical influences of the extracellular environmenton cell migration. Nat. Rev. Mol. Cell Biol. 15, 813–824.
7. ACD/Structure Elucidator, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
8. ACD/Structure Elucidator, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
9. Alagusundara M, Madhu Sudana Chetty.C, Umashankari.K, Attuluri Venkata Badarinath, Lavanya.C and Ramkanth.S, “Microspheres as a novel drug delivery system”, Int.J. of Chem Tech Research, Jan-Sept 2009.
10. Soni et al. Quantification of Ascorbic acid in leaves of *Annona squamosa*. Int J Pharm Pharm Sci, Vol 4, Issue 3, 144-147.
11. Soni et al. Characterization of Azadirachtin from ethanolic extract of leaves of *Azadirachtaindica.. Journal of Pharmacy Research* ,2012,5(1),199-201.
12. P. J. Waller and S. M. Thamsborg, “Nematode control in 'green' ruminant productionsystems,” *Trends in Parasitology*, vol. 20, no. 10, pp. 493–497, 2004.

13. Singh BP, Wayne F. Whitehead Pointed gourd: Potential for temperate climates. J Janick.1999;118:27–35. [Google Scholar]

14. Nadkarni AK. Indian Materia Medica. 3rd ed. Mumbai: Popular prakashan Pvt. Ltd; 1982.pp. 1236–7

15. Kongton S. Chemical study of bioactive constituents from Trichosanthes cucumerina rootand fruit juice. Hong Kong: Thesis, Mahidol University; 2003.

16. Ali N, Mohammed S, Kenoth R, Swamy MJ. Purification, physicochemical characterization, saccharide specificity, and chemical modification of a Gal/GalNAc specific lectin from the seeds of Trichosanthes dioica. Arch Biochem Biophysics. 2004;432:212–21.

17. Sharma RK, Chatterji S, Rai DK, Mehta S, Rai PK, Singh RK, et al. Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. J Med Plants Res. 2009;3:944–8.

18. Mahia Khandaker, Saleha Akter, Mohammad Zafar Imam, Trichosanthes dioica Roxb.: Avegetable with diverse pharmacological properties, Food Science and Human Wellness, Volume 7, Issue 1,2018, Pages 34-48.

19. Goswami S, Pandey A, Tripathi P, Singh A, Rai A. An in vitro evaluation of the anthelmintic activity of Hedychium spichatum rhizomes and Zingiber zerumbet rhizomes on the Pheritima Posthuma model: A comparative study. Pharmacognosy Res. 2011 Apr;3(2):140-2.

20. Himesh Soni *etal*. (2022). Silibin as Potent Inhibitor of COVID -19 Main Protease: In-Silico Docking Approach. Journal of Volume-4, Issue-1 (January-June, 2022)Molecular Pharmaceuticals and Regulatory Affairs.1-7.

21. Himesh Soni, Satish Sarankar, Sarvesh Sharma & Jitender K Malik. Hydroxychloroquine as Potent Inhibitor of COVID -19 Main Protease : Grid Based Docking Approach. EJMO2020;4(3):219–226.

22. Himesh Soni, Dr. V.K. Gautam, Sarvesh Sharma, Jitender K Malik. Rifampicin as Potent Inhibitor of COVID - 19 Main Protease: In-Silico Docking Approach. Saudi J Med PharmSci, September, 2020; 6(9): 588-593.

23. Himesh Soni etal. (2022). Silibin as Potent Inhibitor of COVID -19 Main Protease: In-Silico Docking Approach. Journal of Volume-4, Issue-1 (January-June, 2022)

Molecular Pharmaceuticals and Regulatory Affairs.1-7.

24. Saurabh Soni , Jitender K Malik , Satish K. Sarankar & Himesh Soni. Rutin as a c Potent Inhibitor of Dihydrofolate Reductase: A Computational Design and Docking. EASJ Pharm& Pharmacol; Vol-1, Iss-6 (Nov-Dec, 2019): 130-134.

25. N. Soni, K.R. Pardasani, S. Mujwar, Insilico analysis of dietary agents as hepatoprotectiveinhibitors of insulin like growth factor 1 receptor (IGF1R), J Pharm Pharm Sci, 7 (2015) 191-196.

26. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis, J Comput Chem, 25 (2004) 1605-1612.

27. Ali Esmail Al-Snafi. Glycyrrhiza glabra: A phytochemical and pharmacological review.IOSR Journal of Pharmacy.2018.8;6:1-17.

28. Amritpal Singh Herbal Medicine Dream Unresolved. Ethnobotanical Leaflets 11: 195-198. 2007.

29. Anam Abrar *etal*. Formulation and evaluation of microsphere of antiulcer drug using *Acacia nilotica* gum. Int J Health Sci (Qassim). 2020; 14(2): 10–17.

30. Ando S, Putnam D, Pack DW, and Langer R. PLGA Microspheres Containing Plasmid DNA: Preservation of Super coiled DNA via Cry preparation and Carbohydrate Stabilization. J. Pharmaceut. Sci.1998;88(1): 126– 130.

31. Antonio J. Ribeiro, Catarina Silva, Domingos Ferreira, Francisco Veiga, "Chitosan-reinforced alginate microsphere obtainedthrough the emulsification/internal gelatin technique" European J. of pharmaceutical science, 2005, 31-40.

32. Asija R, Sharma D and Mall KR: Solvent evaporation matrix erosion method: a novel approach for floating microsphere development. Journal of Drug Discovery and Therapeutics 2014; 2:24-29.

33. Bansal H., kaur S. P., Gupta A. K., "Microsphere: Methods of preparation and applications, A comparative study", Int. J Pharm Sci. Rev Res. 2011;10(1):69-78.

34. Bartoszewska S, Sławski J, Collawn JF, Bartoszewski R. Dual RNase activity of IRE1 asa target for anticancer therapies. J Cell Commun Signal. 2023 Sep 18.

35. Beckmann L, Tretbar US, Kitte R, Tretbar M. Anticancer Activity of Natural and Semi- Synthetic Drimane and Coloratane Sesquiterpenoids. Molecules. 2022 Apr

13;27(8):2501.

36. Ben Amar M. Cannabinoids in medicine: a review of their therapeutic potential. J Ethnopharmacol. 2006; 105: 1-25.

37. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. Nucleic Acids Res. Jan 1 2000;28(1):235-242.

38. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. Nucleic Acids Res. Jan 1 2000;28(1):235-242.

39. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. Nucleic Acids Res. Jan 1 2000;28(1):235-242.

40. Bhat AA, Singh I, Tandon N, Tandon R. Structure activity relationship (SAR) and anticancer activity of pyrrolidine derivatives: Recent developments and future prospects(A review). Eur J Med Chem. 2023 Jan 15;246:114954.

41. Changchao Huan *etal*.Research Progress on the Antiviral Activity of Glycyrrhizin and itsDerivatives in Liquorice. Front. Pharmacol., 2021.

42. Cherng, J.M.; Tsai, K.D.; Yu, Y.W.; Lin, J.C. Molecular Mechanisms Underlying Chemopreventive Activities of Glycyrrhizic Acid against UVB-Radiation-Induced Carcinogenesis in SKH-1 Hairless Mouse Epidermis. Radiat. Res. 2011, 176, 177–186

43. Chi H, Qiu Y, Ye X, Shi J, Li Z. Preparation strategy of hydrogel microsphere and its application in skin repair. Front Bioeng Biotechnol. 2023 Jul 24;11:1239183.

44. Chinna Gangadhar B, Shyam Sunder R., Vimal Kumar Varma. M., Sleeva Raju M., Sai Kiran M, Formulation and Evaluation of Indomethacin Microspheres using natural and synthetic polymers as Controlled Release Dosage Forms. International Journal of Drug Discovery, 2(1), 2010,8-16.

45. Dai Y, Zhu C, Xiao W, Huang K, Wang X, Shi C, Lin D, Zhang H, Liu X, Peng B, Gao Y, Liu CH, Ge B, Kaufmann SH, Feng CG, Chen X, Cai Y. Mycobacterium tuberculosis hijacks host TRIM21- and NCOA4-dependent ferritinophagy to enhance intracellular growth. J Clin Invest. 2023 Apr 17;133(8):e159941.D'Angelo S, Morana A, Salvatore A, Zappia V, Galletti P.Protective effect of polyphenolsfrom Glycyrrhiza glabra against oxidative stress in Caco-2 cells. *J Med Food.* 2009;12(6):1326-33.

46. Das S, Basu SP. Cytotoxic activity of methanolic extract of Berberis aristata DC on coloncancer. Global J Pharmacol. 2009; 3(3): 137-140.

47. DeLano WLJCNopc. Pymol: An open-source molecular graphics tool. 2002;40(1):82-92.

48. DeLano WLJCNopc. Pymol: An open-source molecular graphics tool. 2002;40(1):82-92.

49. DeLano WLJCNopc. Pymol: An open-source molecular graphics tool. 2002;40(1):82-92.

50. Dhingra D and Sharma A. Antidepressant-like activity of Glycyrrhiza glabra L in mouse models ofimmobility tests. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2006; 30(3): 449-454.[62].

51. Di Mambro VM, Fonseca MJ.Assays of physical stability and antioxidant activity of a topical formulation added with different plant extracts. *J Pharm Biomed Anal.* 2005 23;37(2):287-95.

52. Don G. Catharanthus roseus. In: Ross IA, ed. Medicinal plants of the world: chemical constituents, traditional and modern uses. Vol 1. Totowa, New Jersey, USA: Humana Press; 2003: 175-196.

53. Dreosti IE. Bioactive ingredients: antioxidants and polyphenols in tea. Nutr Rev. 1996; 54: S51-58.

54. Du Toit Lisa Clare, Pillay Viness and Danckwerts Michael Paul, "Tuberculosis Chemotherapy: current drug delivery approaches", Respiratory Researches, 2006; 1-31.

55. Farah Hamad Farah, et. al., Magnetic Microspheres: A Novel Drug Delivery System, Analytical and Pharmaceutical Research,2016 3 (5), Page 1-10.

56. Feng Z, Su X, Wang T, Sun X, Yang H, Guo S. The Role of Microsphere Structures in Bottom-Up Bone Tissue Engineering. Pharmaceutics. 2023 Jan 18;15(2):321.

57. Franceschelli S, Pesce M, Vinciguerra I, Ferrone A, Riccioni G, Patruno A, Grilli A, Felaco M, Speranza L. Licocalchone-C extracted from Glycyrrhiza glabra inhibits lipopolysaccharide-interferon-γ inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. *Molecule.* 2011 6;16(7):5720-34.

58. Fu X, Ping Q and Gao Y. Effects of formulation factor on encapsulation efficiency and release behavior *in- vitro* of huperzine A-PLGA microspheres. Journal of Microencapsulation 2005; 22:705-714.

59. Fukai T, Satoh K, Nomura T, Sakagami H.Preliminary evaluation of antinephritis and radical scavenging activities of glabridin from Glycyrrhiza glabra.*Fitoterapia.* 2003;74(7-8):624-9.

60. Ghalop SB, Banerjee SK and Throat RM et al: Hollow microsphere a Review. International Journal of Pharmaceutical Science Review & Research 2010; 1:10-15.

61. Go.drugbank.com/drugs/DB11063.

62. Guerfi M, Berredjem M, Dekir A, Bahadi R, Djouad SE, Sothea TO, Redjemia R, Belhani B, Boussaker M. Anticancer activity, DFT study, ADMET prediction, and molecular docking of novel α-sulfamidophosphonates. Mol Divers. 2023 Apr 3.

63. Guzman M. Cannabinoids potential anticancer agents. Nature Rev Cancer. 2003; 3: 745-755.

64. Hadiyal SD, Lalpara JN, Dhaduk BB, Joshi HS. Rational synthesis, anticancer activity, and molecular docking studies of novel benzofuran liked thiazole hybrids. Mol Divers. 2023 Jun;27(3):1345-1357.

65. Harsh Bansal, et. al., Microsphere: Methods of Prepration and Applications; a Comparative Study, International Journal of Pharmaceutical Sciences Review and Research, 2011, 10(1); Article-012.

66. Hasan, M.K.; Ara, I.; Mondal, M.S.A.; Kabir, Y. Phytochemistry, Pharmacological Activity, and Potential Health Benefits of Glycyrrhiza Glabra. Heliyon 2021, 7, e07240.

67. Hasan, S.K.; Siddiqi, A.; Nafees, S.; Ali, N.; Rashid, S.; Ali, R.; Shahid, A.; Sultana, S. Chemopreventive Effect of 18β-Glycyrrhetinic Acid via Modulation of Inflammatory Markers and Induction of Apoptosis in Human Hepatoma Cell Line (HepG2). Mol. Cell. Biochem. 2016, 416, 169–177.

68. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 incancer: A review. J Cell Physiol. 2019 May;234(5):5683-5699.

69. Hassanpour SH, Dehghani M, Review of cancer from perspective ofmolecular, Journal of Cancer Research and Practice (2017).

70. Himesh Soni *etal*. Preformulation Studies of Tramadol HCl: Vital Part of Formulation Design. ***EJBPS,*** *2020, 7;1, 369-373.*

71. Himesh Soni *etal*. Recent Update of Botanical For Wound Healing Activity. IRJP,3(7)(2012)1-7.

72. Hoever, G.; Baltina, L.; Michaelis, M.; Kondratenko, R.; Baltina, L.; Tolstikov, G.A.; Doerr, H.W.; Cinatl, J. Antiviral Activity of Glycyrrhizic Acid Derivatives against SARS-Coronavirus. J. Med. Chem. 2005, 48, 1256–1259

73. Horigome, H.; Homma, M.; Hirano, T.; Oka, K. Glycyrrhetinic Acid Induced Apoptosis in Murine Splenocytes. Biol. Pharm. Bull. 2001, 24, 54–58.

74. Huang P, Wang X, Liang X, Yang J, Zhang C, Kong D, Wang W. Nano-, micro-, and macroscale drug delivery systems for cancer immunotherapy. Acta Biomater. 2019 Feb;85:1-26.

75. Ilango S, Sahoo DK, Paital B, Kathirvel K, Gabriel JI, Subramaniam K, Jayachandran P, Dash RK, Hati AK, Behera TR, Mishra P, Nirmaladevi R. A Review on Annona muricataand Its Anticancer Activity. Cancers (Basel). 2022 Sep 19;14(18):4539.

76. Ilaria Marotti *etal*. Evaluation of licorice (Glycyrrhiza glabra L.) as a novel microgreen from the anti-inflammatory potential of polyphenols. AIMS Agriculture and Food .2021, 6;1: 1-13.

77. Illum Lisbeth, “Chitosan and its use as a pharmaceutical exciepents”, Pharmaceutical Research, Vol. 15, No. 9, 1998; 1326-1327.

78. Jagtap Yogesh Mukund, et. al., Floating microspheres: a review, Brazilian Journal of Pharmaceutical Sciences, 2012, 48 (1), Page:17-30.

79. Jun-Xian Zhou, Markus Santhosh Braun , Pille Wetterauer , Bernhard Wetterauer and Michael Wink .Antioxidant, Cytotoxic, and Antimicrobial Activities of Glycyrrhiza glabra L., Paeonia lactiflora Pall., and Eriobotrya japonica (Thunb.) Lindl. Extracts. Medicines 2019, 6, 43.

80. Kadam N.R and Suvarna V,” Microsphere: A Brief Review”, Asian J. of Biomed. and Pharm. Sci., 2015; 5(47), 13-49.

81. Kadam N.R and Suvarna V,” Microsphere: A Brief Review”, Asian J. of Biomed. and Pharm. Sci., 2015; 5(47), 13-49.

82. Kamal N, Ilowefah MA, Hilles AR, Anua NA, Awin T, Alshwyeh HA, Aldosary SK, Jambocus NGS, Alosaimi AA, Rahman A, Mahmood S, Mediani A. Genesis and Mechanism of Some Cancer Types and an Overview on the Role of Diet and Nutrition inCancer Prevention. Molecules. 2022 Mar 9;27(6):1794.

83. Karuppusamy.C and Venkatesan.P. Preformulation Parameters Characterization to Design, Development and Formulation of Miglitol Loaded Nanoparticles. J. Pharm. Sci. & Res. Vol. 9(3), 2017, 326 .

84. Ketie Saralidze, et. al., Polymeric Microspheres for Medical Applications, Materials, 2010, 3, Page 3537-3564. E Veena Rani, et. al., Preparation and Evaluation of Aspirin Loaded Microspheres by Solvent Evaporation Technique, Journal of Medicine andBiology, 2019, 1 (1), Page 27- 32.

85. Khan, R.; Khan, A.Q.; Lateef, A.; Rehman, M.U.; Tahir, M.; Ali, F.; Hamiza, O.O.; Sultana, S. Glycyrrhizic Acid Suppresses the Development of Precancerous Lesions via Regulating the Hyperproliferation, Inflammation, Angiogenesis and Apoptosis in the Colon of Wistar Rats. PLoS ONE 2013, 8, 1–22.

86. Konovalova, G.G.; Tikhaze, A.K.; Lankin, V.Z. Antioxidant Activity of Parapharmaceutics Containing Natural Inhibitors of Free Radical Processes. Bull. Exp. Biol. Med. 2000, 130, 658–660. 54. Tripathi, M.; Singh, B.K.; Kakkar, P. Glycyrrhizic Acid Modulates T-BHP Induced Apoptosis in Primary Rat Hepatocytes. Food Chem. Toxicol. 2009, 47, 339–347.

87. Kordi M, Borzouyi Z, Chitsaz S, Asmaei MH, Salami R, Tabarzad M. Antimicrobial peptides with anticancer activity: Today status, trends and their computational design. Arch Biochem Biophys. 2023 Jan 1;733:109484.

88. Kumar S, Suresh PK, Vijaybabu MR, Arunkumar A, Arunakaran J. Anticancer effects ofethanolic neem leaf extract on prostate cancer cell line (PC-3). J Ethnopharmacol. 2006;105(12): 246-250.

89. Kunchu K and Ashwani RV et al: Albumin microspheres: An Unique System as drug Delivery Carriers for non steroidal anti-inflammatory drugs (NSAIDS). International Journal of Pharmaceutical Sciences Review and Research 2010; 5:10-17.

90. Lachman L, Lieberman HA and Kanig JL: The Theory and Practice of Industrial Pharmacy. Philadelphia, Lea and Febiger, 1987.

91. Lachman LA, Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy.Varghese Publishng House, Mumbai, India: 1991, 3rd edition; P-414-415.

92. Lateef M, Iqbal L, Fatima N, Siddiqui K, Afza N, Zia-ul-Haq M, Ahmad M.Evaluation of antioxidant and urease inhibition activities of roots of Glycyrrhiza glabra. *Pak J PharmSci.* 2012 ;25(1):99-102.

93. Lee, C.S.; Kim, Y.J.; Lee, M.S.; Han, E.S.; Lee, S.J. 18β-Glycyrrhetinic Acid Induces Apoptotic Cell Death in SiHa Cells and Exhibits a Synergistic Effect against Antibiotic Anti-Cancer Drug Toxicity. Life Sci. 2008, 83, 481–489.

94. Li X, Li X, Yang J, Lin J, Zhu Y, Xu X, Cui W. Living and Injectable Porous Hydrogel Microsphere with Paracrine Activity for Cartilage Regeneration. Small. 2023 Apr;19(17):e2207211.

95. Li Y. The Bioactivities of Phycocyanobilin from Spirulina. J Immunol Res. 2022 Jun 11;2022:4008991.

96. Li YJ, Chen J, Li Y, Li Q, Zheng YF, Fu Y, Li P.Screening and characterization of natural antioxidants in four Glycyrrhiza species by liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *J Chromatogr A.* 2011, 11;1218(45):8181-91.

97. Li, J.Y.; Cao, H.Y.; Liu, P.; Cheng, G.H.; Sun, M.Y. Glycyrrhizic Acid in the Treatment of Liver Diseases: Literature Review. Biomed Res. Int. 2014, 872139.

98. Liener IE. Implications of antinutritional components in soybean foods. Crit Rev Food Sci Nutr. 1994; 34: 31-67.

99. Lin X, Xiang X, Hao L, Wang T, Lai Y, Abudoureyimu M, Zhou H, Feng B, Chu X, Wang R. The role of Aurora-A in human cancers and future therapeutics. Am J CancerRes. 2020 Sep 1;10(9):2705-2729.

100. M.Alagusundaram, Chetty Mudhu Sudana, K. Umashankari, Badrinath Attuluri Venkata, C Lavanya and S. Sampath," Microsphere as a Novel Drug Delivery System-A Review", Int. J. of ChemTech Research, 2009; 1(3), 526-534.

101. Marei, H.E., Althani, A., Afifi, N. et al. p53 signaling in cancer progression and therapy.Cancer Cell Int 21, 703 (2021).

102. MENTALHELP.NET is AMERICAN ADDICTION CENTERS, INC. It is operated by RECOVERY BRAND SLLC.

103. Ming, L.J.; Yin, A.C.Y. Therapeutic Effects of Glycyrrhizic Acid. Nat. Prod. Commun.2013, 8, 415–418.

104. Mishra R. Development and Optimization of Floating Microspheres in Amethopterin.Biosc.Biotech.Res.Comm. 2021;14(4).

105. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* Dec 2009;30(16):2785-2791.

106. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* Dec 2009;30(16):2785-2791.

107. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* Dec 2009;30(16):2785-2791.

108. Morteza-Semnani K, Saeedi M, Shahnavaz B.Comparison of antioxidant activity of extract from roots of licorice (Glycyrrhiza glabra L.) to commercialantioxidants in 2% hydroquinone cream. *J Cosmet Sci.* 2003,;54(6):551-8.

109. Mujwar S, Pardasani K. Prediction of riboswitch as a potential drug target and design of its optimal inhibitors for Mycobacterium tuberculosis.*International Journal of Computational Biology and Drug Design,* 2015;8(4):326-47.

110. Mujwar S, Pardasani K. Prediction of riboswitch as a potential drug target and design of its optimal inhibitors for Mycobacterium tuberculosis.*International Journal of Computational Biology and Drug Design,* 2015;8(4):326-47.

111. Mujwar S, Pardasani K. Prediction of riboswitch as a potential drug target and design of its optimal inhibitors for Mycobacterium tuberculosis.*International Journal of Computational Biology and Drug Design,* 2015;8(4):326-47.

112. Mujwar S, Pardasani KR. Prediction of Riboswitch as a Potential Drug Target for Infectious Diseases: An Insilico Case Study of Anthrax *Journal of Medical Imaging and Health Informatics.* 2015;5(5):7-16.

113. Mujwar S, Pardasani KR. Prediction of Riboswitch as a Potential Drug Target for Infectious Diseases: An Insilico Case Study of Anthrax *Journal of Medical Imaging and Health Informatics.* 2015;5(5):7-16.

114. Mujwar S, Pardasani KR. Prediction of Riboswitch as a Potential Drug Target for Infectious Diseases: An Insilico Case Study of Anthrax *Journal of Medical Imaging and Health Informatics.* 2015;5(5):7-16.

115. Mukherjee M, Bhaskaran N, Srinath R, Shivaprasad HN, Allan JJ, Shekhar D, Agarwal A.Anti-ulcer and antioxidant activity of GutGard. *Indian J Exp Biol.* 2010 ; 48(3):269- 74.

116. Mukherjee, PK. Quality Control of Herbal Drugs: An approach to evaluation of botanicals. 1st ed. New Delhi: Business Horizons, 2002 ,114-124.

117. Muriel J. M., Herbs or natural products that decrease cancer growth. Oncol Nursing Forum. 2004; 31(4): E75.

118. Nadkarni K M. Indian Materia Medica, 3rd ed. Vol 1. Mumbai: Popular Prakashan Ltd;1989.

119. Najam ul Hassan IqraChaudheryAsim.ur.RehmanNaveedAhmed. Polymeric nanoparticles used in tissue engineering. Advances in Polymeric Nanomaterials for Biomedical Applications:Micro and Nano Technologies.2021; 191-224.

120. Namavar Jahromi et al. Comparing The Effects of Glycyrrhiza glabra Root Extract, A Cyclooxygenase-2 Inhibitor (Celecoxib) and A Gonadotropin-Releasing Hormone Analog (Diphereline) In A Rat Model of Endometriosis. nternational Journal of Fertilityand Sterility . 13;1, 2019, 45-50.

121. Obolentseva, G.V.; Litvinenko, V.I.; Ammosov, A.S.; Popova, T.P.; Sampiev, A.M. Pharmacological and Therapeutic Properties of Licorice Preparations (A Review). Pharm. Chem. J. 1999, 33, 427–434.

122. Olsson M, Zhivotovsky B. Caspases and cancer. Cell Death Differ. 2011 Sep;18(9):1441-9.

123. P.M. Dandagi, VS. Mastiholimath, M.B. Patil, M.K. Gupta, Biodegradable microparticulate system of captopril. International Journal of Pharmaceutics. 307, 2006,83-88.

124. P.More *etal*. Recent advances in phytochemical-based Nano-formulation for drug-resistant Cancer. Medicine in Drug Discovery.2021.10;100082.

125. Pandey G, Madhuri S. Some medicinal plants as natural anticancer agents. Pharmacognosy Rev. 2009; 3: 259-263.

126. Pastorino, G.; Cornara, L.; Soares, S.; Rodrigues, F.; Oliveira, M.B.P.P. Liquorice (Glycyrrhiza glabra): A Phytochemical and Pharmacological Review. Phyther. Res. 2018, 32, 2323–2339

127. Patel B., Modi V., Patel K., Patel M., "Preparation and evaluation of ethyl cellulose microspheres prepared by emulsification solvent evaporation method", International Journal for Research in Management and Pharmacy. 2012; 1(1):83-91.

128. Patil SK, Salunkhe VR, Mohite SK. Development and validation of UV spectrophotometric method for estimation of Glycyrrhetinic acid in hydroalcoholic extract of Glycyrrhiza glabra. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2012; 2(4): 617-621.

129. Patrick B. O'Donnell, et. al., Preparation of microspheres by the solvent evaporation technique, Advanced Drug Delivery Reviews 28 (1997) Page 25–42.

130. Pawan Chaware, et. al., 1991.Bioadhesive Microspheres: A Review On Preparation And In-Vitro Characterization, World Journal of Pharmaceutical Research, 4, (2), 423-436.

131. Poonam Patil, rt. al., A Review on Ionotropic Gelation Method: Novel Approach for Controlled Gastroretentive Gelispheres, International Journal of Pharmacy and Pharmaceutical Sciences, 2012, 4 (4), 27-32.

132. Prajapati H, Patel K and Gupta AK: Formulation and evaluation of floating microspheres of baclofen. Int J Pharm Sci & Res 2021; 12(3):1482-94. doi: 10.13040/IJPSR.0975- 8232.12(3).1482-94.

133. Prakash Om, Amit Kumar, Pawan Kumar, Ajeet. Anticancer potential of plants and natural products: a review. Am J Pharmacol Sci. 2013; 1: 104-115.

134. Prasad T, Mahapatra A, Sharma T, Sahoo CR, Padhy RN. Dihydropyrimidinones as potent anticancer agents: Insight into the structure-activity relationship. Arch Pharm (Weinheim). 2023 Jun;356(6):e2200664.

135. Prasanna Kumar Desu, G.Vaishnavi, K. Divya, U.Lakshmi. An Overview on Preformulation Studies. Indo Am. J. Pharm. Sci, 2015;2(10).

136. Pratheeshkumar P, Sreekala C, Zhang Z, et al. Cancer prevention with promising natural products: mechanisms of action and molecular targets. Anti-Cancer Agents Med Chem.2012; 12(10): 1159-1184.

promotional stages of mammary tumorigenesis. Nutr Cancer. 1992; 17: 153-948.

137. Pushkarev VM, Kovzun OI, Tronko MD. Antineoplastic and apoptotic effects of cannabinoids. N-acylethanolamines. J Cancer Res Exp Oncol. 2008; 30(1): 6-21.

138. Ranu Biswas & Kalyan Kumar Sen. DEVELOPMENT AND CHARACTERIZATION OF NOVEL HERBAL FORMULATION (POLYMERIC MICROSPHERES) OF SYZYGIUM CUMINI SEED EXTRACT. International Journal of Applied Pharmaceutics, ISSN: 0975-7058, Vol: 10, Issue: 5, Page: 226-234

139. Ribeiro, Aliny A. L. Herbal Mixture Adsorbed to Polyethylene Glycol Microspheres Induces Apoptotic Effects on Breast Cancer Cells. Current Drug Delivery, Volume 15, Number 2, 2018, pp. 227-234(8)

140. Rodino S, Butu A, Butu M and Cornea PC. Comparative studies on antibacterial activity of licorice, elderberry and dandelion. Digest Journal of Nanomaterials and Biostructures2015; 10(3): 947-9

141. Saied S, Batool S, Naz S. Phytochemical studies of Berberis aristata. J Basic Appl Sci.2007; 3(1): 1-4.

142. Sakarkar DM, Deshmukh VN. Ethnopharmacological review of traditional medicinal plants for anticancer activity. Int J Pharm Tech Res. 2011; 3: 298-308.

143. Saravana Kumar K, et. al., A Review on Microsphere for Novel drug delivery System,Journal of Pharmacy Research, 2012,5(1), Page 420-424.

144. Saurabh K. Sinha, Satyendra K. Prasad, Md Ataul Islam, Shailendra S. Gurav, Rajesh B. Patil, Nora Abdullah AlFaris, Tahany Saleh Aldayel, Nora M. AlKehayez, Saikh Mohammad Wabaidur & Anshul Shakya (2021) Identification of bioactive compounds from Glycyrrhiza glabra as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: a pharmacoinformatics study, Journal of Biomolecular Structure and Dynamics, 39:13, 4686-4700.

145. Selyutina, O.Y.; Polyakov, N.E. Glycyrrhizic acid as a multifunctional drug carrier—From physicochemicalproperties to biomedical applications: A modern insight on the ancient drug. Int. J. Pharm. 2019, 559, 271–279.

146. Serraino M, Thompson LU. The effect of flaxseed supplementation on the initiation and

147. Shah K, Mujwar S, Gupta JK, Shrivastava SK, Mishra P. Molecular Docking and In Silico Cogitation Validate Mefenamic Acid Prodrugs as Human Cyclooxygenase-2

Inhibitor. Assay Drug Dev Technol. 2019;17(6):285-91.

148. Shah K, Mujwar S, Gupta JK, Shrivastava SK, Mishra P. Molecular Docking and In Silico Cogitation Validate Mefenamic Acid Prodrugs as Human Cyclooxygenase-2 Inhibitor. Assay Drug Dev Technol. 2019;17(6):285-91.

149. Shah K, Mujwar S, Gupta JK, Shrivastava SK, Mishra P. Molecular Docking and In Silico Cogitation Validate Mefenamic Acid Prodrugs as Human Cyclooxygenase-2 Inhibitor. Assay Drug Dev Technol. 2019;17(6):285-91.

150. Shah S, Narang R, Singh VJ, Pilli G, Nayak SK. A Review on Anticancer Profile of Flavonoids: Sources, Chemistry, Mechanisms, Structure-activity Relationship and Anticancer Activity. Curr Drug Res Rev. 2023;15(2):122-148.

151. Shah, N., Sheikh, A. and Jain, H. (2021) "Formulation Development and Optimization of Sustained Release Microspheres of Acebrophylline", Journal of Pharmaceutical Research International, 33(33A), pp. 13-28.

152. Sharma K, Bairwa R, Chauhan N, Shrivastava B, Saini NK. Berberis aristata: a review.Int J Res Ayurveda Pharm. 2011; 2(2): 383-388.

153. Sharma KK, Singh B, Mujwar S, Bisen PS. Molecular Docking Based Analysis to Elucidate the DNA Topoisomerase IIbeta as the Potential Target for the Ganoderic Acid; A Natural Therapeutic Agent in Cancer Therapy. Curr Comput Aided Drug Des. 2020;16(2):176-89.

154. Sharma KK, Singh B, Mujwar S, Bisen PS. Molecular Docking Based Analysis to Elucidate the DNA Topoisomerase IIbeta as the Potential Target for the Ganoderic Acid; A Natural Therapeutic Agent in Cancer Therapy. Curr Comput Aided Drug Des. 2020;16(2):176-89.

155. Sharma KK, Singh B, Mujwar S, Bisen PS. Molecular Docking Based Analysis to Elucidate the DNA Topoisomerase IIbeta as the Potential Target for the Ganoderic Acid; A Natural Therapeutic Agent in Cancer Therapy. Curr Comput Aided Drug Des. 2020;16(2):176-89.

156. Shweta Saini, et. al., Microspheres as Controlled Drug Delivery System: An Updated Review, international journal of pharmaceutical science and research,4, 2018, 1760- 1768.

157. Siracusa L, Saija A, Cristani M, Cimino F, D'Arrigo M, Trombetta D, Rao F, Ruberto G.Phytocomplexes from liquorice (Glycyrrhiza glabra L.) leaves-chemical characterization and evaluation of theirantioxidant, anti-genotoxic and anti-inflammatory activity. *Fitoterapia.* 2011 ;82(4):546-56.

158. Sukhdev Singh, Bhupender Sharma, Shamsher S. Kanwar, and Ashok Kumar. LeadPhytochemicals for Anticancer Drug Development. Front Plant Sci. 2016; 7: 1667.

159. Sunil Datt Belwal *etal*. Formulation and Evaluation of Microsphere of Aceclofenac .Journal of Advancement in Pharmacology.2020.1:1;65.

160. Szewczyk M, Abarzua S, Schlichting A, Nebe B, Piechulla B, Briese V, Richter DU. Effects of extracts from Linum usitatissimum on cell vitality, proliferation and cytotoxicity in human breast cancer cell lines. J Med Plants Res. 2014; 8(5): 237-245.

161. The Wealth of India-A dictionary of Indian raw materials and industrial products, Revised edition. Vol X. New Delhi: CSIR; 1982.

162. Thomas Sander, Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil,Switzerland, Email: thomas.sanderidorsia.com

163. Thomas Sander, Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil,Switzerland, Email: thomas.sanderidorsia.com

164. Thomas Sander, Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil,Switzerland, Email: thomas.sanderidorsia.com

165. Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. Nutr Cancer. 1991; 16: 43-52.

166. Thomson M, Ali M. Garlic (Allium sativum): a review of its potential use as an anti-cancer agent. Curr Cancer Drug Targets. 2003; 3(1): 67-81.

167. Tibor Renkecz, et. al., Preparation of Molecularly Imprinted Microspheres by Precipitation Polymerization, Methods in Molecular Biology, 2017, 1575, Page 341-352.

168. Urs Häfeli, et. al., Review: Radioactive Microspheres for Medical Applicationsm, Cleveland Clinic Foundation, Radiation Oncology Department T28, page 1-29.

169. Van Der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthusalkaloids: pharmacognosy and biotechnology. Curr Med Chem. 2004;

11(5): 607-628.

170. Velasco G, Sánchez C, Guzmán M. Towards the use of cannabinoids as antitumour agents. Nature Rev Cancer. 2012; 12(6): 436-444.

171. Venkatesan PC, Manavalan R, and Valliappan K, Selection of better method for the preparation of microspheres by applying Analytic Hierarchy Process. J. Pharm. Sci. andRes. 2009; Vol.1 (3): P- 64-78.

172. Ventura Cinzia A, Tommosini Silvana, CrupiEmanuela, Giannone Ignazio, Cardile Giovanni, "Chitosan Microsphere for Intrapulmonary Administration of Moxifloxacin: Interaction with bio-membrane models and *in vitro*Permeation Studies", Euro. J. of Pharma. And Biopharm., 2007; 68, 235-244.

173. Visavadiya NP, Narasimhacharya AV.Hypocholesterolaemic and antioxidant effectsof Glycyrrhiza glabra (Linn) in rats. *Mol Nutr Food Res.* 2006;50(11):1080-6.

174. Visavadiya NP, Soni B, Dalwadi N. Evaluation of antioxidant and anti-atherogenic properties of Glycyrrhiza glabra root using in vitro models. *Int J Food Sci Nutr.* 2009;60Suppl 2:135-49.

175. Wang J, Ito H, Shimura K. Enhancing effect of antitumor polysaccharide from Astralagus or Radix hedysarum onC3 cleavage production of macrophages in mice. Department of Pharmacology, Mie University School of Medicine, Japan. Mem Inst Oswaldo Cruz. 1991; 86(2): 159-164.

176. Wang, H.; Ge, X.; Qu, H.; Wang, N.; Zhou, J.; Xu, W.; Xie, J.; Zhou, Y.; Shi, L.; Qin, Z.; et al. Glycyrrhizic Acid Inhibits Proliferation of Gastric Cancer Cells by Inducing Cell Cycle Arrest and Apoptosis. Cancer Manag. Res. 2020, 12, 2853–2861.

177. Wu, P.-C., Huang, Y.-B., Chang, J.-S., Tsai, M.-J., Tsai, Y.-H., 2003. Design and evaluation of sustained release microspheres of potassium chloride prepared by Eudragit. Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 19, 115–122.

178. Xiao, X.Y.; Hao, M.; Yang, X.Y.; Ba, Q.; Li, M.; Ni, S.J.; Wang, L.S.; Du, X. Licochalcone A Inhibits Growth of Gastric Cancer Cells by Arresting Cell Cycle Progression and Inducing Apoptosis. Cancer Lett. 2011, 302, 69–75.

179. Xinghang Ma, et. al., Stability Study of Drugloaded Proteinoid Microsphere Formulations during Freeze-drying, Iournal of Drug Targeting, 1994, 2, Page 9-21.

180. Yadav AS, Bhatnagar D. Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors.* 2007;31(3-4):219-27.

181. Yan L, Zhang Z, Liu Y, Ren S, Zhu Z, Wei L, Feng J, Duan T, Sun X, Xie T, Sui X. Anticancer Activity of Erianin: Cancer-Specific Target Prediction Based on Network Pharmacology. Front Mol Biosci. 2022 Mar 17;9:862932.

182. Yousef TA, Alhamzani AG, Abou-Krisha MM, Kanthimathi G, Raghu MS, Kumar KY, Prashanth MK, Jeon BH. Synthesis, molecular docking study and anticancer activity ofnovel 1,3,4-oxadiazole derivatives as potential tubulin inhibitors. Heliyon. 2023 Feb 8;9(2):e13460.

183. Zhang Y, Zhang X, Zhou B. Zinc Protoporphyrin-9 Potentiates the Anticancer Activityof Dihydroartemisinin. Antioxidants (Basel). 2023 Jan 22;12(2):250.

ANNEXURES

CONFERENCE/SEMINAR/WORKSHOP CERTIFICATE

NATIONAL CONFERENCE 2022

On

HEALTHCARE ADVANCEMENT BY OPTIMIZING PHARMACEUTICAL & EDUCATIONAL SECTOR

75 Azadi Ka Amrit Mahotsav

Healthcare Award

WE SUPPORT SUSTAINABLE DEVELOPMENT GOALS

CERTIFICATE of Appreciation

11th June 2022

Venue :
M.P. Council of Science & Technology
Vigyan Bhawan, Nehru Nagar, Bhopal-03

This is to certify that Prof./Dr./Mr./Ms./Mrs. Mohit Saini of Institute of Pharmacy, P.K. University, Shivpuri (M.P.) has Participated as Delegate / Presented Oral / Poster Presentation Entitled A Review on Phytoceuticals for wound Healing activity in the National Conference theme entitled : On Healthcare Advancement By Optimizing Pharmaceutical & Educational Sector.

Organized By

PRISAL
PHARMACEUTICAL ROYAL INTERNATIONAL SOCIETY
www.prisal.in founder@prisal.in

Dr. Rajendra B. Kakde
Advisor-I, Approval Bureau,
All India Council for
Technical Education, New Delhi

Prof. Priti Tagde
President
(PRISAL)
Pharmaceutical Royal International Society

PEOPLE'S UNIVERSITY

MPCST SPONSORED
NATIONAL SCIENCE DAY
28TH FEBRUARY 2024

Organised By

SCHOOL OF PHARMACY AND RESEARCH, PEOPLE'S UNIVERSITY, BHOPAL

CERTIFICATE

This is to certify that Dr/Mr/Mrs/Ms .....MOHIT SAINI.....has actively participated in the deliberations of the MPCST sponsored National Science Day as delegate/participant in Scientific Rangoli/Poster/ Model competition. She/He secured .....—.....position in the National Science Day competitions.

We wish him/her a bright & successful future.

Mrs. Rajni Dubey
COORDINATOR

Dr. Bhaskar Kumar Gupta
PRINCIPAL, SOPR

CERTIFICATE OF APPRECIATION

CAREER POINT UNIVERSITY

INSTITUTION'S INNOVATION COUNCIL (Ministry Education Initiative)

International Conference on Emerging Cutting-Edge Research & Innovations in Pharmaceutical, Life Sciences & Technology (ICERIPLST-2024)

16th March 2024

ICERIPLST/PH/2024/1374

Certificate of Appreciation

This is to certify that Dr/ Mr/ Ms

Mohit Saini

of Institute of Pharmacy, P K University, Shivpuri, Madhya Pradesh

has presented a poster / oral presentation on

Current Scenario on Novel Drug Delivery System: Microsphere

in the **Conference** organized by **Career Point School of Pharmacy, CPU Kota, Rajasthan.**

Prof (Dr) M. K. Gupta	**Mr. Kamal Arora**	**Mr. Lav Sharma**	**Dr. Yogesh Kumar**	**Dr. Rajkumari Thagele**
Vice Chancellor (Off.)	Registrar	Sr. General Manager,	Senior PDF Scientist	H.O.D,
Dean Pharmacy,	CPU, Kota	Amneal Pharmaceuticals Ltd.,	Department of General, Visceral and	CP School of Pharmacy,
CPU, Kota		Ahmedabad Gujarat.	Thoracic Surgery, University Medical Center	CPU, Kota
			Hamburg-Eppendorf, Hamburg, Germany.	

CERTIFICATE OF PUBLICATION

SASPRedu International Pvt. Ltd.
Registered under the Companies Act, 2013 (18 of 2013), Govt. of. India

Website: www.saspredu.com
Email: hr@saspredu.com
Tel & Fax: 03674-267245
Mob: +91-9365665504

SCHOLARS ACADEMIC AND SCIENTIFIC SOCIETY
204, Borhawor, Murajhar, Hojai, Assam, India-782439, www.sassociety.com
(A Society Registered under Society Registration Act XXI of 1860, India)

Publication Certificate

This certificate confirms that **"Mohit Saini, Jitender K. Malik"** have published manuscript titled **"In-Silico Validation of Glycyrrhizin against Proinflammatory Mediator COX-2: Anti-Proliferative Potential"**.

Journal: **South Asian Research Journal of Pharmaceutical Sciences (SARJPS)**
Abbreviated key title: South Asian Res J Pharm Sci
ISSN 2664-6749 (Online) ISSN 2664-4142 (Print)
Publisher: SAR Publication, Bangladesh

Details of Published Article as follow-
Volume : **5**
Issue : **5**
Year : **2023**
Page Number : **206-212**

Date of Issue: **18-10-2023**

Mr. Nazim Hussain
CEO
SASPR Edu International Pvt. Ltd.
Mobile: +91-9365665504, E-mail: hr@saspredu.com


GIS SCIENCE JOURNAL
An UGC-CARE Approved Group II Journal
ISSN NO : 1869-9391 / Website : www.gisscience.net /
Email : editorgsjournal@gmail.com
Certificate of Publication
Paper ID : GSJ/9394
This is to certify that the paper titled
Glycyrrhizin from Glycyrrhiza glabra root: Phytochemical analysis and Characterization
Authored by
Mohit Saini
From
P.K. University, Shivpuri (M.P.)-India.
Has been published in
GIS SCIENCE JOURNAL Volume 9, Issue 12, December 2022.
UGC APPROVED JOURNAL
DOI: 20.18001.GSJ
crossref member
CROSSREF.ORG
THE CITATION LINKING BACKBONE
M Palaniswami
Editor-in-chief
GISSCIENCE
6.1
IMPACT FACTOR

COPY OF PUBLISHED PAPER- 1

SAR Journal of Anatomy and Physiology

Abbreviated Key Title: *SAR J Anat Physiol*
Home page: https://sarpublication.com/journal/sarjap/home
DOI: 10.36346/sarjap.2022.v03i02.001

ISSN 2707-7713 (P)
ISSN 2709-6874 (O)

Review Article

Novel Drug Delivery System Microsphere: A Review

Mohit Saini[1*], Jitender K Malik[2]
[1]Dept. of Pharmaceutic, Institute of Pharmacy, P.K. University, Shivpuri Madhya Pradesh India
[2]Research Scholar, P.K. University, Shivpuri Madhya Pradesh, India

***Corresponding Author:** Mohit Saini
Dept. of Pharmaceutic, Institute of Pharmacy, P.K. University, Shivpuri Madhya Pradesh India

Article History: | Received: 17.03.2022 | Accepted: 23.04.2022 | Published: 29.04.2022 |

Abstract: The concept of targeted drug delivery is designed to attempt to concentrate the drug in the tissues of interest while reducing the relative concentration of the drug in the remaining tissues. As a result, the drug is localized to the targeted site. Therefore, the surrounding tissues are not affected by the drug. Therefore, carrier technology provides an intelligent approach to drug delivery by coupling drugs to carrier particles such as microspheres, nanoparticles, liposomes, niosomes, etc., modulating the release and absorption characteristics drug revenue. Microspheres are typically free-flowing powders made of proteins or synthetic polymers that are biodegradable in nature and ideally have a particle size of less than 200 µm. It is a reliable way to deliver drugs to the target site with specificity, if altered, and to maintain the desired concentration at the site of interest without side effects. Microspheres have received a great deal of attention not only for sustained release but also for targeting anti-cancer drugs to tumors. In the future, by combining various strategies, microspheres will occupy a central place in the delivery of new drugs, especially in the classification of diseased cells, diagnostics, genes and genetic material, safe, targeted and effective in vivo delivery and supplements in miniature versions of diseased organs and tissues in the body.

Keywords: Microspheres, Types of microspheres, Formulation and characterization of microspheres& applications.

INTRODUCTION

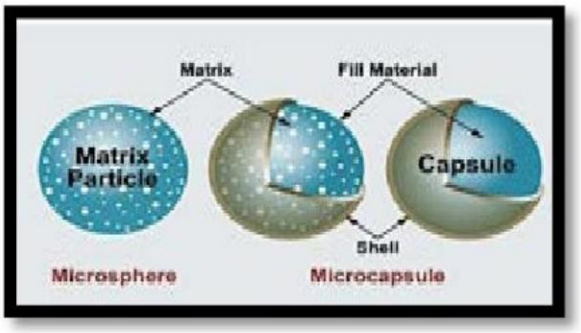
Novel Drug Delivery System

Between the 1940s and 1960s, the concept of microencapsulation technology began as an alternative way to deliver drugs. In the continued search for a more sophisticated system, in the 1980s polymer/membrane technology became known at the forefront. In addition, site-specific targeting and delivery can be achieved with absolute precision by attaching bioactive molecules to liposomes, biopolymers, implants, monoclonal antibodies, and carriers of different particles (eg, nanoparticles and microspheres, etc.).The most desirable and convenient method of drug administration is the oral route because it is easy to administer. However, in many cases, oral administration is not desirable if the drug undergoes significant first-pass degradation in the liver. Therefore, the lack of systemic absorption through the gastrointestinal tract has led to the search for alternative routes of drug delivery such as parenteral, intramuscular, subcutaneous, and intranasal and transdermal. Traditional oral drug administration usually does not result in a significant increase in drug concentration. This often reaches toxic levels, and after a relatively short period of time at therapeutic levels, drug levels eventually drop until re-administration. To achieve maximum therapeutic effect, the drug must be delivered to the target tissue in the optimal amount for the required period, with little toxicity and minimal side effects. Targeted drug delivery, sometimes referred to as smart drug delivery, is a method of delivering a drug to a patient by increasing the drug concentration in a particular part of the bodies compared to other. The goal of a targeted drug delivery system is to stretch, localize, and target lesion tissue and protect drug interactions with. The conventional drug delivery system is the absorption of the drug through the biological membrane, and the targeted release system is the reduction in the frequency of dosing taken by the patient, resulting in a more consistent effect of the drug, a reduction in the side effects of the drug. , And reduced

Citation: Mohit Saini & Jitender K Malik (2022). Novel Drug Delivery System Microsphere: A Review, *SAR J Anat Physiol*, *3*(2), 9-16.

fluctuations in circulating drug levels. The disadvantages of this system are its high cost, which makes it difficult to increase productivity and limits its ability to adjust dosages. There are various types of vehicles used in targeted drug delivery systems, including: The ideal drug delivery vehicle should be non-toxic, biocompatible, non-immunogenic, and biodegradable.

Microspheres are small spherical particles with a diameter in the micron range, typically 1 µm to 1000 µm (1 mm). Microspheres are sometimes called fine particles. They are free-flowing spherical particles made from synthetic proteins or polymers. Microspheres are free-flowing powders made from proteins or synthetic polymers, which are inherently biodegradable. They are made of polymers, waxes, or other protective materials. H. Biodegradable synthetic polymers and denatured natural products such as starch, gum, proteins, fats and waxes. Natural macromolecules include albumin and gelatin. Synthetic polymers include polylactic acid and polyglycolic acid. Microspheres are small and have a large surface area to volume ratio. At the lower end of their size range, they have colloidal properties. The interfacial properties of microspheres are very important and often determine their activity [1].



Microsphere

Microspheres (MS), that are emulsion cells or solid dispersed in a continuous phase, were applied in numerous industries consisting of foods, cosmetics and pharmaceuticals, etc. Using the traditional techniques of emulsion production, the emulsions (or MS) produced are commonly extensively polydispersed over a huge range. It is believed that surfactants play a very important role in the emulsification process. Surfactants reduce interfacial tension and promote emulsion formation. It is presumed that the surfactant stabilizes the emulsion by generating a repulsive force between the droplets [2]. Microspheres are small spherical particles with a diameter in the micron range, typically 1 µm to 1000 µm (1 mm). Microspheres are sometimes called fine particles. They are made of polymers, waxes, or other protective materials. H. Biodegradable synthetic polymers and denatured natural products such as starch, gum, proteins, fats and waxes. Natural macromolecules include albumin and gelatin. Synthetic polymers include polylactic acid and polyglycolic acid. Microspheres are small and have a large surface area to volume ratio. At the lower end of their size range, they have colloidal properties. The interfacial properties of microspheres are very important and often determine their activity [3]. Basically, each particle is a mixture of a drug dispersed in a polymer, and the drug release pattern follows a first order process. The release of the drug is controlled by dissolution or degradation of the substrate. Microspheres provide a ball bearing effect due to their size and shape. Microspheres differ in quality, sphericity, particle uniformity and particle size distribution. You must choose the right microsphere for each unique application. There are many possibilities for fabricating microspheres to control drug administration. Facilitates accurate delivery of small amounts of potent drugs, reduces drug concentrations at sites other than the target site, and protects labile compounds before and after administration and before appearing at the site of action. By binding drugs to carrier molecules, we can change how drugs work in vivo. The behavior of carrier molecules can influence clearance kinetics, tissue metabolism, and cellular drug interactions. The use of these changes in pharmacodynamics may increase the effectiveness of treatment [4]. The purpose of this controlled drug delivery system is to immediately ensure that the amount of therapeutic amount is immediately and reached the treatment level, and maintain the desired drug concentration in the action area [5]. Oral route is convenient and usually occupied route for most drugs. Some medicines that are easily absorbed in G.I.T. having short t1 / 2 are quickly removed from blood circulation. Managed drug delivery systems can avoid problems with existing drug delivery systems, and slowly emit drugs of G.I.T. Maintain a constant concentration in serum for a longer time.

Various advantages and disadvantage of Microsphere are as follows [6, 7]:

ADVANTAGES	DISADVANTAGES
• Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. • Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug. • Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor. • The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles in vivo. • Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intracellularly. • Blood flow determination. • Microspheres provide freedom from drug and recipients incompatibilities especially with buffer. • Microspheres reduce dose dumping. • Microspheres provide the protection of drugs against environment. • Microspheres also mask the taste and odor. • A microsphere avoids the first pass metabolism. • Microspheres can be easily injected in body because of their small and spherical size. • Microspheres enhance the biological half-life and also improve the bioavailability. • Microspheres also reduce the chances of G.I. irritation • Drug discharge in stomach is hindered and that's why local unwanted effects are reduced. • In case of microspheres, better therapeutic effect for short half-life of drugs can be achieved.	• Drug gets difficult to remove after injected. • Sometime non-uniformity of drug content may result while preparation.

Limitations of microspheres [8]

- The controlled release rate of microspheres can vary due to certain factors such as internal or external factors be it food, intestinal transit rate, mucin turnover rate etc.
- There is variation in release from one dosage form to another.
- Low drug intake is done in case of extra-gastrointestinal microspheres.
- In the case of parenteral administration of microspheres, it is difficult to completely remove the carrier from the body.
- Parenteral use of microspheres may interact or form complexes with blood components. Formula release may vary.
- Any loss of integrity in the release sample could lead to potential toxicity.

Materials used in formulation of Microspheres [9-10]

Microspheres used generally are polymers. They are classified into two types.

1. Synthetic Polymers
2. Natural polymers

Synthetic Polymers	Natural polymers
• Non-biodegradable polymers : Poly methyl methacrylate (PMMA), Acrolein, Glycidyl methacrylate, Epoxy polymers • Biodegradable polymers: Lactides, Glycolides & their co polymers Poly alkyl cyano Acrylates, Poly anhydrides.	• **Proteins:** Albumin, Gelatin, Collagen. • **Carbohydrates:** Agarose, Carrageen an, Chitosan, Starch. • **Chemically modified carbohydrates:** Poly dextran, Poly starch.

Formulation of Microsphere

Preparation of microspheres should satisfy certain criteria:-

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersability in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability.
6. Susceptibility to chemical modification

Single Emulsion Technique

The micro particulate carriers of natural polymers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, di acid chloride etc. Heat denaturation is not suitable for thermolabile substances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation.

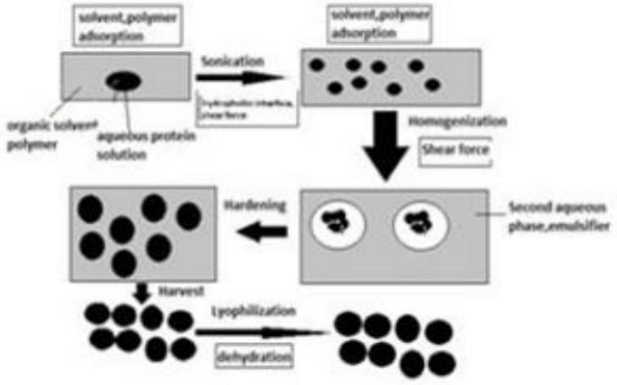


Fig-1: Single emulsion technique by chemical cross-linking

Double Emulsion Technique

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like leutinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/ extraction.

Polymerization Techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization
II. II. Interfacial polymerization.

Both are carried out in liquid phase. Normal polymerization: It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be molded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers. Interfacial polymerization: It involves the reaction of various monomers at the interface between the two

immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.

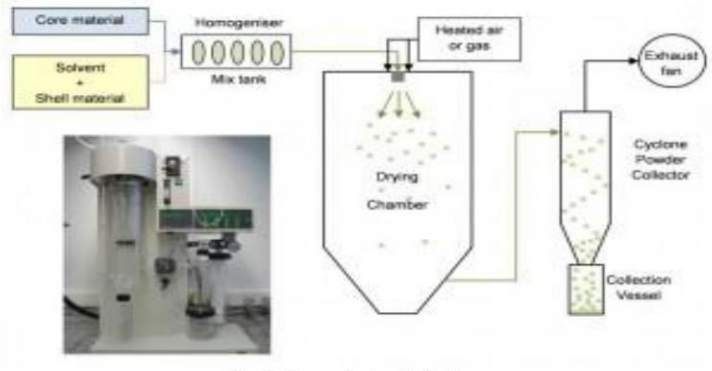


Fig-2: Spray drying technique

Phase Separation Coacervation Technique

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the Polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

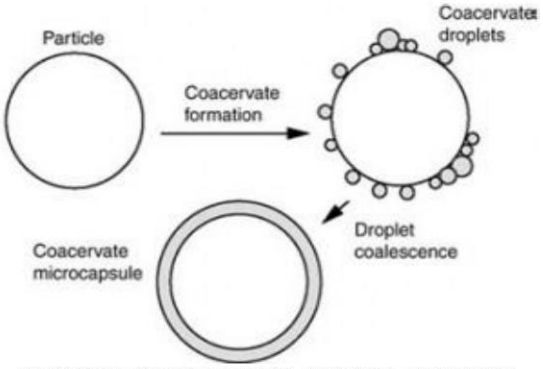


Fig-3: Formation of coacervates around the core material

Spray Drying and Spray Congealing

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 µm. Micro-particles are separated from the hot air by means of the cyclone separator while the

traces of solvent are removed by vacuum drying. One of the major advantages of the process is feasibility of operation under aseptic conditions. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate14 and sulpha-ethylthiadizole15 are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous micro-particles.

Solvent Evaporation

Solvent evaporation method is used for the preparation of micro-particles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer [11].

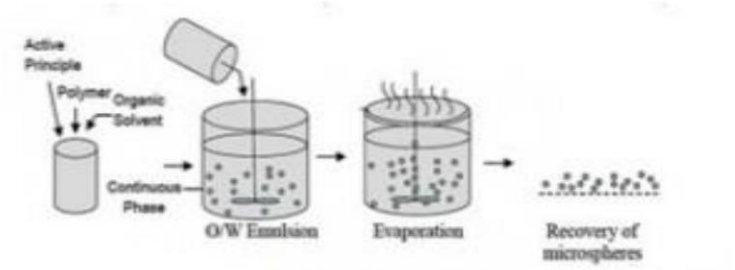


Fig-4: Solvent evaporation technique

Characterization

Characterization of the micro particle carrier is an important phenomenon, helping to design suitable carriers for the delivery of proteins, drugs or antigens. These microspheres have different microstructures. These microstructures determine the release and stability of the carrier [12].

- Particle size and shape determination.
- Electron spectroscopy for chemical analysis
- Attenuated total reflectance Fourier-Transform Infrared Spectroscopy
- Density determination.
- Capture efficiency

Applications of Microspheres

Novel applications for microspheres are discovered every day, below are now a few [13-20]

- **Ophthalmic Drug Delivery:** Microspheres designed with polymers exhibit favorable biological properties such as bio-adhesion, permeation enhancing properties and interesting physicochemical properties, making them unique materials for the creation of ophthalmic drug delivery vehicles such as chitosan, alginate, and gelatin.
- **Oral Drug Delivery:** The ability of microspheres containing polymer to form films permit its use in the formulation of film dosage forms, as an alternative to pharmaceutical tablets. The pH sensitivity, coupled with the reactivity of the primary amine groups, make microspheres more suitable for oral drug delivery applications e.g. Chitosan, Gelatin.
- **Gene Delivery:** Microspheres can be useful oral gene carriers due to their adhesion and transport properties in the gastrointestinal tract. For example, Chitosan, gelatin, viral vectors, cationic liposomes, polycationic complexes.
- **Nasal Drug Delivery:** Polymer-based drug delivery systems such as microspheres, liposomes, and gels have been shown to increase bioavailability and residence time of drugs through

the nasal route by having good bioadhesive properties and easily swelling upon contact with the nasal mucosa. e.g. Starch, Dextran, Albumin, Chitosan+ Gelatin.

- **Intratumoral and Local Drug Delivery:** A polymeric film is created to deliver paclitaxel to the tumor site at a therapeutically appropriate concentration. The mixture of drugs has promising potential for use in the controlled delivery of to the oral cavity. e.g. Gelatin, Chitosan.
- **Buccal Drug Delivery:** Polymers are excellent polymers for buccal delivery as they have mucosal/bioadhesive properties and can act as absorption enhancers (eg chitosan, sodium alginate).
- **Gastrointestinal Drug Delivery:** Internal cavities have prepared in polymer via a de- acidification through added to acidic and neutral media are found buoyant and provided a controlled release of the drug e.g. Eudragit, Gelatin.
- **Transdermal drug delivery:** Polymer having good film-forming properties. The drug release from the devices is pretentious by the membrane thickness and cross-linking of the film. e.g. Chitosan, Alginate.
- **Colonic drug delivery:** Polymer has been used for the precise delivery of insulin into the colon. e.g. Chitosan.

CONCLUSION

This review focuses on recent advances in microsphere formulation, characterization, and applications. In the future, by combining other substances, the microspheres will find the central location and the meaning of providing new drugs, especially the classification of disease cells, diagnosis, genes and raw genetic, safe delivery with additional efficiency. Several microencapsulated pharmaceutical products are currently marketed, such as aspirin, theophylline and its derivatives, vitamins, pancrelipase, antihypertensives, potassium chloride, progesterone, and combinations of oral contraceptives. Microencapsulated KCL is used to prevent gastrointestinal complications associated with potassium chloride. The dispersibility of the microcapsules and controlled release of ions minimizes the possibility that high local salt concentrations can lead to ulceration, bleeding or perforation. Microspheres have also been found to have potential applications as injectable or inhaled products. They have emerged as an exciting new platform for biologists to apply these techniques to the study of biomolecular interactions and cellular processes.

REFERENCE

1. Alagusundaram, M., Chetty, M. S., Umashankari, K., Badarinath, A. V., Lavanya, C., & Ramkanth, S. (2009). Microspheres as a novel drug delivery system: A review. *Int J Chem Tech Res*, *1*(3), 526-534.
2. ul Hassan, N., Chaudhery, I., & Ahmed, N. (2021). Polymeric nanoparticles used in tissue engineering. In *Advances in Polymeric Nanomaterials for Biomedical Applications* (pp. 191-224). Elsevier.
3. Patel, B., Modi, V., Patel, K., & Patel, M. (2012). Preparation and evaluation of ethyl cellulose microspheres prepared by emulsification-solvent evaporation method. *International journal for research in management and pharmacy*, *1*(1), 82-91.
4. Fu, X., Ping, Q., & Gao, Y. (2005). Effects of formulation factors on encapsulation efficiency and release behaviour in vitro of huperzine A-PLGA microspheres. *Journal of microencapsulation*, *22*(7), 705-714.
5. Ghalop, S.B., Banerjee, S.K., & Throat, R.M. (2010). Hollow microsphere a Review. *International Journal of Pharmaceutical Science Review & Research, 1*; 10-15.
6. Asija, R., Sharma, D., & Mall, K.R. (2014). Solvent evaporation matrix erosion method: a novel approach for floating microsphere development. *Journal of Drug Discovery and Therapeutics*, 2; 24-29.
7. Lachman, L., Lieberman, H. A., & Kanig, J. L. (1976). *The theory and practice of industrial pharmacy* (pp. 412-428). Philadelphia: Lea & Febiger.
8. Urs, A. V. R., Kavitha, K., & Sockan, G. N. (2010). Albumin microspheres: an unique system as drug delivery carriers for non steroidal anti-inflammatory drugs (NASIDs). *Int J Pharm Sci Rev Res*, *5*(2), 10-17.
9. Dandagi, P. M., Mastiholimath, V. S., Patil, M. B., & Gupta, M. K. (2006). Biodegradable microparticulate system of captopril. *International journal of pharmaceutics*, *307*(1), 83-88.
10. Chinna Gangadhar, B., & Shyam Sunder, R. (2010). Vimal Kumar Varma. M., Sleeva Raju M., Sai Kiran M, Formulation and Evaluation of Indomethacin Microspheres using natural and synthetic polymers as Controlled Release Dosage Forms. *International Journal of Drug Discovery*, *2*(1), 8-16.
11. NR, K. (2015). Microsphere: A Brief Review. *Asian J Biomed Pharm Sci*, *5*(47), 13-19.
12. Bansal, H., Kaur, S. P., & Gupta, A. K. (2011). Microspheres: methods of preparation and applications: A comparative study. *Int J Pharm Sci Rev Res*, *10*(1), 69-78.
13. Jain, N.K. (2004). Advances in controlled & Novel drug delivery, 03 Edition, 71. CBSpublication. delhi.
14. Nachts, S., & Martin, K. (1990). In: The microsponges a novel topical programmable deliveryformulation, Marcel Dekker Inc. Newyork, 299.

15. Saralidze, K., Leo, H., Koole, Menno, L., Knetsch, W. (2010). Polymeric Microspheres for MedicalApplications, Materials, 3; 3357- 64.
16. Rajput, S., Agrawal, P., Pathak, A., Shrivasatava, N., Baghel, S. S., & Baghel, R. S. (2012). A review on microspheres: methods of preparation and evaluation. *World journal of pharmacy and pharmaceutical sciences*, *1*, 422-38.
17. Meghna, K. S., Pillai, K., Giridas, S., Sreelakshmi, C., & Vijayakumar, B. (2017). Microsphere a drug delivery system–a review. *International Journal of Novel Trends in Pharmaceutical Sciences*, *7*(4), 109-118.
18. Kumar, A., Mahajan, S., & Bhandari, N. (2017). Microspheres: a review. *World J Pharm Pharm Sci*, *14*(6), 724-40.
19. Nikam, V. K., Gudsoorkar, V. R., Hiremath, S. N., Dolas, R. T., & Kashid, V. A. (2012). Microspheres-A Novel drug delivery system: An overview. *International Journal of Pharmaceuticaland chemical sciences*, *1*, 113-128.
20. Parmar, H., & Bakliwal, S. (2010). Different method of evaluation of mucoadhesive microspheres. *International Journal of Applied Biology and Pharmaceutical Technology*, *1*(3), 1164-1165.

COPY OF PUBLISHED PAPER – 2

ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-5 | Issue-5 | Sep-Oct- 2023 | **DOI: 10.36346/sarjps.2023.v05i05.004**

Original Research Article

In-Silico Validation of Glycyrrhizin against Proinflammatory Mediator COX-2: Anti-Proliferative Potential

Mohit Saini[1*], Jitender K. Malik[2]

[1]Research Scholar, P. K. University, Shivpuri (M.P.), India
[2]Department of Pharmaceutic, Faculty of Pharmacy, P. K. University, Shivpuri (M.P.), India

***Corresponding Author:** Mohit Saini
Research Scholar, P. K. University, Shivpuri (M.P.), India

Article History
Received: 05.09.2023
Accepted: 10.10.2023
Published: 13.10.2023

Abstract: ***Background*:** Licorice's scientific name is Glycyrrhiza glabra. It is a well-known medicinal plant that grows in numerous locations throughout the world. It is one of the most ancient and widely utilised plants and has been used for a very long period in both western and eastern nations. The primary source of the triterpenoid saponin, glycyrrhizic acid (also known as glycyrrhizin), which is a sweeter component and around 50 times sweeter than sugar, was the Glycyrrhiza glabra (Fabaceae) root. Glycyrrhetinic acid has been found as the chemical constituent of glycyrrhizin. ***Aim*:** The current work sought to elucidate the molecular basis for glycyrrhizin's antiproliferative activity against the COX-2 enzyme, which functions as a proinflammatory factor in proliferation. ***Method*:** A molecular docking method was employed in the current work to look for COX 2 protein inhibitors. The binding was determined by the Auto Dock software utilising a grid-based docking method. Compounds' 2D structures were constructed using the Merck Molecular Force Field, converted to 3D, and then energetically reduced up to an arms gradient of 0.01. (MMFF). ***Results*:** The molecular docking result revealed that glycyrrhizin showed encouraging docking score. The docking score found to be -7.7 kcal mol^{-1}. ***Conclusion*:** The interaction of ligand hits to targeted site and docking score finding it can be predicted that glycyrrhizin found in the plants *G.glabra* exhibited good inhibitor of *COX 2* protein inhibitors.

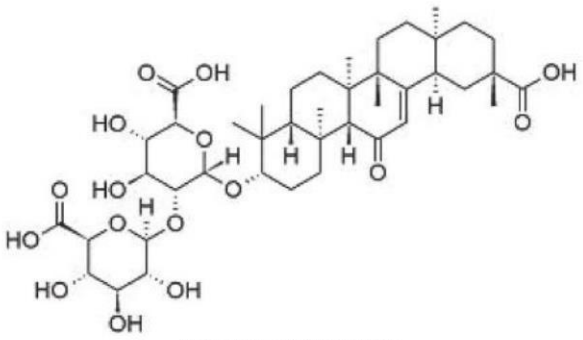
Keywords: Glycyrrhizin, COX 2, in-silico molecular docking & Anti proliferative activity.

INTRODUCTION

Drug designing is a technique for finding and creating novel compounds that have a particular effect on people [1]. A new class of antimicrobial medications must be developed because the number of microbial illnesses that are multidrug resistant is increasing daily [2]. A serious health problem and the second most common cause of death worldwide is cancer. Deregulation of the cell cycle, which impairs cellular differentiation and promotes unchecked cellular proliferation, is the root cause [3, 4]. Therefore, it is essential to create new bioactive compounds whose chemical makeup and mechanisms of action are notably different from those of currently accessible drugs [5]. Discovery of drug is a sluggish, lengthy costly and interdisciplinary approach but the recent discoveries have revolutionised the methods by which researchers manufacture novel therapeutic compounds e.g. The CADD technology reduces the cost of drug design by up to 50%. The I drug-receptor interaction (ii) binding affinity (iii) orientation and approach of drug molecules to the target site are all understood using the molecular docking technique [6]. The major goals of docking studies are accurate structural modelling and accurate activity prediction. It creates a new logical approach to drug design and offers the most optimistic image of drug-receptor interaction.

The sweet flavour of licorice root is primarily due to the triterpenoid glycyrrhizin. This chemical is a part of a combination of potassium, calcium, and magnesium salts of glycyrrhizic acid [7].

CITATION: Mohit Saini & Jitender K. Malik (2023). *In-Silico* Validation of Glycyrrhizin against Proinflammatory Mediator COX-2: Anti-Proliferative Potential. *South Asian Res J Pharm Sci, 5*(5): 206-212.



Structure of Glycyrrhizin

Description of Glycyrrhizin [8, 9]

S. No	Glycyrrhizin Description	
1.	**Mol. Formula**	$C_{42}H_{62}O_{16}$
2.	**Average Molecular Weight**	822.9 g/mol
3.	**IUPAC Name**	S,3S,4S,5R,6R)-6-[(2R,3R,4S,5S,6S)-2-[[(3S,4aR,6aR,6bS,8aS,11S,12aR,14aR,14bS)-11-carboxy-4,4,6a,6b,8a,11,14b-heptamethyl-14-oxo-2,3,4a,5,6,7,8,9,10,12,12a,14a-dodecahydro-1H-picen-3-yl]oxy]-6-carboxy-4,5-dihydroxyoxan-3-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid.
4.	**Class**	Triterpene glycoside
5.	**M.P.**	220 °C
6.	**Partition coefficient**	2.80
7.	**Pharmacology**	It possesses expectorant (antitussive) characteristics in addition to being useful in the treatment of peptic ulcers.
8.	**Mechanism of Action**	Inhibition of Hepatic Apoptosis and Necrosis Anti-Inflammation and Immunity Regulation Antiviral Effects Antitumor Effects Inductive Effect of Liver Enzyme Activity

Experimental work

Ligand Preparation:

2D Structure of ligand (glycyrrhizin) was drawn using ChemSketch [10], the two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structure of ligand (glycyrrhizin) is given below:

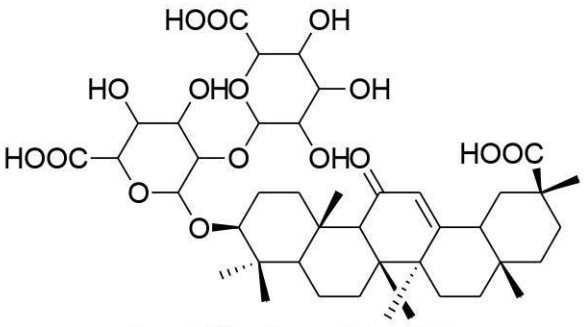


Figure 1: 2D conformer of glycyrrhizin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.375 Å and No. of points considered are 46, 44 and 46 points in the x, y, and z dimensions and 38.042, 2.131and 61.28 as x, y, z centers [11, 12].

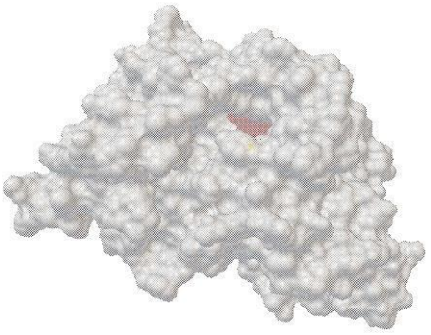


Figure 2: Grid box covering all active sites in receptor

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13-15].

Docking of COX2 with Glycyrrhizin

Crystal structure

The crystal structure of the protein consisting of receptor was downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5ikr.pdb) registered in the Protein data bank was used [16].

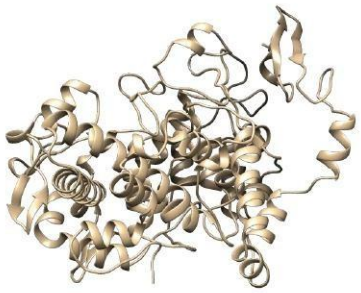


Figure 3: Crystal structure of COX2 protein (PDB ID-5ikr)

Processing of Protein

The downloaded receptor protein is having two chains A, and B, out of which two chain A were selected for the experimental purpose. There was mefenamic acid ligand was present within the macromolecular complex [17].

Molecular Docking Simulation Studies

Docking of glycyrrhizin ligand on COX 2 protein was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [18].

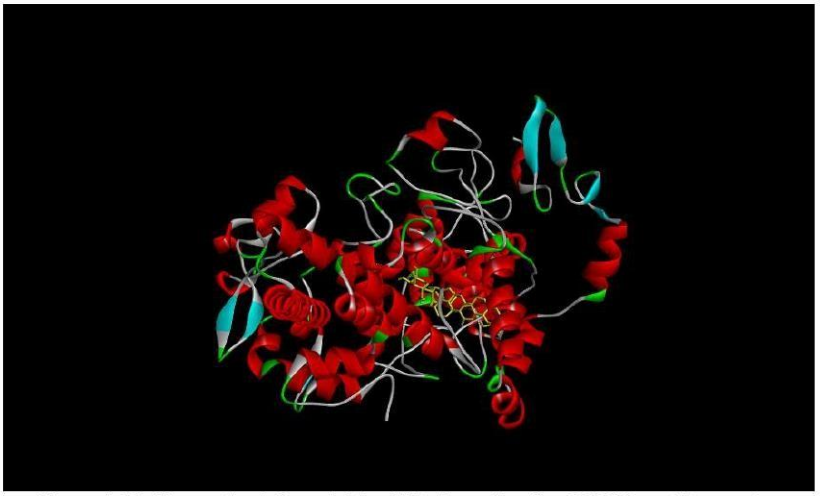


Figure 4: Binding mode of glycyrrhizin within the active site of COX2 protein receptor

Toxicity & ADME-T Studies

The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [19].

RESULTS AND DISCUSSION

Nature has always been a bizarre supply of medical ingredients, providing us with several medicinal plants that yield beneficial phytochemicals. Licorice is a member of the Leguminosae family and has the scientific name *Glycyrrhiza glabra*. The ayurvedic herb G. glabra is commonly used. Numerous licorice chemical components have undergone extensive pharmacological research to determine their potent anticancer, antibacterial, anti-inflammatory, cardioprotective, hepatoprotective, and other pharmacological effects. Gastric and duodenal ulcers have traditionally been treated with liquorice as a prophylactic measure. It is used as an anti-inflammatory medication in dyspepsia during allergic responses. The stereoisomer forms of glycyrrhizin are 18α and 18 β. Due to its anti-inflammatory effects on neutrophil functions, especially the production of ROS, glycyrrhizin is regarded to be the most popular folk remedy (reactive oxygen species).

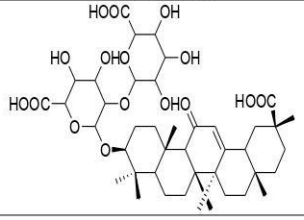
The majority of cancer treatment plans in use today focus primarily on surgical excision of tumour masses. Chemo- and radiotherapies, among other chemical and physical therapies, have significantly slowed the spread of malignant cells. Additionally, these strategies are frequently coupled to improve treatment indices. It is well known that normal cell growth is also inhibited by surgery, chemotherapy, and radiotherapy. A bad quality of life is also a result of the severe side effects and high toxicity of various therapeutic techniques.

The *in-silico* molecular docking investigation was done using autodock in order to clarify the suggested mechanism of glycyrrhizin as an antiproliferative drug. First, based on a review of the literature, a target protein was selected for the molecular modelling investigation. According to a simple definition, molecular docking techniques are used to determine the ideal orientation of a ligand to its molecular target with the least amount of free energy in the formation of a stable complex. When compared to other conventional cancer therapy methods, this computational drug design approach can be claimed to be more thorough, time- and money-efficient, and complete. The possibility for improved output and quality in pharmaceutical research is another advantage of adopting molecular modelling.

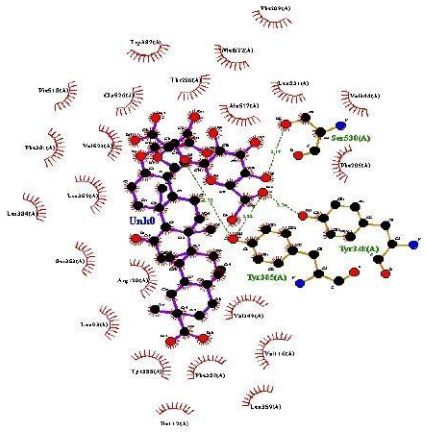
The binding energy between the protein and ligand is a crucial metric generated by molecular docking. This provides information on the binding affinity and efficacy of the protein and ligand-receptor docking. The lower the binding energy value, the higher the binding affinity and docking. This work demonstrates how molecular docking analysis was used to calculate the binding energies of the selected protein COX-2 with glycyrrhizin and amino acid residues.

The bioactive compound glycyrrhizin which exhibits the best binding affinity with Cox 2 having binding energy -7.7 kcal/mol. As per outcome of investigation, glycyrrhizin reduces the multiplication of malignant cells which also results in the death of cancerous cells. At growth phase 1 (G1), it induces apoptosis, and at growth phase 2 (G2), or mitotic phase, it induces cell arrest (M). The docking results were tabulated in table 1. The binding mode of glycyrrhizin within the active site of COX 2 protein receptor showed in figure 4-5. The glycyrrhizin interacts with the Tyr385, and Tyr348 residues of COX2 protein to form a complex structure. Glycyrrhizin showed the good binding affinity with COX-2. Cyclooxygenase-2 (COX-2) is liked with breast cancer. Therefore, it is of interest to design and develop new yet effective compounds against COX-2 from medicinal plants. COX-2 is released by cancer-associated fibroblasts (CAFs), macrophage type 2 (M2) cells, and cancer cells to the tumor microenvironment (TME). COX-2 induces cancer stem cell (CSC)-like activity, and promotes apoptotic resistance, proliferation, angiogenesis, inflammation, invasion, and metastasis of cancer cells. The docking results were produced and shown using pymol. As shown by the test, the docking score for the selected compound is similar enough and thus reflects their maximal activity against COX-2. The findings showed that the investigative molecules had higher energy values on the COX-2 protein, which means that glycyrrhizin have greater affinity and steric compatibility with COX-2 protein. The pharmacokinetic profile of glycyrrhizin reveals that it is having good pharmacokinetic profile without presence of any major toxic effects. The pharmacokinetic and toxicity profiling results of glycyrrhizin were shown in figure 6.

Table 1: Results of docking of glycyrrhizin against COX2 protein

S. No	Compound Name	Structure	Binding Energy (Kcal/mole)	Ki (μM)
1	Glycyrrhizin		-7.7	6.82

Interactions



Complex

Figure 5: Binding interaction of glycyrrhizin with COX2 protein

Toxicity & ADME-T Studies

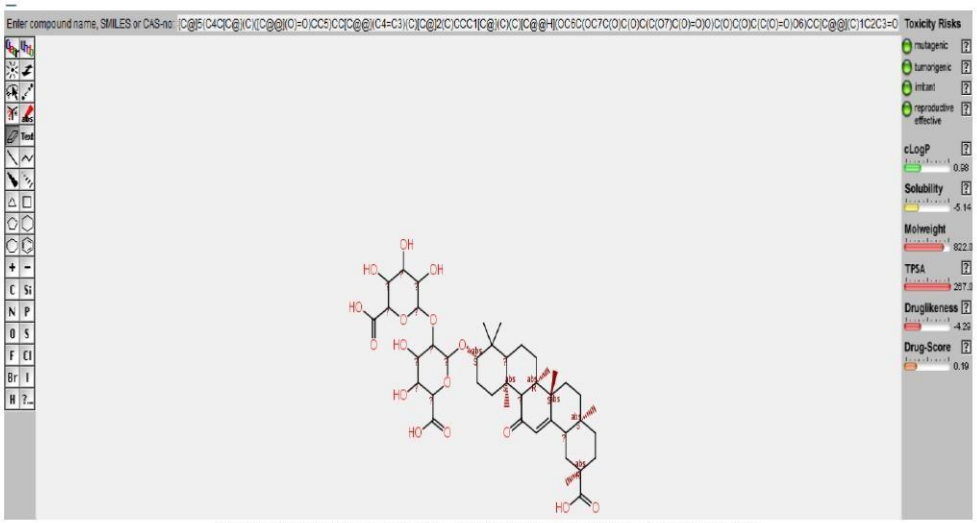


Figure 6: Pharmacokinetic and toxicity profiling of glycyrrhizin

CONCLUSION

The molecular docking of glycyrrhizin with COX2 protein revealed that, it has exhibited the chemical interaction with the amino acids in the active pockets which is showed in Figure.5. Theoretically, the ligand molecule has shown encouraging docking score. The docking result of glycyrrhizin revealed that their docking scores was -7.7 kcal mol^{-1}, and it can be predicted as good inhibitor of COX 2 protein.

REFERENCES

1. Taft, C. A., & Da Silva, V. B. (2008). Current topics in computer-aided drug design. *Journal of pharmaceutical sciences*, *97*(3), 1089-1098.
2. Kakkar, S., Kumar, S., Narasimhan, B., Lim, S. M., Ramasamy, K., Mani, V., & Shah, S. A. A. (2018). Design, synthesis and biological potential of heterocyclic benzoxazole scaffolds as promising antimicrobial and anticancer agents. *Chemistry Central Journal*, *12*, 1-11.
3. Rani, J., Saini, M., Kumar, S., & Verma, P. K. (2017). Design, synthesis and biological potentials of novel tetrahydroimidazo [1, 2-a] pyrimidine derivatives. *Chemistry Central Journal*, *11*(1), 1-11.
4. Hu, Y., & Fu, L. (2012). Targeting cancer stem cells: a new therapy to cure cancer patients. *American journal of cancer research*, *2*(3), 340-356.
5. Kassab, A. E., & Gedawy, E. M. (2013). Synthesis and anticancer activity of novel 2-pyridyl hexahyrocyclooctathieno [2, 3-d] pyrimidine derivatives. *European Journal of Medicinal Chemistry*, *63*, 224-230.
6. Kumar, S., Lim, S. M., Ramasamy, K., Vasudevan, M., Shah, S. A. A., Selvaraj, M., & Narasimhan, B. (2017). Synthesis, molecular docking and biological evaluation of bis-pyrimidine Schiff base derivatives. *Chemistry Central Journal*, *11*(1), 1-16.
7. Pizzorno, J. E., & Murray, M. T. (1999). Textbook of Natural Medicine. 2nd edition. J. Harcourt, eds. Publishers Limited, 768.
8. Chandler, R. F. (1985). Licorice, more than just a flavour. *Canadian Pharmaceutical Journal*, 118, 420-424.
9. Mukharjee, P. K. (2002). Quality control of herbal drugs- an approach to evaluation of botanicals 31st edition. New Delhi, Business Horizons Pharmaceuticals. Publications; pp 183-197.
10. ACD/Structure Elucidator, version 2018. 1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
11. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, *30*(16), 2785-2791.
12. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.

13. Malik, J. K., Soni, H., Sharma, S., & Sarankar, S. (2020). Hydroxychloroquine as potent inhibitor of COVID-19 main protease: Grid based docking approach. *Eurasian Journal of Medicine and Oncology*, *4*(3), 219-226.
14. Soni, H., Gautam, D., Sharma, S., & Malik, J. (2020). Rifampicin as potent inhibitor of COVID-19 main protease: In-silico docking approach. *Saudi Journal of Medical and Pharmaceutical Sciences*, *6*(9), 588-593.
15. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
16. Soni, S., Malik, J. K., Sarankar, S. K., & Soni, H. (2019). Rutin as a potent inhibitor of dihydrofolate reductase: A computational design and docking. *EAS J. Pharm. Pharmacol*, *1*, 130-134.
17. Soni, N. U. P. U. R., Pardasani, K. R., & Mujwar, S. O. M. D. U. T. T. (2015). Insilico analysis of dietary agents as anticancer inhibitors of insulin like growth factor 1 receptor (IGF1R). *J Pharm Pharm Sci*, *7*(9), 191-196.
18. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.
19. Thomas, S. Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil, Switzerland, Email: thomas.sanderidorsia.com

COPY OF PUBLISHED PAPER – 3

Glycyrrhizin from Glycyrrhiza glabra root: Phytochemical analysis and Characterization

Mohit Saini* Amit kumar & Jitender K Malik

Dept. of Pharmaceutics, Institute of Pharmacy, P.K. University, Shivpuri (M.P.)-India.

Correspondence Author*: Mohit Saini

Research Scholar, P.K. University, Shivpuri (M.P.)-India.

Abstract

Glycyrrhiza glabra is the scientific name for licorice. It is a well-known medicinal plant that can be found growing in many different regions of the world. It is one of the oldest and most commonly used plants that has been utilised in both western and eastern countries for a long time. Glycyrrhiza glabra (Fabaceae) roots were the main source of the triterpenoid saponin, glycyrrhizic acid (glycyrrhizin), which is a sweeter component and around 50 times sweeter than sugar, and is widely used as a sweetening addition in the food industry. Glycyrrhizin's chemical makeup has been identified as glycyrrhetinic acid. The goal of the current study was to determine the root aqueous extract of G. glabra's preliminary phytochemical analysis. Glycyrrhizin's isolation and characterisation were expanded upon.

Key words: *Glycyrrhiza glabra*, glycyrrhizin, TLC, HPLC, UV & IR.

Introduction

Triterpenoids represent a diverse, physiologically appealing type of terpenoids and have been found in both terrestrial and marine living creatures. They also have a wide structural range of secondary metabolites with more than 100 carbon skeletons. Triterpenes, steroids, limonoids, quassinoids, and triterpenoidal and steroidal saponins are among the more than 30,000 isolated and recognised molecules that make up this class of natural products. Three monoterpenes are referred to as triterpenes, and these compounds have 30 carbons arranged in six isoprenyl units[1]. Glykos, which means sweet in ancient Greek, and rhiza, which means root, are the roots of the word glycyrrhiza. In India, Glycyrrhiza glabra is generally known as mulaithi. Licorice is another name for it in the Mediterranean and some parts of Asia[2]. The plant's dried, peeled or unpeeled root and stolon are what make up this substance (Leguminosae). Both the food and pharmaceutical industries make extensive use of this plant. Glycyrrhizin (2-20%), a triterpenoid

saponin glycoside that is composed of glycyrrhizic acid salts in both potassium and calcium, is the primary component of licorice. It is 50 times sweeter than sucrose and is safe for use in treating diabetes[3]. Predictably, the plant has not been required as a prophylaxis for stomach and duodenal ulcers, or for dyspepsia as an anti-inflammatory during allergic reactions[4]. It is employed as a herbal remedy as a laxative, contraceptive, galactogogue, anti-asthmatic, and antiviral agent[5]. Previous research has shown that it is effective for treating a variety of conditions, including anaemia, gout, sore throats, tonsillitis, flatulence, sexual dysfunction, fever, coughs, skin conditions, acidity, leucorrhoea, bleeding, jaundice, and bronchitis[6].

Fig1: Root of *G.glabra*

The triterpenoid component glycyrrhizin is mostly accountable for the licorice root's sweet flavour. This substance is a component of a mixture of glycyrrhizic acid potassium, calcium, and magnesium salts. [7].

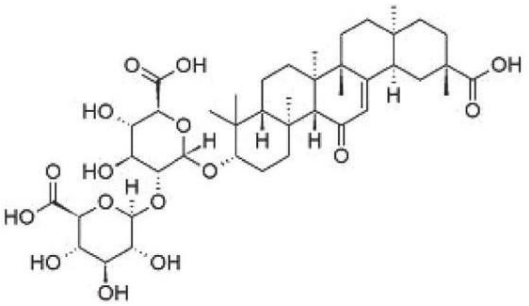


Fig:2 Structure of Glycyrrhizin

Description of Glycyrrhizin [8-9]

S.No.	Glycyrrhizin Description	
1.	**Mol. Formula**	$C_{42}H_{62}O_{16}$
2.	**Average Molecular Weight**	822.9 g/mol
3.	**IUPAC Name**	S,3S,4S,5R,6R)-6-[(2R,3R,4S,5S,6S)-2-[[(3S,4aR,6aR,6bS,8aS,11S,12aR,14aR,14bS)-11-carboxy-4,4,6a,6b,8a,11,14b-heptamethyl-14-oxo-2,3,4a,5,6,7,8,9,10,12,12a,14a-dodecahydro-1H-picen-3-yl]oxy]-6-carboxy-4,5-dihydroxyoxan-3-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid.
4.	**Class**	Triterpene glycoside
5.	**M.P.**	220 °C
6.	**Partition coefficient**	2.80
7.	**Pharmacology**	It is effective in the treatment of peptic ulcer and also has expectorant (antitussive) properties.
8.	**Mechanism of Action**	Inhibition of Hepatic Apoptosis and Necrosis Anti-Inflammation and Immunity Regulation Antiviral Effects Antitumor Effects Inductive Effect of Liver Enzyme Activity

MATERIAL AND METHOD

Plant material collection

The root was purchased from local market at Shivpuri (M.P.) and authenticated. They were dried in shade for several days at room temperature and then grinded as powder.

Extraction

50 grams of the powdered root was extracted by hot percolation (Soxhlet apparatus) with 250 ml of water till exhaustion. The extract was filtered and concentrated by evaporation under vacuum.

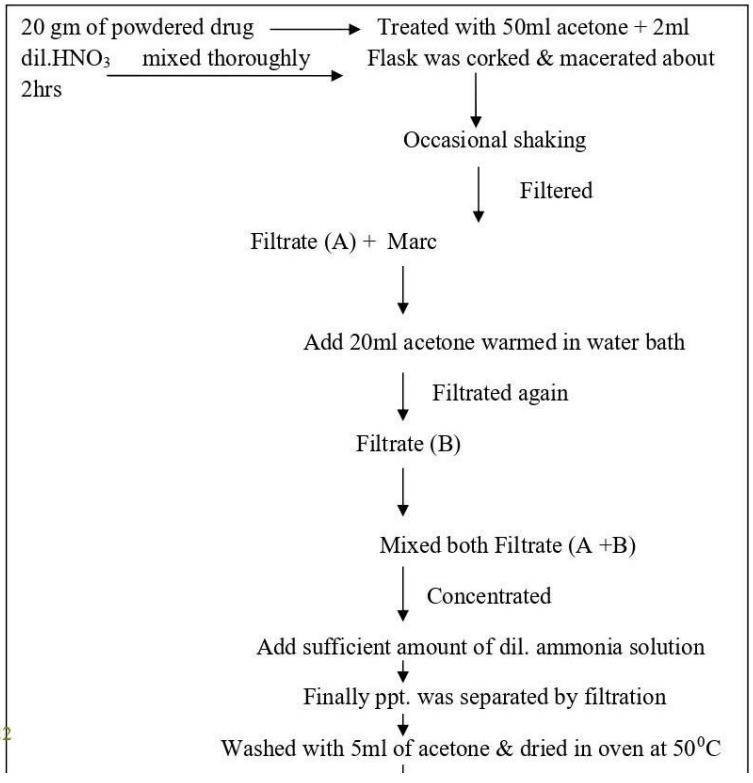
PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

Physico-chemical parameters were determined as per standard procedures[10].

Preliminary qualitative test

The extract was subjected to preliminary qualitative phytochemical investigation[11-16].

Isolation of Glycyrrhizin



General and Physical Properties: Appearance, color, solubility and melting point of the isolated constituents will be determined.

TLC chromatography

A pre-coated aluminium sheet with silica gel GF254 and the following mobile phases were used to compare isolated glycyrrhizin to standard glycyrrhizin using TLC technique. Toluene, ethyl acetate, and glacial acetic acid (12.5: 7.5: 0.5), with anisaldehyde-sulphuric acid used as a spray reagent[17–18].

Spectrophotometric analysis

Methanol was used to dissolve the isolated Glycyrrhizin, and its UV absorption peaks were measured. The Shimadzu 1700 UV spectrophotometer was used for the spectrophotometer-based analysis[19].

HPLC analysis

A LC-100 CyberlabTM, Salo Torrace, Millburry, MAO 1527, USA, with an LC-UV-100 UV detector was used for the HPLC analysis. For the chromatographic separations, a C-18 (CAPCELL) HPLC-packed column (4.6 mm I.D. x 250 mm), type MG 5 m, and number AKAD/05245 were utilized. Phosphoric acid accounted for 1% of the mobile phase: 60:40 acetone The column temperature was 25°C and the flow rate was 0.2 mL/min. The UV detection wavelength was 251 nm, and the injection volume was 25 µl.

IR analysis

IR spectral data was acquired using a Bruker (AT-IR).

Result and Discussion

Liquorice has traditionally been prescribed as a preventative treatment for gastric and duodenal ulcers. It is utilized in dyspepsia during allergenic reactions as an anti-inflammatory agent.

Glycyrrhizin can be found in stereoisomer forms of 18 and 18. Glycyrrhizin is thought to be the most widely used folk medicine for its anti-inflammatory effects on neutrophil functions, including the generation of ROS (reactive oxygen species)[20]. Thus, the current examination investigated the different phytochemicals and physiochemical boundaries found in fluid root remove. The normalization boundaries were misfortune on drying at 100-105^{0}C , complete debris esteem, corrosive insoluble debris esteem, water dissolvable debris esteem was classified in table1. Table 2 shows that the aqueous root extract contained a number of phytoceuticals. Glycyrrhizin was extracted from the aqueous root of G.glabra with a yield of 7.91 percent w/w. The melting point of isolated glycyrrhizin was 221°C, which is the same as the one that was previously reported for glycyrrhizin. Table 3 summarizes the general properties of isolated glycyrrhizin. Qualitative TLC chromatography analysis of isolated glycyrrhizin revealed Rf values that were roughly comparable to those of standard glycyrrhizin (table 4 and Fig. 4). The UV range of glycyrrhizin in methanolic arrangement shows two significant assimilation groups at 204 and 251nm, which demonstrates the presence of triterpenoid structure (Fig:5). For the purpose of quality control of herbal medicines, it is recommended to obtain chemical fingerprints through chromatographic methods. As a result, the chromatographic fingerprint should be taken into consideration when evaluating the quality of herbal medicines around the world due to the numerous components they contain[21]. The HPLC analysis was carried out with a LC-100, CyberlabTM, Salo Torrace, Millburry, MAO 1527, and USA with LC-UV-100 UV detector. The chromatograms of the standard and isolated glycyrrhizin showed, respectively, RT 8 and 7.58min (Fig. 6 and 7). Figure 8 and table 6 show the results of the IR analysis. When compared to the standard, the isolated compound's infrared spectrum revealed the same number of functional group presences.[22-47]

Conclusion

The purpose of this study was to evaluate the physiochemical and phytochemical analyses of G.glabra's aqueous root extract. The solvent precipitation method was used to further isolate glycyrrhizin from the root of G. glabra. Various chromatographic and analytical methods were used to further characterize glycyrrhizin. These measures have huge appositeness for the food and drug industry. For the quantification of glycyrrhizin in the G. glabra root, this newly

developed method proved to be straightforward, effective, dependable, and cost-effective, as this study demonstrated. The procedure is quick, easy, and very specific for glycyrrhizin.

Table: 1 Physiochemical properties of aqueous root extract of *G.glabra*

S.No	Ash values	Observation in (w/w)
1	Total ash	4.7%
2	Acid insoluble ash	0.55%
3	Water soluble ash	6.55%
4	LOD	5.5%

Table No 2: Phytochemical analysis of aqueous root extract of *G.glabra*

S.No.	Test	Aqueous root extract
1	**Tests for sterols** 1. Salkowski's Test 2. Libermann Burchard's Test	 + +
2	**Test for glycosides** 1. Baljet's Test 2. Brontrager Test	 + -
3	**Tests for saponins**	

	1. Foam Test	+
4	**Test for carbohydrates** 1. Molish's Test 2. Barfoed's Test 3. Benedict's Test	 - + -
5	**Tests for alkaloids** 1. Mayer's Test. 2. Wagner's Test. 3. Dragendorff's Test	- - - -
6	**Tests for flavonoids** 1. Ferric chloride Test. 2. Shinoda Test. 3. Alkaline Reagent Test. 4. Lead Acetate Test.	 - + - -
7	**Tests for tannins** 1. Ferric chloride Test. 2. Gelatin Test	 - -
8	**Test for amino acid and protein** 1. Biurete test	 +

Table 3: General Physical Properties of isolated Glycyrrhizin

S.No.	Physical properties	Inference
1.	Appearance	powder
2.	Color	Yellowish brown
3.	Solubility	Chloroform, methanol & slightly soluble in Pet.ether
4.	Melting point	221^{0}C

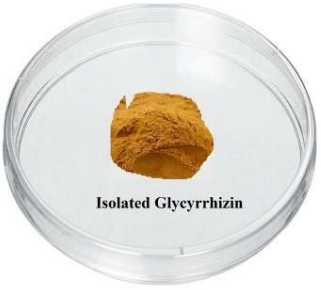


Fig 3: Isolated Glycyrrhizin

Table 4: TLC chromatography Analysis of Glycyrrhizin

Solvent System	R_f(standard)	R_f(isolated sample)
Toluene: ethyl acetate: glacial acetic acid (12.5:7.5:0.5)	0.43 (Dark violet purple spot)	0.41 (Dark violet purple spot)

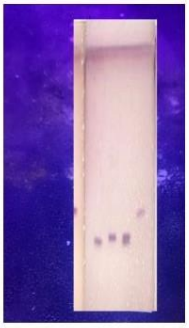


Fig 4: TLC chromatogram of isolated compound (Glycyrrhizin)

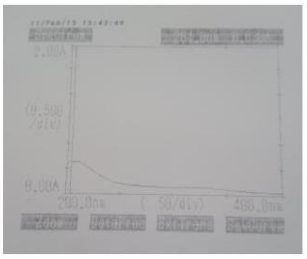


Fig 5: UV Scan of isolated compound (Glycyrrhizin)

Table: 5 HPLC Analysis of Glycyrrhizin

S.No.	Sample	Height	Area	Conc.	RT	Inference
1.	Standard Glycyrrhizin	49442	1251681.2	96.7	8.00	Glycyrrhizin
2.	Isolated Glycyrrhizin	32582	417227.2	29.0598	7.58	Glycyrrhizin

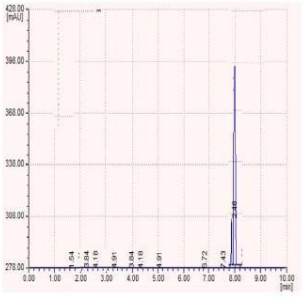


Fig 6:HPLC chromatogram of Std Glycyrrhizin

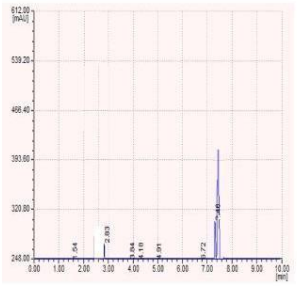


Fig 7:HPLC chromatogram of Glycyrrhizin (isolated)

Table: 6 IR Analysis of Glycyrrhizin (isolated)

cm^{-1}	**Functional Group**
3343	O-H (stretch)
3355	O-H (stretch)
3755	Aromatic
2874	CH stretch
1639	C=O stretch
1620	C=C
1364	C-O-C

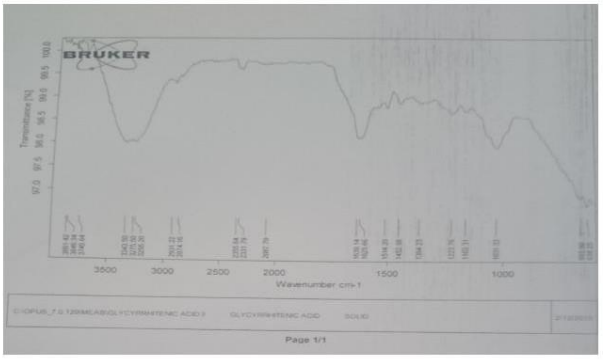


Fig 8: IR spectra of Glycyrrhizin (isolated)

Reference

1. Mahato SB, Nandy AK, Roy G. Triterpenoids. Phytochemistry. 1992;31(7):2199-249.
2. Chopra RN, Nayar SL, and Chopra IC. *Glossary of Indian Medicinal Plants*. New Delhi,India, NISCAIR, CSIR;2002.
3. Reid D. A Handbook of Chinese Healing Herbs. I edition, Singapore. Periplus. 2001.
4. Ammosov S, Litvinenko V. Triterpenoids of Plants of *Glycyrrhiza* L. and *Meristotropis* Fisch. Et Mey Genuses. *Pharm Chem J*. 2003; 37: 83-94.
5. Saxena S. *Glycyrrhiza glabra*: Medicine over the millennium. *Nat product red*. 2005; 4:358-367.
6. Sheth Ashok. The Herbs of India. 1st Edition, Vol.2. Gujrat. India. *Hi Scan Pvt. Ltd.*; 566.
7. Pizzorno JE, Murray MT. Textbook of Natural Medicine. 2nd edition. J. Harcourt, eds. Publishers Limited. 1999; 768.
8. Chandler R. F. Licorice, more than just a flavour. Canadian Pharmaceutical Journal. 1985; 118: 420-424.
9. Mukharjee P K. Quality control of herbal drugs- an approach to evaluation of botanicals 31st edition. New Delhi, Business Horizons Pharmaceuticals. Publications; 2002; pp 183-197.
10. Khandelwal K R. Practical Pharmacognosy. 3rd edition. Pune: Nirali Prakashan. 1996; pp 165.

11. Jitender K., Evaluation of anti-inflammatory activity of Gymnema sylvestre leaves extract in rats. International Journal of Green Pharmacy: April-June 2008, 114-115.

12. Development and Optimization of fixed dose Antihypertensive combination drugs using double layer sustained release Microsphere Technology. *Asian Journal of Chemistry:* 2011, Vol -23 (9), 3883-3886

13. Himesh soni and Jitender K Malik Isolation, characterization and radical scavenging activities of rutin isolated from leaves of Annona squamosa. Journal of Pharmacognosy and Phytochemistry 2022; 11(2): 222-228.

14. Himesh Soni, Evaluation of Leaves of Aqueous Extract of Coleus Aromaticus and Methanolic Extract of Annona Squamosa Extracts on Cell Viability, *American J. Pharm Tech Res*. 2012 Vol. 2(4), 936-944.

15. Himesh Soni, Jitender Malik, Abhay Pratap Yadav and Bhavana Yadav.Characterization of Rutin isolated by leaves Annona squamosa by Modern Analytical Techniques. ejbps, 2018, 5; 484-489.

16. Mukul Tailang & Ashok Sharma. Phytochemistry-Theory and Practical. First edition, Delhi. Birla Publication Pvt.Ltd; 2008,173.

17. Li L., Yang, Z., He-shui, Y., Hong-zhi, H., Li-ping, K., Man, C., Jiang-ming, C., Li-yan, Y., Xin-boo, S., Bai-ping, M.A. Preparation of glycyrrhetinic acid monoglucuronide by selective hydrolysis of glycyrrhizic acid via biotransformation, *Chinese Herbal Medicines*. 2012; 4: 324-328.

18. V Rajpal. Standarization of Botanicals.Vol-1.First edtion, New Delhi, India. Eastern Publishers; 2008,123.

19. Monica Damle. Glycyrrhiza glabra (Liquorice) - a potent medicinal herb. International Journal of Herbal Medicine 2014; 2(2): 132-136.

20. S Jain, Evaluation of hydro-alcoholic extract of leaves of Boerhaavia diffusa for anxiolytic activity in rats, African Journal of Pharmacy and Pharmacology, 2013, Vol. 7(18), *1071-1074.*

21. Sourabh Jain, Nanosuspension of vasicine from Adhatoda vasica: Isolation and characterization, Drug invention today, 2013, Vol. 5(1), 32-38.

22. Pathophysiological and therapeutic effect of arthritic ailments- a brief review. *European Journal of Biomedical and Pharmaceutical sciences:* 2016, Vol. 3(12), 179-186.

23. Kumari Pooja, Overview on: - characteristic of isoflavones & its biological activity *International Journal of Biology, Pharmacy and Allied Science*, March, 2017, Vol. 6(3), 447-467.

24. Amit Kumar, In- Silico Analysis to Access the Antibacterial Effect of Genistein: Molecular Docking Approach, EAS Journal of Pharmacy and Pharmacology, Published By East African Scholars Publisher, Kenya, Sept- Oct 2019, Vol 1(5), 125-129.

25. Amit Kumar, Preliminary Phytochemical Evaluation and Antioxidant potential of Aerial parts of *Desmodium gangeticum* Asian Journal of Medical and Health Research 2019, Vol 4(10), 1-11.

26. Amit Kumar, Characterization of Flavonoids in Aqueous extract of *Desmodium gangeticum* by RP-HPLC Global Academic Journal of Pharmacy and Drug Research, 2019; Vol 1(1) 1-4.

27. Preliminary Pharmacognostical Standardization of *Ipomoea turpethum*, European Journal of Biomedical and Pharmaceutical sciences, 2019, Vol. 6(13), 391-397.

28. Deovrat Kumar, Amit Kumar and Jitender K. Malik, Preformulation Studies of Drotaverine HCl: An Integral Part of Formulation Design, European Journal of Biomedical and Pharmaceutical sciences, 2019 Vol 6(13), 304-307.

29. Preliminary Pharmacognostical Standardization of *Ipomoea turpethum*, European Journal of Biomedical and Pharmaceutical sciences, 2019, Vol. 6(13), 391-397.

30. Efficacy of Anti-Inflammatory Potential of *Desmodium gangeticum* Extracts, Advanced Research in Chemistry and Applied Science, Nov 2019 Vol 1(1). 19-23.

31. Pharmacognostical & TLC Fingerprinting of Kiratatikta, EAS Journal of Pharmacy and Pharmacology, Dec.2019, Vol 1(6), 170-177.

32. Process Evaluation and In-vitro drug release study of fast dissolving uncoated tablets of Drotaverine HCl., European Journal of Scientific Exploration Dec.2019, Vol 2(6), 1-8.

33. Amit Kumar, Formulation and evaluation of fast dissolving uncoated tablets of Drotaverine HCl, World Journal of Pharmaceutical Research, Jan 2020, Vol 9(1), 1716-1727.

34. Pharmacognostical & TLC Fingerprinting of *Andrographis paniculata* (Kalmegh), Saudi Journal of Medical and Pharmaceutical Sciences, Published By Scholars Middle East Publishers, Dubai, United Arab Emirates, Feb 2020, Vol. 6 (2), 160-167.

35. Tiwari P. A Comprehensive Review on Botanical as Anti-ulcer Therapeutics: Prospective Avenues of Biocompatible Drug Discovery, Scholars International Journal of Tradicinal and complementary Medicine, Published by Scholars Middle East Publishers, Dubai, United Arab Emirates, Feb 2020, Vol. 3 (2), 27-32.

36. Preliminary phytochemical analysis & TLC Fingerprinting of *Desmostachya bipinnata* , Saudi Journal of Medical and Pharmaceutical Sciences, Published By Scholars Middle East Publishers, Dubai, United Arab Emirates, March 2020, Vol. 6 (3), 297-305

37. Jitender K. Malik; Bhavana Yadav; Phytography & Phytopharmacology of Genus cassia, Journal of Advances in Bio- pharmaceutics and Pharmacovigilance, March 2020, Vol. 2(2), 1-9.

38. Sneha singh, Amit Kumar, Analytical Method Development and Validation for Estimation of Silymarin in Tablet Dosage form by HPLC, Asian Journal of Applied Chemistry Research, May 2020, Vol. 5(3), 22-31.

39. Jitender K malik, Vivekanand K., Pharmacological Activities of Martynia (Martynia annua Lin.): A Brief Review, Asian Plant Research Journal, May 2020, Vol. 4(2): 46-51.

40. Amit Kumar, Tramadol HCL: Preformulation Studies of Imperative Part of Formulation Design, Scholars Academic Journal of Pharmacy, September, 2020; 9(9): 257-262.

41. Jitender k malik, himesh Soni, Phyto-Pharmacological Potential of Zizyphus jujube: A Review. Scholars International Journal of Biochemistry, Feb 2021,Vol 4(2): 1-5.

42. Amit kumar, Pharmacognostical Exploration of Saccharum officinarum, Scholars International Journal of Traditional and Complementary Medicine, Published by Scholars Middle East Publishers, Dubai, United Arab Emirates, April 2021, Vol- 4(4), 53-60.

43. Shikha Sharma, Dinesh Chandra, Preliminary Phytochemical Screening and HPTLC Fingerprinting of Leaf Methanolic Extract of Spermacoce Hispida, EAS Journal of Pharmacy and Pharmacology, Published By East African Scholars Publisher, Kenya, Jan-Feb: 2022 Vol 4(1), 22-25.

44. Amit kumar, Recent Progression in Wound healing Technologies, SAR Journal of anatomy and Physiology, Jan-Feb: 2022 Vol 3(1), 01-08.

45. Mohit saini, Jitender K Malik, Novel Drug Delivery System Microsphere: A Review, SAR Journal of Anatomy and Physiology, Published by South Asian Research Publication, Mar-Apr, 2022, Vol-3(2), 9-16.

46. Vivek Kumar & Jitender K. Malik., Neuro-pharmacology of Phytoceuticals: A Brief Review on Depression, SAR Journal of Psychiatry and Neuroscience, Published by South Asian Research Publication, Mar-Apr, 2022, Vol-3(1), 6-14.

47. Shikha Sharma, Jitender K. Malik & Dinesh Chandra, Pharmacognostic Evaluation and Antioxidant Potential of *Spermacoce hispida*, Glob Acad J Pharm Drug Res; Published

by Global Academic Journal's Research Consortium (GAJRC), Sep-Oct- 2022, Vol-4 (4); 83-88.

P.K.UNIVERSITY
SHIVPURI (M.P.)

University Established Under section 2f of UGC ACT 1956 Vide MP Government Act No 17 of 2015

Ref no PhD/PKU/2024/PhD Cell/Intimation/362 Date 31/10/24

An Intimation Letter

To
Mr.Mohit Saini
Research Scholar
Faculty of Pharmacy
P.K.University.

Greetings from P.K.University. Mr.Mohit Saini, (2021-22) your **Phd. Pre-Submission Viva voce (PSDC)** is scheduled on **09/11/2024 at 11:00 am** in P.K.University ,Dr.A.P.J.Abdul Kalam Conference Hall., Guru Vashista Administrative Block. Make sure to attend and present in ICT mode of presentation.Equip you to accompany ICT gadgets / Laptop / Pendrive etc.

31/10/2024

All the best
Dr Nalla Bhaskar
Dean Research & I/c Ph.D Cell
P.K.University
Thanra village Karera Tehsil
Shivpuri - M.P

ADDRESS: VILL: THANRA, TEHSIL: KARERA, NH-27, DIST: SHIVPURI, M.P. 473665,
MOB: 7241115088, Email:registrar.pkuniversity@gmail.com

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

भारत सरकार / GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
पर्यावरण , वन एंव जलवायु परिवर्तन मंत्रालय
मध्य क्षेत्रीय केंद्र / CENTRAL REGIONAL CENTRAL
10. चैतम लाइन्स, इलाहबाद -211002 / 10, CHATAM LINES, ALLAHABAD-211002

सा०भा.व.स.म.झे.के./ प्रशा./2022-23/01800 **दिनांक/ Date: 18/11/23**

Mr. Mohit Saini

C/O Faculty of Pharmacy, P K University, Shivpuri, M P

विषय / subject: Identification and authentication of plant specimen /पादप नमूना पहचान एवं प्रमाणीकरण के सन्दर्भ में।

महोदया,

आपके पत्र,दिनांक- (भारत कोश भुगतानसं. 2406230006345, दिनांक 05/10/2023) जो की पादप नमूनों के पहचान एंव सत्यापन के सन्दर्भ में है, आपके द्वारा भेजे गए नमूनों को निमन्लिखित नाम से प्रमाणित किया जाता है।

With reference to your latter, vide dated nil (Bharat Kosh transaction ref. no. 2406230006345, dated 05/10/2023) regarding authentication and identification of the plant specimen, the specimen is identified and authenticated as under:

S. No.	Name of plant species	Family
01	*Glycyrrhiza glabra* Herb.	Fabaceae

साधनयवाद/ Thanking you,

(आरती गर्ग/Arti Garg)
वैज्ञानिक -एवं प्रधान कार्यालय/
Scientist- E & Head of Office