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Molecular Docking and *In Vitro* Evaluation of Luteolin and Piroxicam Reveal Synergistic Anticancer Potential

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Abstract

The present study investigates the antioxidant and cytotoxic properties of natural phytochemicals and NSAIDs, focusing on their potential anticancer effects against selected cancer cell lines. Luteolin, a dietary flavonoid with known antioxidant and anticancer activities, and piroxicam, a Non-Steroidal Anti-Inflammatory Drug (NSAID) with reported anticancer potential, were examined both individually and in combination against MMP-9. Molecular docking revealed that the piroxicam–luteolin complex demonstrated stable interactions with key residues, including GLU241, ALA242, LEU243, TYR245, MET247, PRO245, HIS226, GLN227, ALA189, LEU188, LEU222, TYR248, and ARG249, with a binding energy of –6.89 kcal/mol, indicating favorable binding affinity. Antioxidant activity assays revealed that luteolin and piroxicam alone exhibited IC_{50} values of 22.85 ± 0.080 µM and 20.512 ± 0.04 µM, respectively. Notably, their combination reduced the IC_{50} to 10.89 ± 0.34 µM, suggesting a synergistic enhancement of antioxidant capacity. Similarly, MTT assays demonstrated that luteolin and piroxicam individually displayed cytotoxic effects with IC_{50} values of 198.3 ± 0.088 µM and 175.5 ± 0.129 µM, while their combination yielded a significantly lower IC_{50} of 73.3 ± 0.25 µM, confirming a synergistic effect in inhibiting cancer cell proliferation. Furthermore, intracellular ROS estimation revealed effective reduction in ROS levels by luteolin and piroxicam individually, with amplified effects observed upon their combined treatment. These findings indicate that the luteolin–piroxicam combination offers superior antioxidant and cytotoxic activity compared to either compound alone. Thus, this synergistic interaction highlights a promising strategy for developing safe, natural, and effective anticancer therapies, warranting further validation through in vivo studies.

Keywords: Luteolin; Piroxicam; Antioxidants; Reactive Oxygen Species (ROS); Molecular Docking.

1. INTRODUCTION

Cancer is a highly complicated disease that influences a large number of people and is a prime cause of death in the world (9.7 million cancer deaths in 2022), with about 78 % of cases diagnosed in individuals aged 55 and older. The most common types of fatal cancers vary between men and women, with lung, stomach, liver, colon, and breast cancer being the most frequent. Worldwide, cancer deaths are projected to rise, with an estimated 12 million deaths expected annually by 2030 (Sainz *et al.,* 2012).

Free radicals are normally reactive oxygen species (ROS) and reactive nitrogen species (RNS), which oxidize cellular proteins, nucleic acids, and lipids. Lipid peroxidation is a process where free radicals cause damage to polyunsaturated fatty acids. This process involves the propagation of oxidative damage. It can be terminated by enzymes such as glutathione reductase, glutathione peroxidase, and superoxide dismutase (Schattler *et al.,* 1998) or antioxidants present in the body that scavenge free radicals. (Cheeseman & Slater, 1993). While the body has antioxidant defences to manage these free radicals, an excess can lead to oxidative and nitrosative stress. This chronic stress is linked to several diseases, including cancer, highlighting the importance of maintaining a balance in the body's redox system. It is investigated that ROS may cause the breaking of the DNA strand, and oxidative damage to the nucleotides, causing mutagenesis, resulting in cancer. Cancer cells have high levels of reactive oxygen species (ROS), which can lead to DNA damage and cell death. While a certain amount of ROS is necessary for normal cell functions, excessive ROS from external factors or metabolic changes can promote cancer. High ROS levels cause oxidative stress, damaging proteins, lipids, DNA and mitochondria (Pizzino *et al.,* 2017), with DNA being particularly vulnerable. This damage can lead to genomic instability and cancer progression, while high ROS levels can harm cancer cells, they also have potential anticancer

effects. Recent studies on treatment called NCX4040 (a nitric oxide donor) generates ROS, causing oxidative damage that can destroy tumor cells (Sinha *et al.,* 2022). Thus, Oxidative stress and inflammation are related to cancer and apoptosis tumor cells (Reuter *et al.,* 2010).

A moderate accumulation of ROS can support tumor growth, (Moloney & Cotter, 2018), while excessive ROS or insufficient clearance leads to oxidative stress, (Perillo *et al.,* 2020), causing damage to DNA and increasing the risk of mutations and genome instability, which can promote cancer. Guanine is particularly vulnerable to oxidation, resulting in products like 8-oxoguanine that linked to tumorigenesis (Burrows & Muller, 1998), (C. Li *et al.,* 2022). The base excision repair pathway is crucial for repairing oxidative DNA damage, if it fails, the likehood of mutation rises can cause tumor induction (Boiteux *et al.,* 2017). additionally, cancer cells can adapt to higher ROS levels by enhancing their antioxidant defences, which further support cancer progression. Thus, a moderate increase in ROS is seen as beneficial for cancer transformation.

Excessive generation of reactive oxygen species (ROS) is linked to cancer development and progression (Circu & Aw, 2010), (Feng *et al.,* 2020). High ROS levels are associated with various malignancies. Factors such as adaptation to low oxygen, metabolic changes, oncogenic mutation, and activation of pro-tumor signaling contribute to tumor formation. Hypoxia induced ROS control the expression of MMP-2 and MMP-9. It also promotes proliferation, migration and invasion of glioblastoma. Thus, it has been specially noted as a significant factor in this process.

Excessive concentration of reactive oxygen species can lead to cell-cycle arrest and apoptosis. To counteract this, cancer cells activate the transcription of antioxidants enzymes (Perillo *et al.,* 2020). The nuclear erythroid 2-related factor (NRF2) play a crucial role in regulating antioxidants response in these cells (Sporn & Liby, 2012). NRF2 is often overexpressed in cancer, promoting cell survival by regulating the antioxidant system. Normally NRF2 is degraded by KEAP1, but under oxidative stress, it separates from KEAP1, moves to the nucleus, and activates antioxidant response elements (ARE) in target genes (Kansanen *et al.,* 2013). These genes include those for various antioxidant enzymes, such as NAD(P)H Quinone dehydrogenase 1 and catalyse (Ma, 2013). Thus, cancer cells prevent themselves from excessive ROS.

Reactive oxygen species (ROS) can cause oxidative DNA damage, leading to double-stranded breaks and the creation of mutagenic 8-oxo-7-hydroxy-2-deoxyguanosine (8-oxodG). This compound is a significant contributor to spontaneous mutagenesis, as it can cause the conversion of guanine to thymine by pairing with cytosine and adenine (Sallmyr *et al.,* 2008), (Oka & Nakabeppu, 2011). The build-up of 8-oxodG in cellular genome is linked to the development of cancer.

Iron is a major source of ROS production and plays a significant role in cell death across various organism and pathological conditions (Dixon & Stockwell, 2013). it is considered as a risk factor for developing several cancers due to iron-induced oxidative stress (Toyokuni, 2016). The clinical impact of excess iron-induced ROS in cancers, emphasizing the connection between iron-induced ROS and carcinogenesis. The relationship between oxidative DNA damage caused by excess iron in the liver and the development of liver cancer (hepatocellular carcinoma). The reducing therapeutic iron levels and lowering ROS can improve liver health and decreases HCC risk in liver cancer patients (Kato *et al.,* 2007).Antioxidants help mitigate this damage by breaking the chains formed by these free radicals either by donating a hydrogen atom or an electron. Many of the investigations suggested that vegetables, fruits, and plants contain natural substances such as flavonoids, which have an antioxidant effect and can reduce the potential stress generated by reactive oxygen species. Approximately 4000 flavonoids have been found to date. (AQIL *et al.,* 2006) Flavonoids are known for their protective effects in biological systems due to their ability to transfer electrons to free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases. The common flavonoids included in DPPH and MTT assay study were Luteolin, Apigenin, and Quercetin. They have significant health benefits in various studies, such as luteolin has potential use as a chemopreventive agent against chromium-induced cancer by scavenging reactive oxygen species (ROS) and modulating cell signalling in human bronchial epithelial cells (Pratheeshkumar *et al.,* 2014). It may also have therapeutic benefits for cognitive dysfunction in Alzheimer's disease (Fu *et al.,* 2014), and can positively influence liver carcinogenesis by reducing mast cell recruitment (Balamurugan & Karthikeyan, 2012). Apigenin is a natural flavonoid known for its antioxidative properties and chelating redox-active metals. Apigenin's antioxidative activities are linked to its ability to donate hydrogen ions and electrons, which helps to stop the production of free radicals and prevent oxidative damage by scavenging reactive oxygen species (ROS), (Abdulla *et al.,* 2017). The antioxidant mechanism of apigenin, highlighting its ability to enhance bioavailability and inhibit oxidative enzymes. It modulates various signalling pathways, including NF-kB, Nrf2, MAPK, and PI3/Akt, which are involved in both enzymatic and non-enzymatic antioxidant activities. The major in-vitro methods for assessing Apigenin's antioxidant potential include DPPH, ORAC and ABTS. (Kashyap *et al.,* 2022). There is limited information available on Apigenin's antioxidant properties and discussion on its effects and mechanisms of action. Quercetin has been studied for its biological effects, including antioxidants, anti-inflammatory,

antitumor, and its ability to induce apoptosis (Y. Li *et al.,* 2016). Quercetin can inhibit cancer cell growth by causing cell cycle arrests at G2/M or G1 phase and promoting the activity of enzymes that reduce reactive oxygen species (ROS) in cells (Seufi *et al.,* 2009). It activates ROS-scavenging enzymes like SOD, CAT, and glutathione peroxidase for the reduction of intracellular ROS level (N. Li *et al.,* 2014). Pure Quercetin have higher antioxidant activity. Due to the contribution of hydroxyl groups. The radical inhibitory and metal reducing activity of quercetin decreases when cations are chelated. It utilized three methods including DPPH. The metal ions significantly alter the chemical properties, affecting its antioxidant activity. Quercetin can scavenge free radicals or reduce Fe (III) in a concentration and time dependent manner (Dolatabadi *et al.,* 2014).

Various assays are employed to assess the antioxidant activity of herbal extracts and phenolic compounds, utilizing different radicals and methods to analyze antioxidant effects and determine oxidation products. The most potent method involves using a stable free radical, DPPH, to assess how well antioxidants can neutralize reactive species. The ability of antioxidants to reduce DPPH is a key feature of this method, as a single electron of the nitrogen atom in DPPH is reduced by hydrazine by taking a hydrogen atom from the antioxidants. The DPPH radical is intensely coloured and stable; due to this property, its solution is commonly used. It is identified that the UV-vis spectrum of DPPH shows two distinct bands due to π-π^* transitions with the unpaired electron contributing significantly to the visible band (O. Chen *et al.,* 2009). When DPPH is mixed with a hydrogen atom donor substance solution, its violet colour fades, indicating the formation of the reduced DPPH radical (DPPH-H) (Yapıcı *et al.,* 2021). This colour change from violet to pale yellow occurs due to radical reduction by antioxidants, and can be measured using UV-vis spectroscopy and is commonly used to assess the antioxidant capacity of substances like herbal extracts and phenolic compounds (Xie & Schaich, 2014).

The DPPH test is used to estimate the total content of reductants in plant extracts, indicating the antioxidant capabilities of phenolic compounds and their capacity is quantified (Gulcin, 2020), (Gülçin, 2011).

$$XO^* + ROOH \longrightarrow XOH + ROO^*$$

This method is known for being simple, sensitive, fast, and reproducible, making it a convenient choice for evaluating the antioxidant potential of various compounds and herbal extracts. The concentration referred to as $IC_{50,}$ indicates its efficiency or inhibitory capacity. The IC_{50} values are essential for comparing the radical scavenging capacities of various antioxidants.

The MTT assay, developed in 1983, is widely used to assess cell viability and metabolic activity (Mosmann, 1983).The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) consists of a positively charged tetrazole ring surrounded by aromatic rings. When reduced by metabolically active cells, MTT is converted into a violet-blue insoluble molecule called formazan (Berridge *et al.,* 2005), (Stockert *et al.,* 2018). This reaction allows for colorimetric measurement of cell metabolic activity. While mitochondria are often associated with MTT reduction, (Surin *et al.,* 2017), (Stockert *et al.,* 2018). Various studies have found formazan in multiple cellular organelles, including the endoplasmic reticulum, lipid droplets, plasma membranes, nucleus, and microsomes (Stockert *et al.,* 2012) (Bernas & Dobrucki, 2000), (Y. Liu *et al.,* 1997). In an MTT assay, the IC50 value represents the concentration of a drug or compound needed to inhibit a biological process by 50% and indicates the potency of the drug.

The present study deals with in vitro investigation of natural phytochemicals for their antioxidant and cytotoxic effects against cancer cell lines to determine their anticancer effects. Further analysis in in-vivo conditions can provide safe, natural and effective treatment against cancers.

2. METHODOLOGY

2.1 Molecular Docking analysis of MMP-9

Molecular docking studies were performed to evaluate the binding interactions of selected flavonoids (Quercetin, Luteolin, and Genistein) and NSAIDs (Ketorol and Piroxicam), both individually and in combination, against matrix metalloproteinase-9 (MMP-9) based on our previous studies (Singh *et al.,* 2024). The docking experiments were carried out using AutoDock Tools 1.5.6. The three-dimensional crystal structure of MMP-9 was retrieved from the Protein Data Bank (PDB) and prepared by removing water molecules, adding polar hydrogen atoms, and assigning Kollman charges. The ligands were obtained from the PubChem database in SDF format and converted into PDBQT files after energy minimization using MMFF94 force field. Gasteiger charges were assigned, and torsional degrees of freedom were defined for each ligand. For docking, a grid box was constructed to cover the active site of MMP-9, with dimensions large enough to accommodate ligand flexibility and ensure comprehensive exploration of the binding pocket. The Lamarckian Genetic Algorithm (LGA) was employed as the search method, with a population size of 150, maximum number of evaluations set to 2.5 × 10^6, and 100 independent docking runs for each ligand. Docking results were ranked based on binding free energy (ΔG, kcal/mol). The most stable complexes were selected for further analysis. Protein–ligand interactions were visualized using PyMol. Comparative docking of combinations of flavonoids and NSAIDs was performed to assess potential synergistic binding interactions within the active site of MMP-9.

2.2 DPPH assay

Free radicals are unstable molecules that can damage DNA, contributing to aging and diseases like cancer and inflammation. Antioxidants can neutralize free radicals and may help prevent these health issues. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is commonly used to test antioxidant activity because it changes colour from purple in methanol to yellow when it reacts with antioxidants, indicating the reduction process. The DPPH purple colour in methanol has maximum absorption at 517 nm, that decreases in yellow colour when it reacts with hydrogen to produce the reduced DPPH-H species. The produced electrons consequent decolorization are stoichiometric.

To measure the antioxidant activity of flavonoids (Luteolin, Genistein, and Quercetin) and NSAIDs (Ketorol and Piroxicam) using the DPPH radical scavenging test. A small amount (0.5 mg/mL) of flavonoids and NSAIDs solution was mixed with 10 % (v/v) ethanol to obtain 100 µL were mixed to a test tube using a micro syringe and 1mL DPPH solution (100 µM) in 99.8% (*v*/*v*) ethanol and 1 mL of 96% (*v*/*v*) ethanol, then vortexed and incubated for 30 minutes. The change in colour was measured at 517 nm to determine how well flavonoids and NSAIDs can neutralize free radicals. The antioxidant activity was also tested with gallic acid (0.05 mg/mL) and Trolox (1 mg/mL) for comparison. The percentage of DPPH radical inhibition was calculated by following expression to assess antioxidant effectiveness. Antioxidant Activity (%) = [Abs Control – Abs Sample / Abs Control] × 100

The final results are shown as IC 50 values, which indicate the concentration of antioxidant or radical-scavenging agent needed to reduce the initial radical amount by 50 %. Linear regression analysis was used to determine these values from the concentration versus activity graphs. The spectrophotometric tests were performed in triplicate on both the samples and reference substances, and the experiments were repeated over three days to ensure accuracy.

2.3 MTT assay

Many flavonoids can inhibit cancer cell growth. The MTT assay was used to monitor cell development and changes, showing by the flavonoids and NSAIDs, which was prominent in phytochemical and antioxidant tests, also reduce human breast cancer cell survival. The study focused on the anticancer potential of Flavonoids (Luteolin, Genistein, and Quercetin) with MCF-7 a human breast cancer cell line. The results compared to the NSAIDs (Ketorol, and Piroxicam), indicating that higher concentrations of the flavonoids increased cell death, suggesting its potential as an anticancer agent.

The testing of the cytotoxic effects of flavonoids and NSAIDs on breast cancer cells (MFC-7). The samples were dissolved in DMSO and applied to cells cultured in 96-well plates. After 24 hours, the medium was replaced, and cells were incubated for an additional 24 or 48 hours with different concentrations of the samples. The cytotoxicity was measured using the MTT assay, which involves adding MTT solution (5 mg/mL), incubating 3 hours, and then processing the plates further with 10 % SDS buffer (100 µL) were added to each well, incubate overnight then absorbance was determined at 570 nm with the help of microplate reader. The study aimed to evaluate the potential of these substances to kill cancer cells.

2.4 ROS Assay

Intracellular ROS levels were quantified using the Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, UK) with the fluorogenic dye H2DCFDA, following the manufacturer's protocol. Breast cancer cells (25,000/well) were seeded in 96-well black-wall plates (Corning, USA) and incubated overnight. The next day, cells were washed with HBSS (150 µl; Gibco, UK) and incubated with staining buffer (100 µl; 20 µM H2DCFDA in HBSS) for 40 min at 37 °C. After washing, HBSS (100 µl) was added, and fluorescence was measured using a POLARstar Omega reader at 485 nm excitation and 535 nm emission. For treatment-induced ROS measurement, compounds (flavonoids and NSAIDs) were added along with HBSS, and fluorescence was recorded after the desired incubation time.

The viability of treated MCF-7 cells was expressed as a percentage of control cell viability. Each test was repeated three times, and results are shown as mean ± SD. Data analysis was performed using GraphPad Prism software, and statistical significance among groups was determined with ANOVA and Tukey's post hoc test.

3. RESULTS AND DISCUSSION

3.1 Molecular docking of flavonoids and NSAIDs

Molecular docking of the Quercetin, Luteolin, Genistein, Ketorol and Piroxicam was performed individually as well as in combination of one flavonoid and one NSAID. These flavonoids and NSAIDs were selected based on our previous analysis conducted separately for inhibition of MPP-9 (Singh *et al.,* 2024). The docking of piroxicam-luteolin combination gave the highest negative binding energy of -6.89 kcal/mol, indicating the effective inhibition of MMP-9 at the active site of the enzyme. To further explore the inhibition potential and to evaluate the antioxidant effect of the best flavonoids and NSAIDs *in vitro,* DPPH assay, MTT assay, and ROS assay were performed both individually and in combinations.

Table 1: Amino acids interaction, Hydrogen bond formation, and Binding energies of MMP9-flavonoid-NSAID complex

S. No.	Combination of NSAID and Flavonoid	Amino Acids Interaction	Binding Energy (Kcal/mol)
1	Piroxicam-Luteolin	GLU241, ALA242, LEU243, TYR245, MET247, PRO245, HIS226, GLN227, ALA189, LEU188, LEU222, TYR248, ARG249	-6.89 kcal/mol

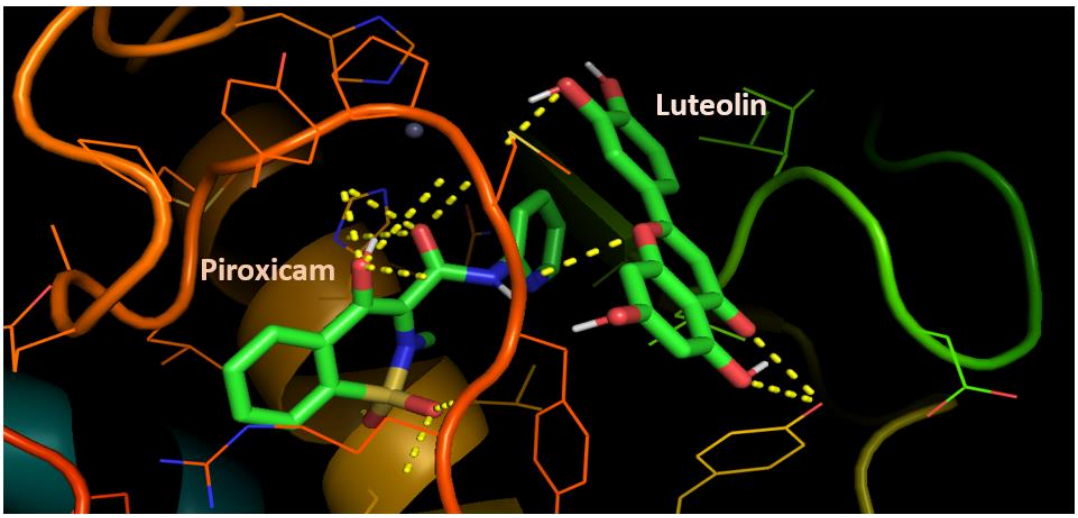

Fig 1: Molecular docking view of Piroxicam – Luteolin combination

3.2 DPPH Assay

The antioxidant activity of the tested samples was determined, and the 50% inhibitory concentration (IC_{50}) values were calculated to identify the most potent flavonoids and non-steroidal anti-inflammatory drugs (NSAIDs) demonstrating effective inhibition of MMP-9. Among the compounds screened, the flavonoids and NSAIDs exhibiting the most favourable binding energies in combination docking studies were further assessed for their antioxidant potential when used together (Table 13). In individual analyses, luteolin and piroxicam displayed highly significant antioxidant effects, with IC_{50} values of 22.85 ± 0.080 µM and 20.512 ± 0.04 µM, respectively. However, when tested in combination, luteolin and piroxicam produced a markedly reduced IC_{50} value of 10.89 ± 0.34 µM, indicating a substantially enhanced antioxidant capacity compared to their individual effects. This notable reduction in IC_{50} highlights the synergistic interaction between luteolin, a naturally occurring flavonoid with well-documented antioxidant and anticancer properties, and piroxicam, an NSAID known for its anti-inflammatory and potential anticancer effects. The observed synergy suggests that the combined administration of luteolin and piroxicam may significantly improve the mitigation of oxidative stress conditions commonly associated with cancer progression. Such findings emphasize the therapeutic potential of integrating natural compounds with conventional pharmacological agents to enhance overall efficacy, reduce required dosages, and potentially minimize side effects, thereby offering a promising strategy for developing novel combination therapies targeting oxidative mechanisms in cancer.

Table 2: Inhibitory concentration (IC50) values of best flavonoids and NSAIDs in DPPH assay

S. No.	Sample Name	Inhibitory Concentration (IC_{50}) Value (µM)
1	Ascorbic Acid	27.73 ± 0.018
2	Quercetin	65.46 ± 0.055
3	Luteolin	22.85 ± 0.080
4	Genistein	2798 ± 0.056
5	Ketorol	1248 ± 0.041
6	Piroxicam	20.512 ± 0.04
7	Luteolin and Piroxicam	10.89 ± 0.34

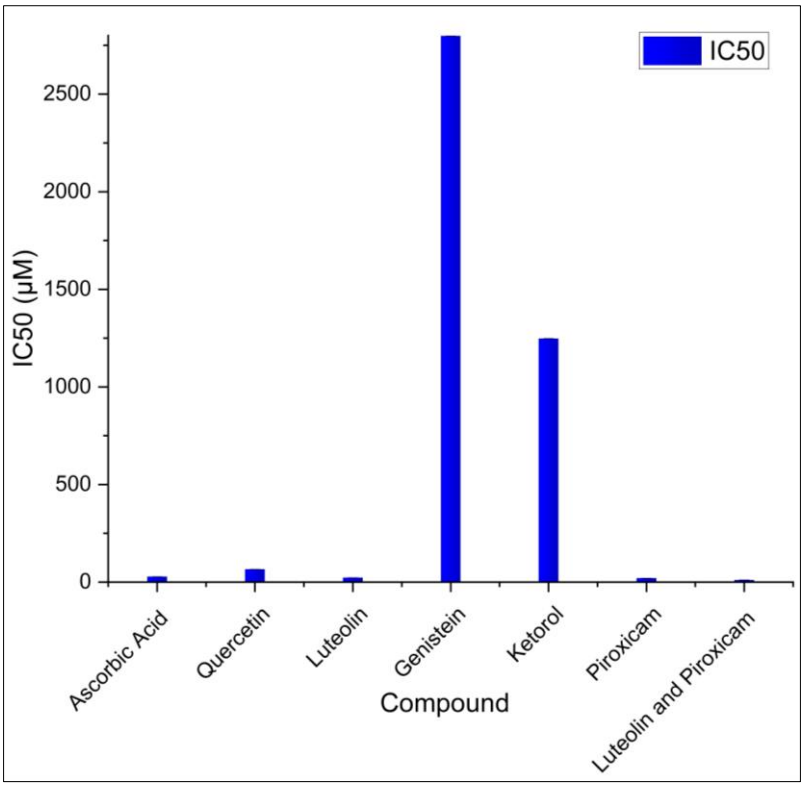

Fig 2: IC50 values of all considered compounds (Flavonoids and NSAIDs) in DPPH assay

3.3 MTT Assay

The MTT assay is a widely used, sensitive, and reliable colorimetric technique for evaluating cell viability, proliferation, and activation. It functions on the principle that mitochondrial dehydrogenase enzymes in metabolically active cells can convert the yellow, water-soluble compound 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into insoluble dark blue formazan crystals. The quantity of formazan produced is directly proportional to the number of viable cells, making this assay a robust quantitative measure of cytotoxicity. In this study, flavonoids and NSAIDs exhibiting the most favourable docking binding energies were selected for evaluation against a human breast cancer cell line. All tested compounds demonstrated the ability to inhibit cancer cell proliferation to varying degrees. Notably, luteolin and piroxicam emerged as the most potent agents, showing individual IC_{50} values of 198.3 ± 0.088 µM and 175.5 ± 0.129 µM, respectively. Further assessment of their combined effect revealed a remarkably reduced IC_{50} value of 73.3 ± 0.25 µM, indicating a pronounced synergistic cytotoxic effect. This substantial decrease in IC_{50} suggests that the luteolin–piroxicam combination significantly enhances the inhibition of cancer cell proliferation compared to either compound alone. The results highlight the potential of integrating natural flavonoids with conventional pharmacological agents to improve therapeutic outcomes, reduce required doses, and potentially minimize toxicity. Such synergistic combinations could represent a promising approach for the development of more effective anticancer treatment strategies targeting cell proliferation mechanisms.

Table 3: **Inhibitory concentration (IC50) values of best flavonoids and NSAIDs in MTT assay**

S. No.	Sample Name	Inhibitory Concentration (*IC₅₀*) Value (µM)
1	Quercetin	1458 ± 0.107
2	Luteolin	198.3 ± 0.088
3	Genistein	524.5 ± 0.103
4	Ketorol	1306 ± 0.058
5	Piroxicam	175.5 ± 0.129
6	Luteolin and Piroxicam	73.3 ± 0.25

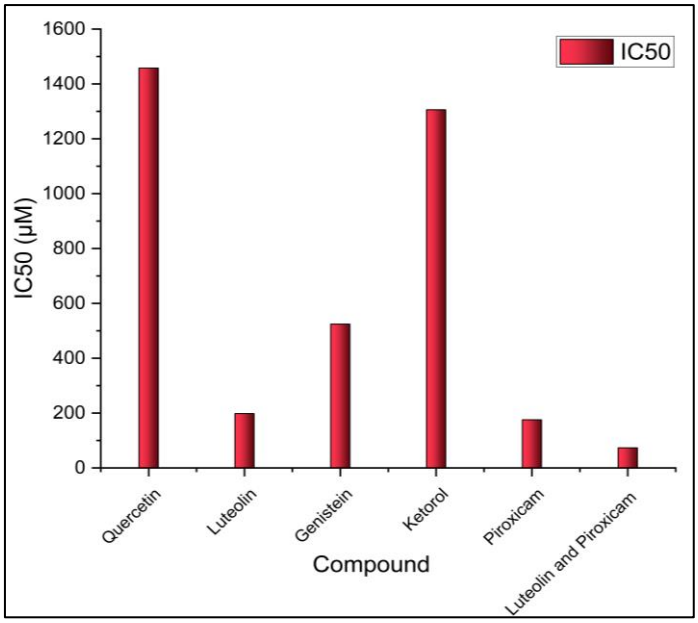

Fig 3: IC50 values of all considered compounds (Flavonoids and NSAIDs) in MTT assay

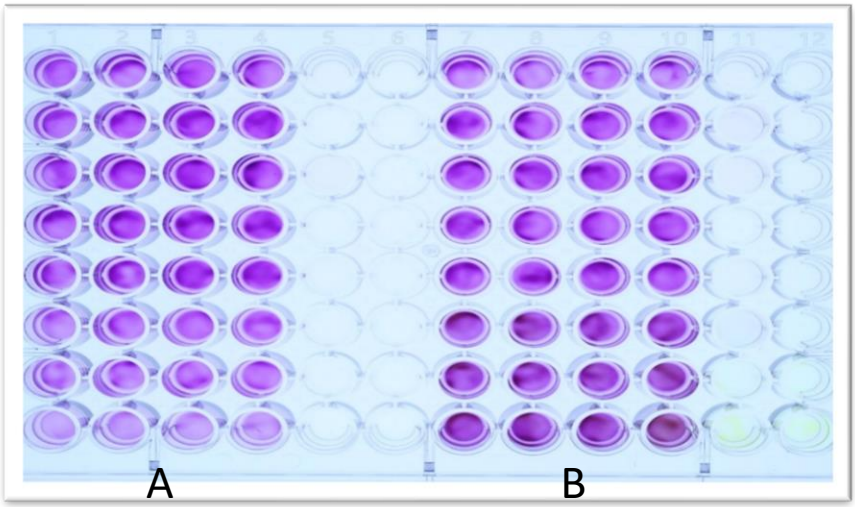

Fig 4: MTT Assay for (A) Luteolin (B) Piroxicam

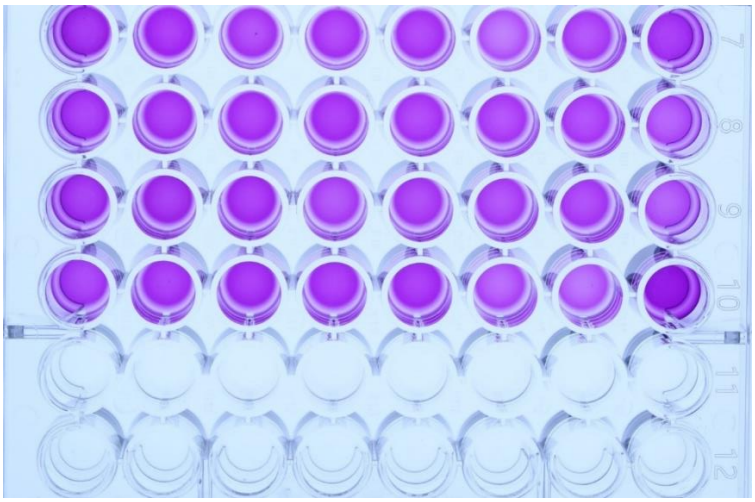

Fig 5: Luteolin-Piroxicam Combination MTT Assay

3.4 ROS Assay

The intracellular ROS levels were estimated in the absence and presence of best flavonoids and NSAIDs, and also in the presence of the best combination of luteolin and piroxicam. The percentage reduction in ROS levels compared to control were evaluated based on the fluorescence recorded (Table 15). Effective reduction in ROS was observed in luteolin and piroxicam individually. This reduction was observed to be amplified when these two compounds were given in combination.

Table 4: ROS reduction efficiency analysis of best flavonoids and NSAIDs

S. No.	Sample Name	ROS reduction (%)
1	Quercetin	20
2	Luteolin	58
3	Genistein	21
4	Ketorol	36
5	Piroxicam	48
6	Luteolin and Piroxicam	73

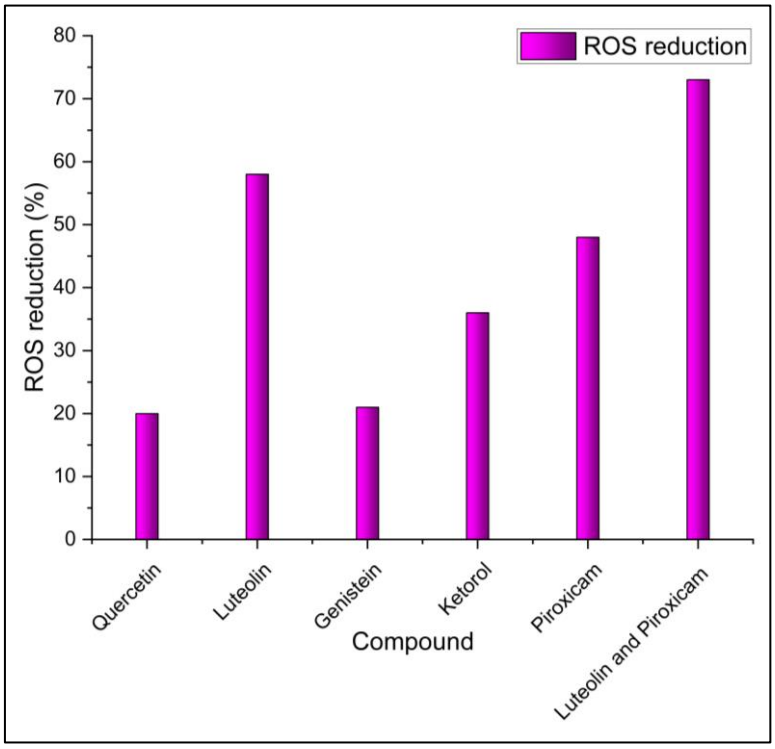

Fig 6: ROS reduction by considered compounds (Flavonoids and NSAIDs)

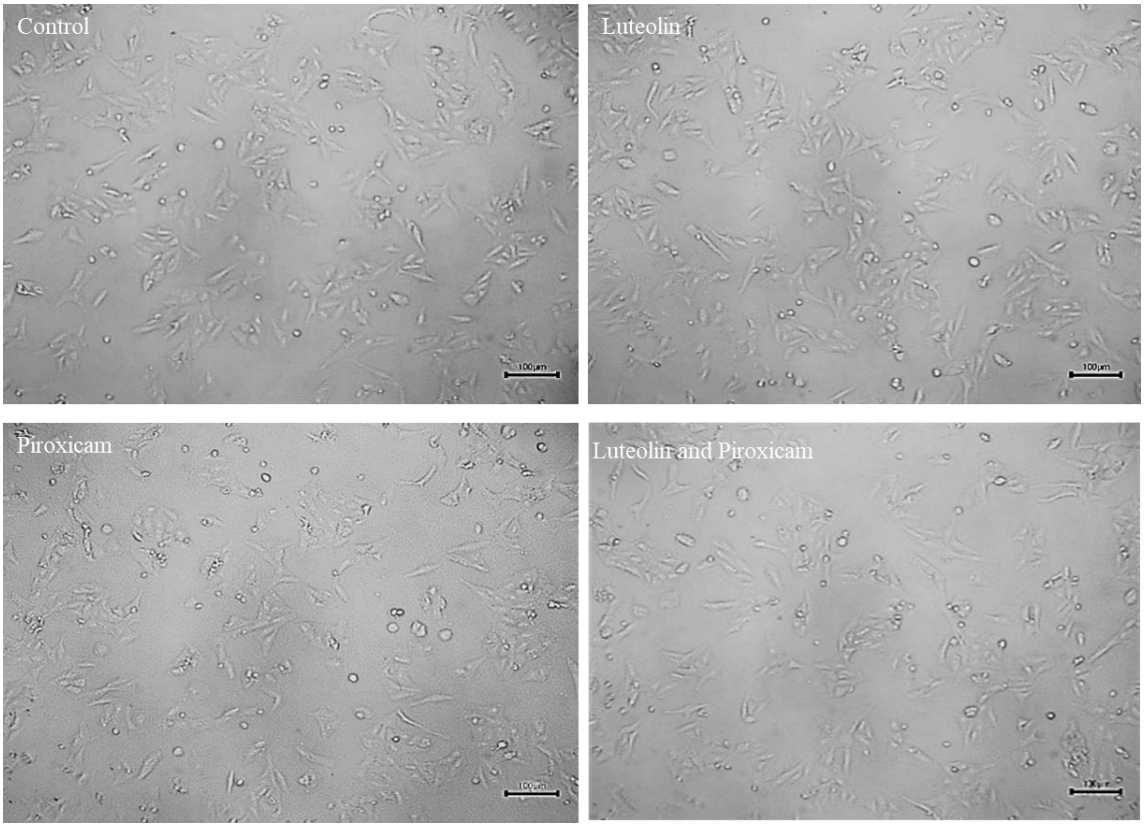

Fig 7: Comparative evaluation of luteolin and piroxicam, treated individually and in combination, on MCF-7 cells, revealing enhanced reduction in cell population with the combination treatment in comparison with control.

4. CONCLUSION

The present investigation demonstrates that the combination of luteolin, a natural flavonoid, and piroxicam, a widely used NSAID, exerts synergistic antioxidant and cytotoxic effects against cancer cell lines. Molecular docking confirmed favorable binding interactions of the luteolin–piroxicam complex with key residues, supporting their strong binding affinity. In vitro assays further revealed that while both compounds individually exhibited significant antioxidant and cytotoxic activities, their combination markedly reduced IC_{50} values, thereby enhancing their overall efficacy. The synergistic reduction in intracellular ROS levels further highlights their ability to modulate oxidative stress, a critical factor in cancer progression. Collectively, these results suggest that the luteolin–piroxicam combination holds considerable promise as a safe, natural, and effective anticancer strategy. However, as this study was limited to in vitro analysis, further in vivo validation and mechanistic studies are essential to fully establish its therapeutic potential and clinical applicability in cancer treatment.

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