Pharmedico Publishers
International Journal of Pharmaceutical Science and Medicine
Volume 2, Issue 1 (2024): 33-36
http://dx.doi.org/

RESEARCH ARTICLE

ISSN: 2584-1610 (Online)

PHYTOCHEMICAL INVESTIGATION OF *CLITORIA TERNATEA* LINN. ROOT EXTRACT

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Article History
Received: 22 November 2023
Revised: 24 December 2023
Accepted: 10 January 2024
Published: 25 March 2024

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ABSTRACT: The present investigation was undertaken to explore the various phytoconstituents of *Clitoria ternatea* Linn. The root extract and its bioactive constituents were also isolated for qualitative tests, unequivocal method was used for detection of carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, proteins, glycosides, saponins, sugars and fixed oils. Carbohydrates, alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids and proteins were qualitatively revealed but, tannins, saponins, sugars and fixed oils were absent. The present results point to a diverse phytochemical composition of the *Clitoria ternatea* root extract, underscoring the therapeutic potential of this plant source. It requires additional research to determine the pharmacological properties, and therapeutic value of these bioactive compounds.

Key Words: *Clitoria ternatea*, phytochemicals, root extract, carbohydrates, flavonoids

I. INTRODUCTION

The Butterfly pea (*Clitoria ternatea*) flower with its eye-catching hues of blue is a botanical wonder growing widely and globally acknowledged for its rich traditional medicinal uses in traditional medicine systems [1]. Although the visual and therapeutic properties of the aerial parts of *Clitoria ternatea* have gained appreciation, the roots of the plant are under investigated, despite their significance in the medicinal history of this plant [2]. Existing literature survey suggests that *Clitoria ternatea* roots are rich in alkaloids, flavonoids, saponins, tannins and terpenoids along with other bioactive compounds [3]. The properties of roots containing characterized compounds are pharmacological including analgesic, anti-inflammatory, antimicrobial, and neuroprotective effects due to the diversity of phytochemicals [4]. The complex interactions of these bioactive elements highlight the importance of the roots in traditional therapies and current pharmacology [5]. For centuries, *Clitoria ternatea* roots have served as alternative medicine as a traditional remedy for stomach issues, coughing, asthma, and nerve conditions. Decoctions, infusions and poultices made from the roots are using since centuries for their presumed aphrodisiac, rejuvenating and remedial elements [6].

Pharmacological research on *Clitoria ternatea* roots has been gathering momentum with the discovery of more functions and the traditional uses beginning to receive scientific validation in contemporary pharmacopoeia [7]. They have shown definitive results in preclinical studies as a chronic disease fighting naturally occuring substance, the effective and traditional monotherapic potential of these roots in treating diseases like cancer, diabetes, and neurodegenerative disorders made their potential appear as a subject of intense modern sciences [8].

This reveals that there are various pharmacological aspects of *Clitoria ternatea* roots that need to be explored further in terms of their bioactive constituents and their mode of therapeutics which might be beneficial for the total therapeutic advancement of medicinal science [9]. This provides the environment for an extensive review of *Clitoria ternatea* roots, emphasizing the phytochemical complexities and therapeutic importance of this lemony flower and calls for further investigations to discover its untapped curative efficacy.

2. METHODS

In this study the methodology was applied for the identification of phytochemical composition and pharmacological activities of *Clitoria ternatea* root.

1. Collection and Authentication of Plant Material: *Clitoria ternatea* root samples were carefully collected from a local park situated near Dinara. MP.

2. Preliminary Phytochemical Analysis:

To predict the chemical reagents that are present in the plant, ethanolic extract was prepared.

(A) Test for Carbohydrates:

Molisch Test: Concentrated hydrogen peroxide and a solution of naphthol in alcohol were added to the ethanolic extract (few drops). The appearance of a violet ring on the interface was a positive result for the presence of carbohydrates [10].

Fehling Test: Fehling A and B solutions were boiled with the test solution. Presence of reducing sugars was inferred by the appearance of a yellow to brick red precipitate [10].

Barfoed's Test: Boiled and treated with Barfoed's Reagent, the appearance of red precipitate represented the quantity of monosaccharides present in the samples [10].

Iodine Test: The test solution was added to diluted iodine solution. The fact that the solution turned blue and the blue color faded upon heating, but re-appeared upon cooling, before finally disappearing confirmed the presence of starch [10].

Tannic Acid Test: A solution of tannic acid (20%) was added to the test mixture. Precipitation indicated the starch [10].

(B) Test for Proteins:

Biuret Test: test solution added with NaOH solution (4%) and $CuSO_4$ solution (1%) The change in color to blue or purple shows proteins are present [11].

Million's Test: The addition of Million's reagent has given white precipitate and the white ppt has turb into brownish red ppt on heating, which confirms the presence of Proteins [11].

Xanthoproteic Test: The test solution turned yellow on heating and orange on addition of NH_4OH solution demonstrating also the presence of tyrosine and tryptophan-containing proteins [11].

Test for Protein containing Sulphur: A solution of100 mg of the test protein was taken, to which 1 ml of lead acetate solution and NaOH solution (40%) was added. Boiling to give PbS suggests the presence of a sulfate-containing protein [11].

Precipitation Test: A drop of the test solution was reacted with $HgCl_2$ solution, $CuSO_4$ solution, and 5% lead acetate solution to check precipitation. The reaction was observed (white colloidal precipitate was formed) indicating the presence of proteins [11].

(C) Test for Amino Acids:

Ninhydrin Test: ninhydrin was heated with solution test. The emergence of a purple or blue hue meant that amino acids were present in the solution [12].

Test for Tyrosine: Addition Million's reagent to the test solution resulted in the solution to turn dark red indicating the presence of tyrosine [12].

Test for Cysteine: Test Solution was boiled with NaOH and lead acetate. The presence of cysteine was verified by formation of a black lead sulphate precipitate [12].

(D) Test for Steroids and Triterpenoids:

Salkowski Reaction: The extract was treated with strong sulphuric acid. If red color of steroid side group is displayed then it is an evidence of steroid presence; and yellow color is visible then it is triterpenoids [13].

Libermann-Burchard Test: The solution was made to react with Acetic anhydride well boiled and kept for cooling. The presence of steroids was shown by a brown ring, whereas the presence of acids was indicated by a deep red hue, when powerful sulfuric acid was applied [13].

(E) Test for Alkaloids: The residue of the ethanolic extract was shaken with dilute HCl and filtered. The filtrate was subjected to the following measures:-

Mayer's Test: An orange-brown precipitate was confirmed which indicated presence of alkaloids [14].

Hager's Test: A precipitate was formed with Hager's reagent which confirmed the presence of alkaloids [14].

Wagner's Test: The presence of alkaloids was verified by the formation of a yellow precipitate using Wagner's reagent [14].

(F) Test for Tannins & Phenolic Compounds:

$FeCl_3$ Test: Deep blue-black colour with addition of 5% $FeCl_3$ solution indicates presence of phenolic compounds [15].

Lead Acetate Test: Its lead acetate test produced white precipitate indicating phenolic nature of the isolates [15].

Acetic Acid Test: A Red color was formed that showed, the presence of phenolic compounds in the sample solution followed by addition of Acetic acid [15].

HNO_3 Test: A few ml of dilute HNO_3 to the test solution to give reddish yellow color, confirm the presence of phenolic compounds [15].

(G) Test for Glycosides:

Legal's Test: Test solution give pink to crimson colour with pyridine and sodium nitroprusside which may be a positive indication for the presence of cardiac glycosides [16].

Keller Killani Test: Add glacial acetic acid, $FeCl_3$ and concentrated HCl created reddish brown colour at the interface *i.e.* deoxy sugar is present [16].

(H) Test for Flavonoids:

Lead Acetate Test: The presence of flavonoids was indicated by the production of a yellow precipitate upon addition of the lead acetate proof [17].

NaOH Test: The yellow colouration obtained with NaOH solution disappeared by addition of acid indicating the presence of flavonoids [17].

(I) Test for Anthraquinone Glycosides:

Borntrager's Test: The test solution was acidified with dilute H_2SO_4, then cooled and filtered. Benzene or chloroform was added, agitated, and the organic layer was separated. The organic layer took on a pinkish-red hue upon adding ammonia, indicating the presence of anthraquinone glycosides [18].

Modified Borntrager's Test for C-glycosides: The test mixture was treated 5—$FeCl_3$ solution and an equal volume of dilute HCl, boiled, cooled, and shaken with benzene or any other organic solvent. The existence of anthraquinone glycosides was confirmed when an organic layer was treated with an ammonia solution, causing it to become pinkish-red [18].

(J) Test for Saponin Glycosides:

Foam Test: A wet moist solution made by shaking the dry powder or test solution. Presence of saponin glycosides were confirmed by the persistence of foam formed [19].

(K) Test for Cyanogenetic Glycosides:

Sodium Picrate Test: A paper strip was impregnated with Picric acid (10 %) Na_2CO_3. They took a conical flask and added the powdered drug and water, and they let the filter paper strip be exposed to the vapours. The presence of cyanogenetic glycosides in the extract was detected when the filter paper turned brick red or maroon color [20].

3. RESULTS AND DISCUSSION

Ethnobotanical uses of *Clitoria ternatea* Linn., preliminary phytochemical analysis of its ethanolic extract. contained the specific presence and absence of different phytochemicals. The results are summarized in the table below:

Table 1: Preliminary Phytochemical Analysis of *Clitoria ternatea* Linn.

Phytochemical Test	*Ethanolic Extract*	*Result (Positive/Negative)*
Carbohydrates	+	Positive
Alkaloids	+	Positive
Flavonoids	+	Positive
Steroids and Triterpenoids	+	Positive
Tannins	-	Negative
Proteins	+	Positive
Glycosides	+	Positive
Saponins	-	Negative
Sugars	-	Negative
Fixed Oils	-	Negative

The roots tested positive for various phytochemicals such as carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, proteins and glycosides whereas tannins, saponins, sugars and fixed oils were absent (Table 1). These phytochemicals may therefore provide some support molecularly for the traditional medicinal uses of *Clitoria ternatea* from which no other known bioactive constituents has been successfully isolated from the plant. Alkaloids and flavonoids are known for their effective antioxidant, anti-inflammatory and antimicrobial activities, and are commonly considered as a few basic bioactive in *Clitoria ternatea* plants seeking to be valuable in caring for different suffering. Its anti-inflammatory activities are modulated by the presence of steroids and triterpenoids, and due to proteins and glycosides, it is nutritional and cardioprotective. The lack of tannins and saponins may lead to a decreased astringency and foaming characteristics, which could affect the specific uses of this plant extract in herbal medicine. Together, these results point to the medicinal property *of Clitoria ternatea* and will aid in driving detailed pharmacological studies to uncover the healing effects of *Clitoria ternatea*.

4. CONCLUSION:

Preliminary phytochemical analysis of *Clitoria ternatea* Linn. roots showed an assortment of biologically active compounds: carbohydrates, alkaloids, flavonols, steroids, terpenoids, and saponins. In this long chain of compounds, yet heretofore no one analyzed exactly what they were used for *Clitoria ternatea* was said to be efficacious against many different diseases. But as you can see here in table one of their appendix, of these compounds. As its active compounds are very numerous, all these reactive substances indicate plant-twin antioxidant and inflammatory, anti-bacterial activities high above what it seems to be given by an outsider listening. The absence of tannins, saponin, sugars or fixed oils better explains the distribution of compounds found inside it than previous analyses had done (see Table 1). In sum, the present work lays a foundation for future research into the possible clinical uses (and potential drugs derived from these constituents) of *Clitoria ternatea* Linn.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to Cite this article:
Yadav M, Kumar GP, Singh G, Kumar S. Phytochemical investigation of *Clitoria ternatea* linn. root extract. International Journal of Pharmaceutical Science and Medicine 2024; 2(1): 33-36.