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Screening of various extracts of flowers of *Clitoria ternatea* L.

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Abstract

Clitoria ternatea L. (Aparajita) family Fabaceae is a herbal remedy used in India. Common applications comprise anti-inflammatory, antipyretic, analgesic, larvicidal, insecticidal, bactericidal, anxiolytic, antidepressant, hepatoprotective, antispasmodic, and sedative characteristics for such plant's root and constituents. Phytochemicals have recently received much attention as bringing new natural antioxidant sources. As a result, the phenolic content, flavonoid content, and saponin content of various extracts of *Clitoria ternatea* L. *flowers* were determined and evaluated in the ongoing study. The Folin-Ciocalteau method was used to evaluate total phenol content, the Aluminum chloride colorimetric method was also used to determine total flavonoid concentration, and the diosgenin spectrophotometric method was being used to determine total saponin content. The current examination found carbohydrates, glycosides, protein, alkaloids, flavonoids, tannin, saponin, and terpenoids. In milligrams of Gallic Acid Equivalent, the amount of total phenol per gram of dry weight was measured (G.A.E.). With 123.16 mg/g of phenol, methanol extract has the highest concentration in roots. The quantity of flavonoids in each gram of dry weight was estimated in milligrams of Rutin Equivalent (RE). The methanol extract would have the highest total flavonoid concentration, measured at 166.67 mg/g. The concentration of saponin in each gram of dry weight was evaluated in mg of Disogenin Equivalent (D.E.). As per the study, the petroleum ether extract had the highest maximum saponin content of 29.989 mg/g. The higher phenolic, flavonoid, and saponin content of the plant's crude extracts showed considerable antioxidant action and required continued evaluation for successful use in both modern and traditional medical systems.

Clitoria ternatea L. is mentioned as a vital herb in all Vedic scriptures. It is a gorgeous twing herb and a common species of flowering plants found everywhere around India, especially in southern India[13,15]. *Clitoria ternatea* is a plant that has a broad range of ethnic medicinal use. The plant has been mostly found in India, Sri Lanka, Malaysia, Burma, and the Philippines. It's a common garden flower found worldwide in tropical and subtropical climates[2,7]. White and blue flowered variants are the two primary varieties based on the colour of the petals.

Fig. 1: Flower of *Clitoria ternatea* L.

Clitoria is claimed to be a good "Medhya" (brain-toning) medication that is primarily used to cure "Masasika" roga (mental disease). Still, it is also helpful in the treatment of hectic fever, acute bronchitis, asthma, and as a snakebite and scorpion sting remedy[4]. The use of dyes and colourants derived from plant extracts as a substitute for synthetic colourants is becoming more popular. As a result, the presence of anthocyanin in *Clitoria* flowers indicates the existence of anthocyanin, which has several *Clitoria* advantages. It's a pretty perennial climber with delicate twining stems that grow up to 2-3 meters tall[1,3,6]. Given the therapeutic value of this widely available plant species, the experiment was planned with the goal of qualitative and quantitative evaluation. The active metabolites in various Clitoria ternatea extracts were also attempted to be identified.

Plant material :

Clitoria ternatea L. flowers were taken in January 2020 from the forest department's Vindhya herbal nursery in Bhopal, M.P. The plants used in the study were properly washed under running tap water and then rinsed in distilled water before being allowed to dry at room temperature. The plant material was then shade dried for 3 to 4 weeks without being contaminated. An electronic grinder was used to grind dried plant material. The aroma, colour, texture, and taste of powdered plant material were all evaluated. Plant material was dried and stored in an airtight container for phytochemical analysis.

Chemical reagents :

All the chemicals used in this study were obtained from Sigma-Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), S.R.L. Pvt. Ltd. (Mumbai, India) and SD Fine-Chem. Ltd. (Mumbai, India). All the chemicals and solvents used in this study were of analytical grade.

Preparation of solvent extract by cold maceration :

The cold maceration method was used to extract plant components. Almost 500 gms

of powder were extracted three times using different organic solvents, petroleum ether, ethyl acetate, and methanol, each for five days. The extract was filtered with Whatman No. 1 filter paper to eliminate any unextractable stuff in the extraction solvent, such as cellular components and other insoluble elements. The extract was transferred to a beaker and evaporated; excess moisture was removed, and the extract was stored in an airtight container until it was utilised. Finally, the dried extracts' percentage yields were calculated[10].

Phytochemical investigation :

A.Qualitative analysis :

Qualitative phytochemical analysis of *Clitoria ternatea* l. flower extract was done and the presence of some phytoconstituents was obtained, then quantitative phytochemical analysis was done[5,8,9,12].

B. Quantitative phytochemical analysis Spectrophotometric Quantification of Total Phenolic Content :

The Folin Ciocalteu reagent was used to determine the quantity of total phenolic in extracts. As a benchmark, gallic acid was utilised, and total phenolic was represented as mg/g gallic acid equivalent (G.A.E.). Gallic acid concentrations of 20-100 mg/ml were produced in methanol. In methanol, plant extracts with concentrations of 0.1 and 1 mg/ml were produced, and 0.5ml of each sample was added to the test and combined with 2.5ml of a 10 fold dilute folinCiocalteu reagent and 2ml of 7.5 percent sodium carbonate. The tubes were covered with parafilm and left at room temperature for 30 minutes before spectrometrically reading the absorbance at 760 nm. All tests were carried out in trplicate. Reduced substances, such as polyphenols, make the folin Ciocalteu reagent sensitive. As a result of the reaction, they turn blue. This blue colour was spectrophotometrically measured. For precision, the experiment was repeated three times, with results represented as mean + standard deviation in terms of phenol content (Gallic acid equivalent, G.A.E.) per gram of dry weight[11,16].

Spectrophotometric quantification of Total Flavonoid content :

Total flavonoids were measured by a colourimetric assay. An aliquot of a diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution and mixed for 6 min before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. The absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g D.W.) through the calibration curve of rutin. All samples were analyzed in three replications.[17,18].

Spectrophotometric Quantification of Total Saponin content :

To prepare the standard curve, 50, 62.5, 75, 87.5, 100, 112.5 and 125.5 µg/ml of the standard saponin solution were placed into test tubes and the volumes were made up to aqueous methanol (80%, 0.25 mL). Standard saponin solution prepared by dissolving 10 mg of diosgenin in a mixture of methanol (16 mL) and distilled water (4 mL). To the aliquots for

each tube, vanillin reagent (8%, 0.25 mL) was added and sulphuric acid (72% v/v, 2.5 mL) was added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60°C water bath. After 10 mins incubation, the tubes were cooled in an ice-cold water bath for 3 – 4 mins. The absorbance was measured at 544 nm against the reagent blank. 0.1 g of freeze-dried sample was dissolved in aqueous methanol (80%, 0.1 mL). 0.25 mL of aliquot was taken for spectrophotometric determination for total saponins at 544 nm [14].

Steroids, tannins, triterpenoids, Glycosides, Flavonoids, Phenolic Compounds, Alkaloids, Saponins, reducing sugar, and carbohydrates were found in the methanol extract but very few phytoconstituents were found in the petroleum ether and ethyl acetate extracts in a qualitative phytochemical analysis of *Clitoria ternatea* L. flower extract. During the screening process, carbohydrates, glycosides, protein, alkaloids, flavonoids, tannin, saponin, and terpenoids all exhibited positive results, indicating that they are responsible for the therapeutic potential of the selected plant's roots. Additional analytical assays for phytochemical ingredient quantification were also performed on the extract.

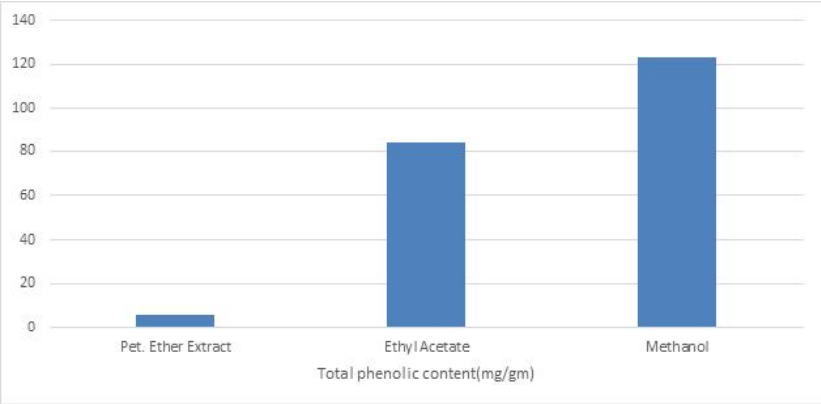
Table-1 shows the phenolic content of *Clitoria ternatea* L. flower. In milligrams of Gallic Acid Equivalent, the amount of total phenol in per gram of dry weight was tested (G.A.E.). Methanol extract of roots has a phenolic content of 123.167 mg/g, which is higher than petroleum ether extract (5.83 mg/g) and ethyl acetate (83.33 mg/g). The flavonoid content of *Clitoria ternatea* L. flowers is shown in Table 2. The amount of flavonoids in each gram of dry weight was determined in milligrams of Rutin Equivalent (RE). Total flavonoid content followed a similar pattern, with methanol extract having the highest value of 166.67mg/g, followed by petroleum ether extract (7.33mg/g) and ethyl acetate (71.0mg/g).

Table-3 shows the saponin content of *Clitoria ternatea* L. flowers. The amount of saponin in each gram of dry weight was measured in mg of Disogenin Equivalent (D.E.). Petroleum ether extract has a saponin concentration of 29.989 mg/gm, which is higher than ethyl acetate (6.889 mg/gm) or methanol (2.00 mg/gm). Graphs 1, 2, and 3 show the phenolic, flavonoid, and saponin content, respectively. It offers the information in a clear and concise manner, making the reading easy to follow. The quantitative estimation of total Saponins, Flavonoids, and Phenols in flower has been discovered, which is useful information for the pharmaceutical industry's drug preparation and emphasises the need for more intensive research in this medicinal plant because the compounds play an important role in healthcare.

Quantitative phytochemical analysis of *Clitoria ternatea* L. flower extract:-

Table-1. Total Phenolic content of *Clitoria ternatea* L. flower Extract

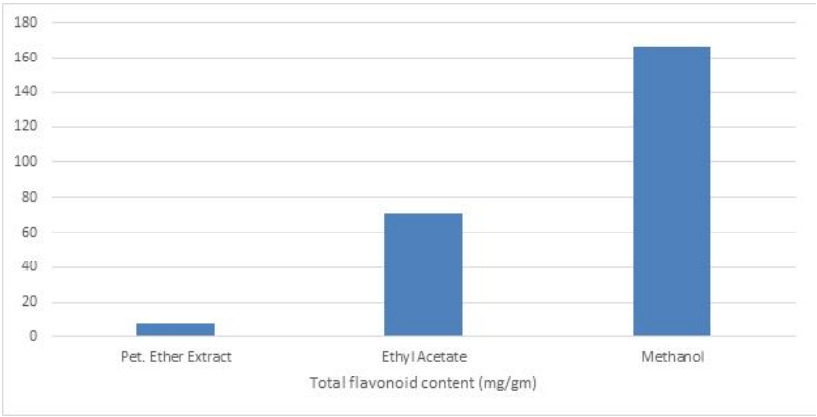
Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)		
	Pet. Ether Extract	Ethyl Acetate	Methanol
Absorbance Mean ± SD	0.093±0.003	0.249±0.001	0.328±0.001
TPC	5.833	83.833	123.167



Graph 1: Total Phenolic content of *Clitoria ternatea* L. flower Extract

Table-2. Total Flavonoid content of *Clitoria ternatea* L. flower Extract

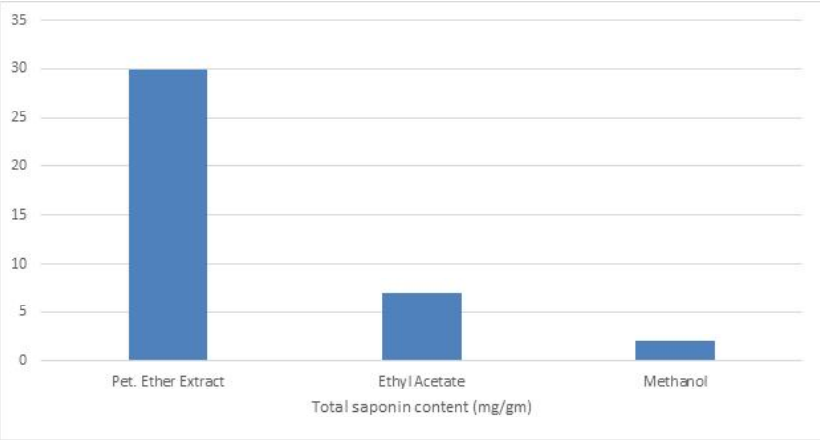
Extracts	Total Flavonoid content (mg/gm equivalent Rutin)		
	Pet. Ether Extract	Ethyl Acetate	Methanol
AbsorbanceMean ± SD	0.099±0.001	0.163±0.001	0.258±0.001
TFC	7.333	71.00	166.667



Graph 2: Total Flavonoid content of *Clitoria ternatea* L. flower Extract

Table-3. Total Saponin content of *Clitoria ternatea* L. flower Extract

Extracts	Total Saponin content (mg/gm equivalent diosgenin)		
	Pet. Ether Extract	Ethyl Acetate	Methanol
AbsorbanceMean ± SD	0.114±0.001	0.045±0.003	0.031±0.001
TSC	29.989	6.889	2.00



Graph 3: Graphical representation of Total saponin content in *Clitoria ternatea* L. flower Extract

The presence of these compounds in the plant validated and supported the use of the flower as natural colours in the food and beverage sector. As a result, these findings revealed that Clitoria flower pigments are naturally present in plants with high anthocyanin output. Furthermore, because the flower can be cultivated throughout the year, it can quickly become a market supply of food colorant. Roots offer a wide range of pharmacological applications due to their high concentration of phenols, flavonoids, and saponins, all of which have diverse effects. This research could be useful as a phytopharmacological technique for standardising Clitoria basic components and finished formulations. Traditional healers, the herbal business, and the general public who ingest Aparajit phytochemicals will benefit greatly from this research.

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