DETECTION AND ESTIMATION OF PHYTOCHEMICALS NECESSARY FOR THE TREATMENT OF GYNECOLOGICAL DISORDERS FROM CLITORIA TERNATEA L. ROOT

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Abstract: In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. According to WHO, up to 80% of the population in the developing countries depend on traditional medicine for their primary health care need. Traditional medicine has been practiced by the traditional healers without knowing the actual phytochemical ingredients present in the plant samples used by them. Phytochemical analysis is the first and foremost step in drug designing. The present investigation was aimed to analyze some of the phytochemicals present in the roots of *Clitoria ternatea* L. The plant *C. ternatea* L. is a perennial legume belongs to the family Fabaceae. The plant has been used to treat some gynecological disorders by the traditional medicine men. Therefore phytochemicals which are related to gynecological disorders were tested both qualitatively and quantitatively. Qualitative analysis was done by thin layer chromatography. Estimation of the phytochemicals was done following standard protocols. The present study detected phytochemicals such as flavonoids, alkaloids, polyphenols and saponins. Estimation of these phytochemicals showed highest amount of polyphenols and lowest amount of saponins. From this analysis it concluded that the plant sample has phytochemicals which are necessary to treat gynecological disorders.

Keywords: Phytochemicals; Clitoria ternatea; gynecological disorders

1. Introduction:

The use of plants by man to treat common ailments is time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various diseases[1]. According to WHO, up to 80% of the population in the developing countries depend on traditional medicine for their primary health care need. Traditional medicine has been practiced by the traditional healers without knowing the actual phytochemical ingredients present in the plant samples used by them. It is imperative that any crude drug for pharmacological or pharmaceutical use needs to be subjected to study for botanical identity. Phytochemical investigation is the first and foremost step in any kind of drug designing. The present investigation was aimed to study some of the phytochemicals present in the roots of the plant *Clitoria ternatea* L. (Plate-1.1). The plant, *Clitoria ternatea* L. belongs to the family Fabaceae (Plate-1.1) is a vigorous, strongly persistent, herbaceous perennial legume. Almost all parts of this plant have been reported to have medicinal properties. Flowers of this plant has been using in a number of Hindu religious purposes since the ancient times. Scientific evaluation of some of the medicinal properties has been investigated by number of workers. Some of these medicinal properties are anthelmintic[2,3,4], anti-hyperglycemic[5,6], anti-diarrheal[7], anti-oxidant[8], hepatoprotective [9,10], immunomodulatory [11], anti-histamic [12]; cholinergic activity [13], anti-cancer [14,15] etc. The medicinal value of this plant is found in the texts of Ayurveda and the plant has been used in many religious purposes in Hindu religion. The plant has been used against many medicinal properties by the traditional medicine men found in India and abroad. The plant has been claimed to have properties for the treatment of infertility, worm infestation, skin disease, tonsillitis, appetizer, digestant, vermicide, cough, asthma etc. It can be assumed that many of the medicinal properties of this plant have not been yet investigated. In many parts of Assam root of this plant has been traditionally used for the treatment of infertility, leucorrhoea and other gynecological disorders. Therefore this study was aimed to investigate the phytochemicals present in the roots of the plant mainly helpful in reproductive functions by both qualitative and quantitative way.

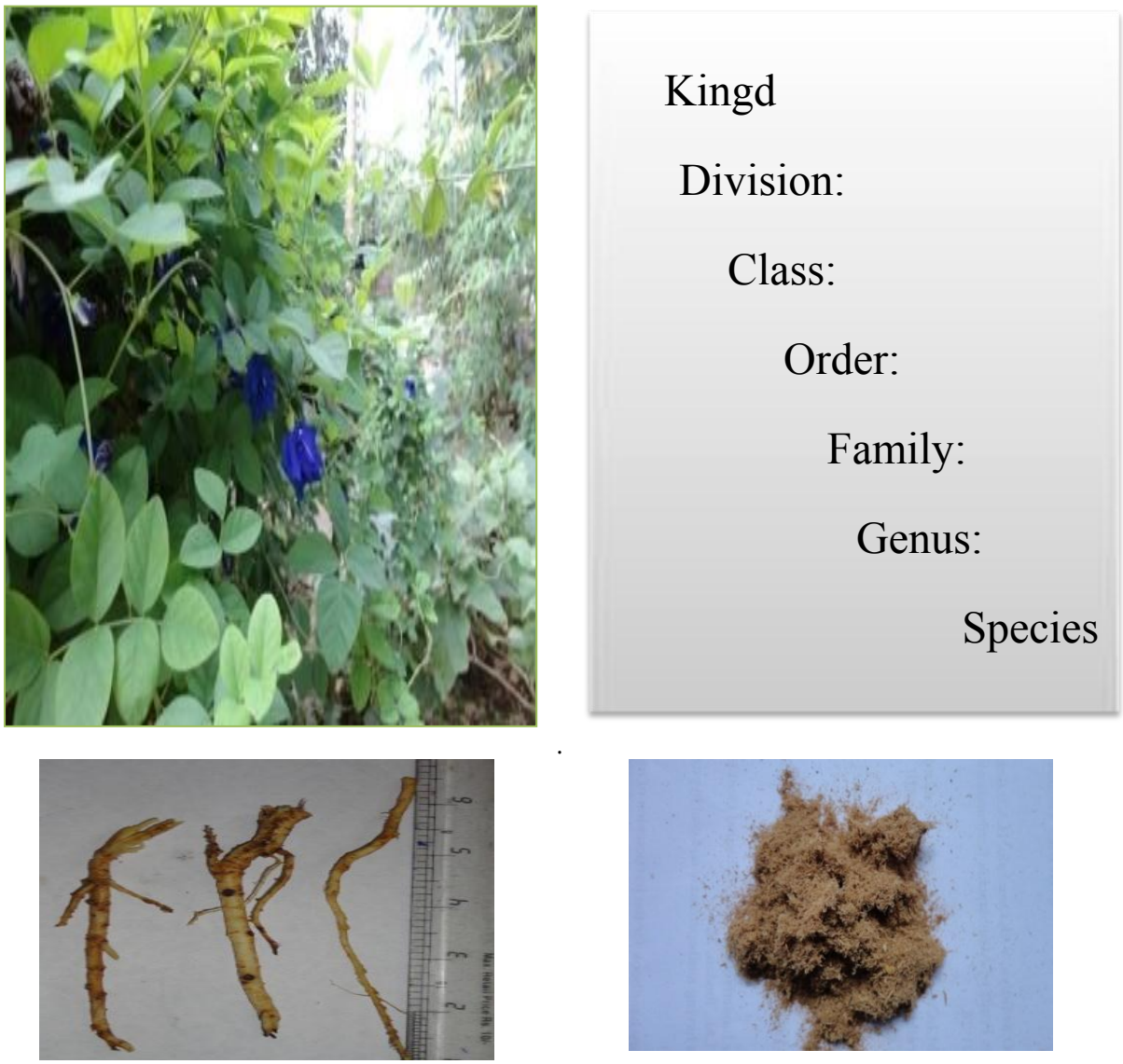

Figure 1.1: Plant species used in the experiment. (Upper left: Whole plant of *Clitoria ternatea* L.. Upper right: Systematic position of *Clitoria ternatea* L. Lower left: Root of the plant. Lower right: Root powder used in the study.)

2. Materials and methods:

2.1. Plant sample collection and identification:

Roots of *Clitoria ternatea* L. were collected from the local household gardens. The roots were washed properly and dried in shed. Dried roots were grinded in a mechanical grinder to make powered form which was used in the present investigation. For scientific identification of the plant, whole plant was collected, prepared herbarium and submitted in the department of Botany, Gauhati University, Assam. A voucher no-09193 was collected against the submitted herbarium for future references.

2.2. Qualitative analysis of phytochemicals:

For qualitative detection of some of the phytochemicals supporting reproductive functions, thin layer chromatography (TLC) was done using standard protocols. TLC was done for the confirmed detection of flavonoids, alkaloids, phenols and saponins as follows:

A. TLC for flavonoid: One gram of root powder was extracted with 10 ml methanol on water bath (60°C/5 min). The filtrate was condensed by evaporation, added a mixture of water and EtOAc (10:1) and mixed thoroughly. The EtOAc phase thus retained is used for chromatography. The flavonoid spots and Rf values were separated using chloroform and methanol (19:1) solvent mixture. The flavonoid spots were recorded under ultraviolet (UV-254 nm) light [16].

B. TLC for alkaloids*:* The powdered root of *C. ternatea* L. was wetted with a half diluted NH_4OH and lixiviated with EtOAc for 24h at room temparature. The organic phase was separated from the acidified filtrate and basified with NH_4OH (p^H 11-12). It was extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using a solvent mixture chloroform and methanol (15:1). The alkaloid spots and Rf values were recorded under visible light after spraying with Dragendorff's reagent [16].

C. TLC for phenols: The powdered root was lixiviated in methanol on rotary shaker (180 thaws/ min) for 24h. The condensed filtrate was used for chromatography. The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The spots and Rf values were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80^oC/10min [17].

D. TLC for saponins*:* Two grams of powdered root was extracted with 10 ml 70% EtOH by refluxing for 10 min. The filtrate is condensed, enriched with saturated *n*-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The spots and Rf values were recorded by exposing chromatogram to the iodine vapour [16].

2.3. Estimation of phytochemicals:

Detected phytochemicals were estimated with standard protocols. Detailed method of estimation of the phytochemicals is as follows:

A. Estimation of total flavonoid: 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was evaporated into dryness over a water bath and weighed to a constant weight [18].

B. Estimation of total alkaloids: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [17].

C. Estimation of total phenols: 2 g of the sample were defatted with 100 ml of diethyl ether using a soxlet apperatus for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm [19].

D. Estimation of total saponin: 20 g of plant powder was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-BuOH was added. The combined n-BuOH extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight; the saponin content was calculated as percentage [20].

3. Results and Discussion:

Phytochemical detection is one of the most important steps in drug designing. As the plant under study is an important Ayurvedic medicine, it is very much essential to know the phytochemical ingredients present in it. In the present study, some of the phytochmicals useful in the reproductive process was targeted and the detailed results of the detection of these phytochemicals are as follows:

3.1. Qualitative analysis of phytochemicals:

In the present study detection of flavonoids, alkaloids, phenols and saponins were done. All these phytochemical groups are important for normal reproductive functions. Results are shown in the plate no-3.1(A-D) and in the Table-3.1. TLC of flavonoids, alkaloids, phenols and saponins showed different Rf values as well as different spots on the TLC plates. Presence of coloured spots on the plates indicated the presence of the phytochemical in the plant material. Different spots were observed for one group of phytochemical indicated the presence of different kinds of plant chemicals from each group. For the detection of flavonoid group, ultraviolet light (UV-254nm) was used as developing agent and 5 coloured bands were observed in UV-detector (plate-3.1A). The alkaloid group was detected with Dragendorff's reagent and 4 coloured bands were observed in visible light (plate-3.1B). For the detection of phenol group, Folin-Ciocalteu's reagent was used and 8 bands were developed (plate-3.1C). The saponin group was detected with Iodine vapour and 5 bands were recorded for this group (plate-3.1D). This study resembles the study of Uma *et al*., 2009[21], who reported the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone in *C. ternatea* L. roots.

Table. 3.1: TLC profile of the detection of various Phytochemicals in *C. ternatea* L. roots. Table shows different developing agents used for different phytochemical groups for the development of spots. Number of spots as bands were recorded for each phytochemical groups, from which Rf values were calculated.

Phytochemical Group	Developing Agent	No of Spots	Rf Values
Flavonoids	Ultraviolet Light (UV254nm)	5	0.33; 0.63; 0.71; 0.79; 0.88
Alkaloids	Dragendorff's Reagent	4	0.74; 0.86; 0.93; 0.98
Phenols	Folin-Ciocalteu's Reagent	8	0.15; 0.2; 0.37; 0.46; 0.53; 0.6; 0.66; 0.8
Saponins	Iodine Vapour	5	0.07; 0.19; 0.21; 0.29; 0.43

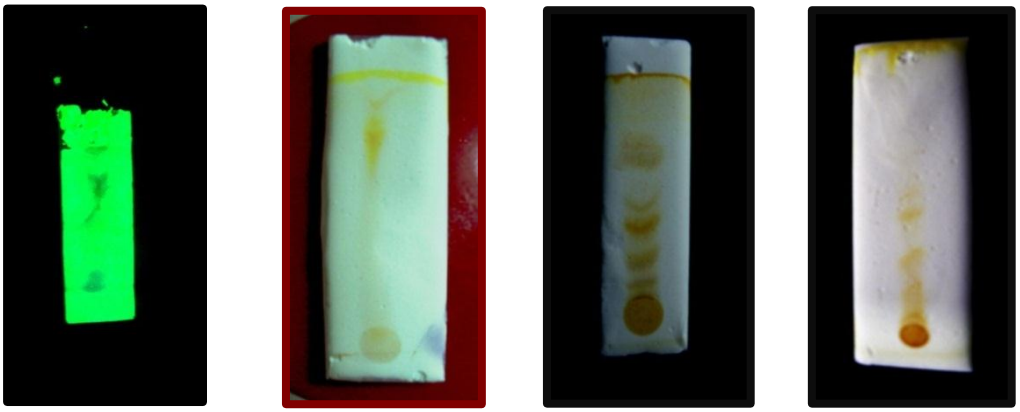

Figure 3.1: TLC spots of different phytochemical groups analysed in the study. Development of different bands on the TLC plates after spraying different developing agents confirmed the presence of particular phytochemical

group. 5 no of spots were recorded for the flavonoid group (A). 4 bandes were recorded for the alkaloid group (B), 8 bands were observed for the phenol group (C) and 5 spots were seen for the saponin group (D).

3.2. Quantitative Analysis Phytochemicals:

Quantitative estimation of the four phytochemical groups, total alkaloid, total flavonoids, saponins and total phenols were done using standard protocols. Results were expressed in mean ± SE. Total % of the alkaloids found was 7.2 ± 0.05, that of flavonoids was 5 ± 0.12, polyphenols was 7.9 ± 0.13 and that of saponins was 0.31 ± 0.14. Results showed highest % of Total polyphenols among all the phytochemicals estimated in the present study. Lowest % of saponins was observed in the study.

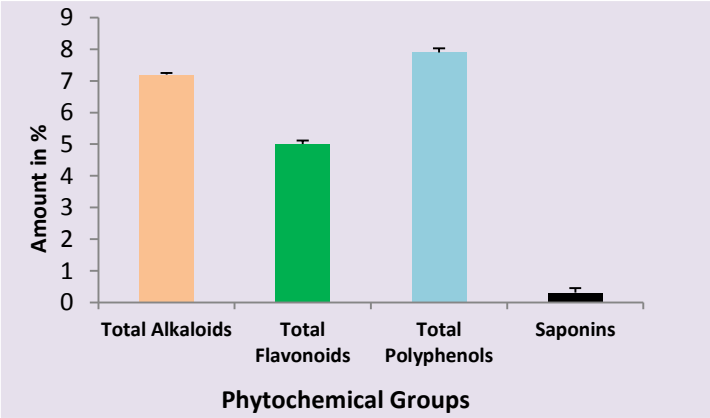

Figure 3.2: Quantitative estimation of phytochemicals. Results were expressed in % from the dried plant sample. Highest amount of polyphenols were recorded among all the groups, while saponin group showed lowest amount.

Alkaloid such as dopamine is used in the treatment of male sexual dysfunctions [22] as well as maintains the hypothalamo-pituitary-ovarian system in aging female rats [23]. Another alkaloid Yohimbine is used as an aphrodisiac. It is helpful in male sexual disorders [24]. These data suggest that alkaloids have some fertility enhancing properties along with its other functions. Flavonoids are low molecular weight bioactive polyphenols and are one type of phytoestrogen. Flavonoid is a group of phytoestrogen which is useful in cases of reproductive abnormalities and many workers have put reports on this group of phytoestrogen to have reproductive effects. Saponins have been shown to have both positive and negative effects on reproduction. Saponins can be used in male infertility and it has been reported to increase sperm number in mice [25]. The negative effects of saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties [26, 27]. All these reports suggest that the phytochemical groups under study have useful in reproductive abnormalities. As already mentioned, the plant has been used as a fertility enhancing medicine, against leucorrhoea, menstrual problems etc. presence of these phytochemicals indicated probable effects of the root of the plant on reproduction.

4. Conclusion:

The present investigation reported the presence of some of the phytochemicals necessary for the treatment of reproductive abnormalities. Estimation of the groups showed different concentrations and highest amount was recorded for the total polyphenols. From this study it can be concluded that the root of the plant is having some phytochemical groups helping for the treatment of some gynecological disorders. Further study is necessary for the confirmation of the medicinal value of the plant for the treatment of gynecological disorders.

5. Abbreviations:

EtOAc:	Ethyl Acetate.
EtOH:	Ethanol.
G:	Gram.
Rf:	Retention factor.
NaOH :	Sodium Hydroxide.

n-BuOH:	n-Butanol.
NH_4OH:	Ammonium Hydroxide.
RT:	Room Temperature.
TLC:	Thin Layer Chromatography

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