RESEARCH ARTICLE:QUALITATIVE AND QUANTITATIVE ESTIMATION AND INORGANIC ELEMENTAL ANALYSIS OF ETHYLACETATE LEAF EXTRACT OF *CLITORIA TERNATEA* LINN

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ABSTRACT

Clitoria ternatea Linn ,commonly known as butterfly pea belongs to the Family Fabacea.It is a medicinal plant possessing many bioactive phytochemical compounds that can be effectively used for the treatment of various ailments.The present study involves the qualitative and quantitative estimation and elemental analysis from ethyl acetate leaf extract of *Clitoria ternatea*.The qualitative test of phytochemical constituents revealed that the leaves of *Clitoria ternatea* containsteroids,flavonoids,terpenoids,phenolic compounds.saponins,tannins,proteins and aminoacids.The quantitative observation showed that the total flavonoids was found 6.28 mg QE/g and total phenol was showed in 41.7 mg GAE/g.These results showed that the leaves had a good quantity of flavonoids and phenols.The inorganic composition of plants was determined using ICP-OES .The amount of Calcium,Copper,Iron,Magnesium and Zinc in plants were determined using ICP-OES.Hence ,the present study is concluded that *Clitoria ternatea* ethyl acetate leaf extract showed significant biological activities and ,that can be used in the pharmaceutical industry as a source of therapeutic agent for new natural herbal medicine.

INTRODUCTION

Plants can synthesize a wide range of bioactive compound that performs significant biological activities.Medicinal plants are used as a valuable source of ingredients that can be used in drug development.The products derived from the plants are one of the vital sources to combat serious disease across the world where traditional medicinal methods play a key role to cover the basic health requirements. The medicinal value of plant lies in the phytochemical constituents of the plant which shows various physiological effects on human body.Therefore,through phytochemical screening one could detect the various important compounds which may be used as the bases of modern drugs for curing various diseases

Clitoria ternatea Linn is rich medicinal with blue and white flowers commonly called Aparajita and Shankhapushpi belongs to the family Fabaceae.it is also known as butterfly pea.It is a perennial climber with slender downy stem,found throughout the tropical regions of the country being cultivated in gardens everywhere and often also found growing over hedges and thickets..Leaves is an important part of plant contains sitosterol,clitorin,kaempferol-3-monoglucoside,kaempferol-3-rutinoside,kaempferol-3-neohesperiodoside etc.*Clitoria ternatea* contains several valuable secondary metabolites which include alkaloids,flavonoids,terpenoids ,tannins,,phenols.This research is aimed at determining the qualitative and quantitative phytochemical components and inorganic elemental analysis in leaf extract of *Clitoria ternatea* as indices of producing plant secondary metabolites which have medicinal values as well as application in industries.

MATERIALS AND METHODS

Plant material

Leaves of *Clitoria ternatea* plant were collected from Orchard botanical arden,pattambi,kerala. .They were authenticated by Dr Ranjusha A P, Department of Botany,NSS College ,ottapalam and were given specimen No.18365

Preparation of extract

The leaves of plant were shade dried and then powdered in mechanical grinder for preparation of extract.The powdered materials of leaves were Soxhlet extracted with ethylacetate for approximately 2 days .The extract was evaporated to dryness.The extract ,on removal of solvent in vaccum ,gave a dark greenish brown semisolid residue/The powdered material or the extracts of the leaves are used for the experimental procedure.

Preliminary phytochemical screening of plant extracts

Preliminary phytochemical screening was done to identify different constituents present in extracts i.e. carbohydrates, proteins, lipids, flavanoid, tannins, glycosides, alkaloids, essential oils etc. All the extracts of *Clitoria ternatea* Linn leaves were subjected to preliminary phytochemical screening.

1. Chemical test of alkaloids

Test	Procedure
a) Mayer's test	2 ml of the extract was treated with 2 ml of Mayer's reagent. Noted for the presence of a creamy precipitate.
b) Hager's test	2 ml of the extract was treated with 1-2 ml of Hager's reagent. Observed for the presence of yellow precipitate.
c) Wagner's test	2 ml of the extract was treated with 1-2 ml of Wagner's reagent. Observed for presence of reddish brown precipitate.

Table No 1: Chemical tests for alkaloids

2. Chemical test of glycosides

Test	Procedure
a) Baljet's test	Mixed 2-3 ml of sample in 2 ml sodium picrate solution. Observe a yellow to orange color.
b) Borntrager's test	To a little quantity of sample solution added water and CHCl3 Separated the organic layer and shaken with dilute ammonia. It was then observed for the development of a deep red color in the lower portion and green color in the upper portion which changed to blue and violet.

Table No 2: Chemical test for glycosides

3. Chemical test of phenolic compounds and tannins

Test	Procedure
a) Ferric chloride test	Mixed 2 ml of the test solution with few ml of 5% ferric chloride solution. Observed for the presence of blue color.
b) Lead acetate test	Mixed 2 ml test solution with 1 ml of lead acetate solution. Noted for the presence of bulky white precipitate.
c) Decolourisation test	The extract dissolved in water was treated with dilute potassium permanganate solution. Noted for the decolourisation of potassium permanganate.

Table No 3: Chemical test for phenolic compounds and tannins

4. Chemical test of flavanones and flavonoids

Test	Procedure
a) Aqueous sodium hydroxide test	Aqueous sodium hydroxide solution was added to the few ml of the extract and the presence of yellow coloration of the solution was noted.
b) Filter paper test	The filter paper was wetted with small quantity of alcoholic solution of the extract. That filter paper was exposed to ammonia vapours and noted the yellow color.

Table No 4: Chemical test for flavanones and flavonoids

5. Chemical test of volatile oil `

Test	Procedure
a) Filter paper test	Filter paper is not permanently stained with volatile oil.

Table No 5:Chemical test for volatile oil and fat

6. Chemical test of carbohydrates

Test	Procedure
a) Molisch's Test.	1 ml of the test solution was mixed with 2 ml of Molisch's reagent, shaken the mixture and added 1 ml of concentrated sulphuric acid along the sides of the test tube. Observed for the presence of violet ring at the junction of two solutions.
b) Fehling's Test	Boiled 1 ml of test solution with 1 ml Fehling's solution A and 1 ml Fehling's solution B on a water bath. Observed for the presence of red residue at the bottom of test tube.
c) Benedict's Test	Mixed 2 ml of the Benedict's reagent with 2 ml of the test solution. Boiled in a water bath. Observed for presence of red precipitate was noted.

Table No 6:Chemical test for carbohydrates

7. Chemical test of proteins and amino acids

Test	Procedure
a) Million's Test	2 ml of the extract was mixed with 2 ml of Million's reagent and boiled. Observed for the presence of white precipitate, which on warming turn into a red colored solution.
b) Ninhydrin Test	The extract (few ml) was treated with two drops of ninhydrin solution and heated on a water bath and then the presence of violet color was noted.

Table No 7:Chemical test for proteins and amino acids

8. Chemical test of terpenoids

Test	Procedure
a) Salkowski's Test	The extract (few ml) was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red color in the chloroform layer and greenish yellow fluorescence in the acid layer.

Table No 8:Chemical test for terpenoids

9. Chemical test of sterols

Test	Procedure
a) Liebermann – Burchard's test	Mixed 2 ml of the test extract with 1 ml of Chloroform and 1 ml acetic anhydride. Then added 1 drop of concentrated sulphuric acid. Observed for the development of a deep red color in the lower portion and green color in the upper portion which changes to blue and violet.
b) Salkowski's Test	The residue was dissolved in chloroform and equal volume of concentrated sulphuric acid was added to it and observed for the red color in the lower layer.

Table No 9:Chemical test for sterols

10. Chemical test of saponin

Test	Procedure
a) Foam or Froth Test	A small quantity of extract was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed

Table No 10:Chemical test for saponins

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Estimation of Total Phenolic Content

Materials required:

Folin-Ciocalteu- phenol reagent, 7 % sodium carbonate solution, Extract, Standard Gallic acid, Deionized water.

Procedure:

The total phenolic content (TPC) of the extract was determined by using Folin-Ciocalteu assay. 1 ml of the sample (1 mg/ml) was mixed with 1 ml of Folin –Ciocalteu phenol reagent. Then 10 ml of 7 % sodium carbonate solution was added to the mixture followed by the addition of 13 ml of deionized water and mixed thoroughly. The mixture was kept in the dark for 90 minutes. After which the absorbance was read at 760 nm. Standard Gallic acid solutions (20,40,60,80 and100µg) were done mentioned as above. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The total phenol content was expressed as µg/ mg of extract.

Estimation of Total Flavanoid Content

Materials required:

Standard Quercetin, Aluminium chloride solution (10 %), Sodium nitrite solution (5 %), Sodium hydroxide solution (1 M), Distilled water, Extract.

Procedure:

The total flavanoid content of the extract was determined by using aluminium chloride colorimetric method. 1 ml of extract was mixed with 4 ml of distilled water in 10ml volumetric

flask. To the flask was added 0.3 ml of sodium nitrite (5 %). After 5 minutes, 0.3 ml aluminium chloride solution (10 %). After 5 minutes, 2 ml of I M sodium hydroxide was added. The volume was made upto10ml with distilled water and mixed. Standard solutions of quercetin (20, 40, 60, 80 and 100 µg) were prepared in the same manner. Absorbance of both test and standard were determined against the blank at 510nm with an UV/visible spectrophotometer. The total flavanoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution. The total flavanoid content was expressed as µg/ mg of extract.

ELEMENTAL ANALYSIS

The plant is found to contain various minerals that are important for several biochemical functions of the body. The plant can be used as a source of important minerals. Mineral elements are inorganic substances and they yield no energy, they are necessary for several biological processes that are essential to life.

The analytical techniques for estimation of elements are based on atomic spectrometry with single element detection. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is advantageous as the technique can estimate many elements at a time. Due of this, ICP-OES is the most widely used analytical technique for elemental determination.

Procedure:

0.1g of sample weighed and digested using con.HNO_3 & H_2O_2 in microwave digester. After digestion, the content was diluted to 25 ml with distilled water. Estimation of elements was performed using Inductively Coupled Plasma with Optical Emission Spectroscope (ICP-OES, Agilent Technologies 700 series, US).The Microwave digested sample was aspirated into ICP-OES to estimate elements viz., Calcium (Ca), Iron (Fe), Zinc (Zn), Magnesium (Mg) and Copper (Cu)

RESULT AND DISCUSSION

Preliminary phytochemical analysis indicated the presence of Flavanoids,steroids,saponoins,Glycosides,tannins,Phenolic compounds,proteins and amino acids,carbohydrates,terpenoids in ethylacetate leaf extract leading to the medicinal properties of plant.

The results of phytochemical screening in ethylacetate extracts of leaf are presented in the table:

Sl. No	Chemical constituents	Ethyl acetate extract
1	Steroids	++
2	Glycosides	+
3	Saponins	+
4	Flavanoids	+ +
5	Tannins	+
6	Phenolic compounds	+
7	Proteins & Amino acids	+
8	Alkaloids	+
9	Carbohydrates	+
10	Terpenoids	+

Note: (++) indicate abundance (+) indicate presence, (-) indicates absence

Table No 11: Qualitative phytochemical analysis of extracts

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Estimation of Total Phenolic Content

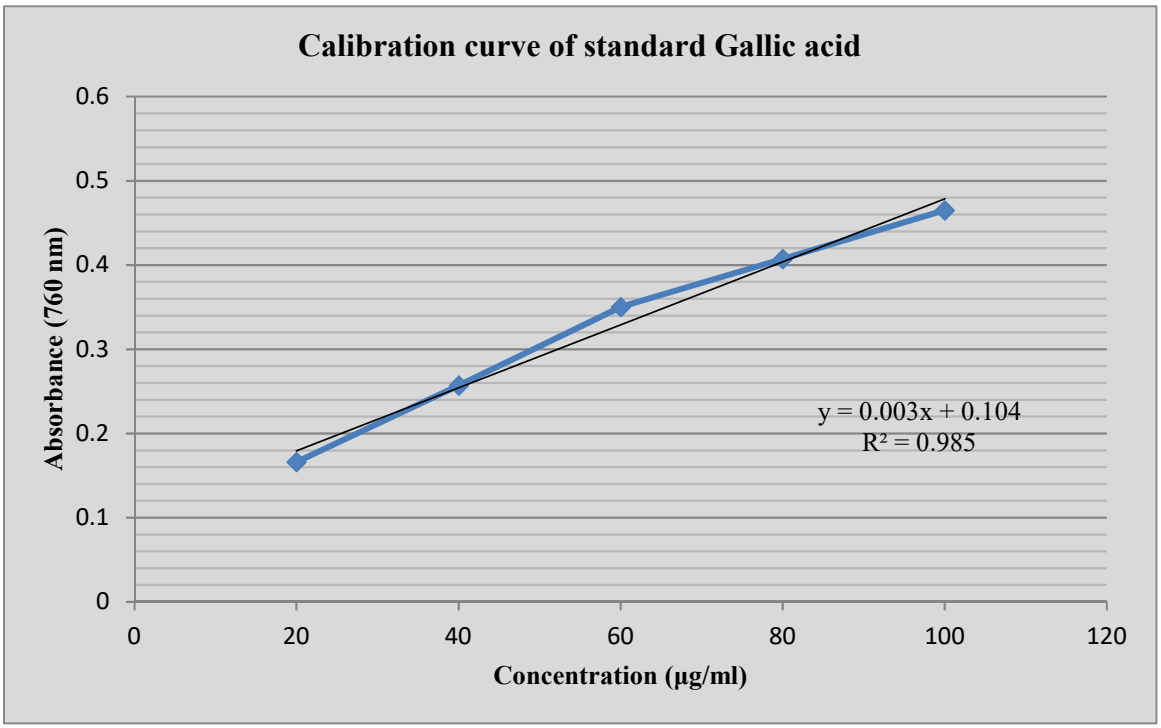
Phenolic compounds are of great importance as cellular support because they form the integral part of cell wall structure by polymeric polyphenols.Bioactive polyphenols protect the human body from the oxidative stress which may cause many diseases including cancer,cardiovascular problems and ageing.

The results of Quantitative estimate of total phenolic contents in ethylacetate extracts of leaf are presented in the table:

Sl.No	Concentration (µg/ml)	Mean absorbance (760nm)
1	20	0.166
2	40	0.257
3	60	0.350
4	80	0.407
5	100	0.465
6	*Clitoria ternatea* Linn ethylacetate extract	0.262

Values were expressed as Mean ± SEM, n=3

Table No : 12 Absorbance of standard gallic acid and extract at 760nm



Graph No 1: Calibration curve of standard gallic acid

Total phenolic content of ethylacetate extract of *Clitoria ternatea* Linn:

Sl. No.	Extract (100 µg/ml)	Concentration of phenolic content in µg/ml of sample
1	Ethyl acetate extract (100 µg/ml)	41.7

Table No 13: Total phenolic content of extract

Estimation of Total Flavanoid Content

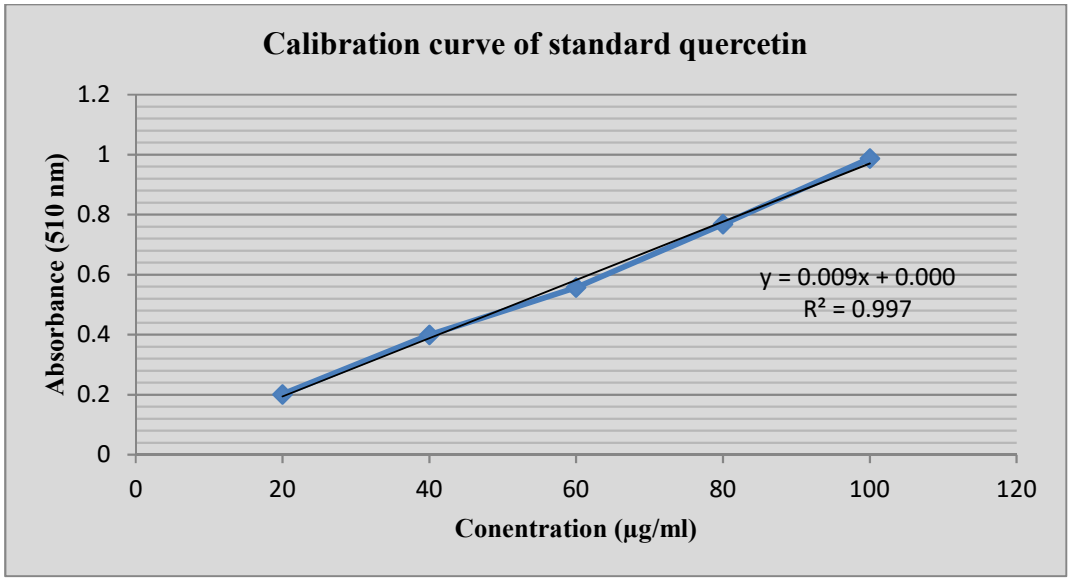
Flavanoids have been reported to possess many useful properties,including anti inflammatory,oestrogenic,enzyme inhibition,anti microbial,anti allergic,antioxidant a cytotoxic anti tumour activity.

The results of Quantitative estimate of total flavonoid content in ethylacetate extracts of leaf are presented in the table:

Sl.No	Concentration (µg/ml)	Mean absorbance (510 nm)
1	20	0.201
2	40	0.399
3	60	0.557
4	80	0.768
5	100	0.987
6	*Clitoria ternatea* Linn ethylacetate extract	0.609

Values were expressed as Mean ± SEM, n=3

Table No 14: Absorbance of standard quercetin and extract at 510nm



Graph No 2: Calibration curve of standard quercetin

Total flavanoid content of ethylacetate extract of *Clitoria ternatea* Linn:

Sl. No.	Extract (100 µg/ml)	Concentration of flavanoid content in µg/ml of sample
1	Ethyl acetate extract (100 µg/ml)	6.28

Table No 15: Total flavanoid content of extract

ELEMENTAL ANALYSIS

The amount of calcium,copper,iron,magnesium,Zinc in plants determined by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

SI.No	Sample (mg/kg)	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Zn (mg/kg)
1	*Clitoria ternatea* Linn	135.925	1.6775	3.77	12.64	0.625

Table No 16: Inorganic Elemental Analysi

CONCLUSION

Clitoria ternatea leaves are a great source of natural bioactive compounds introducing additional health benefits to humans and the prevention of various diseases .*Clitoria ternatea* is a plant with a variety of ethnic medicinl uses.The qualitative analysis of *Clitoria ternatea* shows the presence of bioactive compounds such as steroids,tannins,alkaloids,phenols,proteins and aminoacids,carbohydrates,saponins,glycosides ,saponins and terpenoids.The quantitative estimation of total flavonoids and total phenols in leaves is reported in this study which is very important for the pharmaceutical industry.The amount of inorganic elements like calcium,copper,iron,Magnesium,Zinc in leaf were determined by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).The parameter which are reported here can be considered as enough to identify and decided the authenticity of the more medicinally valuable plant of *Clitoria ternatea* Linn in herbal industries.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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