



**PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT
OF LEAVES OF *CLITORIA TERNATEA* L.**

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ABSTRACT

Medicinal plants are of great importance to the health of individuals and communities. A large number of plants are claimed to possess the anti-diabetic, anti-fertility, anti-hyperlipidaemic, anti-inflammatory, anti-cancer, hepatoprotective and immunomodulatory activities in the traditional therapeutic systems. It is now believed that nature has given the cure of every disease in one way or another. *Clitoria ternatea* a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, goitre, mucous disorders and leprosy. The ethanolic extract of leaves of *C.ternatea* was investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *C.ternatea* acts as a source of therapeutic agent.

KEYWORDS: *Clitoria ternatea*, phytochemical screening, ethanol extract, anti-diabetic.



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INTRODUCTION

Plant medicines were regarded as highly important in the lives of our ancestors since they did not have any alternative therapy. Their dependence on the plants in their surroundings made them to acquire the knowledge about the medicinal properties of many plants by trial and error. They were also aware of the commercial value of these plants. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals which possess protective or disease preventive properties. Some phytochemical studies have been shown to possess antioxidant activities, improving the effects of oxidative stress. They also have complementary and overlapping mechanisms of action in the body, including modulation of detoxifying enzymes, stimulation of the immune system, modulation of hormone mechanism and antibacterial and antiviral effect. Some of the most important phytochemicals includes alkaloids, flavonoids, tannins and phenolic compounds^{1,2,3}. Phytochemicals with biological activity have great utility as pharmaceuticals and pharmacological actions. Many people are aware that eating plant based foods add much needed fiber, vitamins and minerals to the diet but what is less well known is the many benefits of the phytochemicals.

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. Since herbal medicines are prepared from materials of plant origin they are prone to contamination, deterioration and variation in composition. A lot of analytical techniques have been developed for quality control of drugs from plant origin. Therefore it is very important to undertake phytochemical investigations along with biological screening to understand therapeutic dynamics of medicinal plants and also to develop quality parameters. *Clitoria ternatea* Linn (family: Fabaceae) is a perennial twining herb found in India, China, Philippines and Madagascar but

has been introduced to Africa, Australia and America It is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations⁴. It is commonly called "Shankpushpi". In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent⁵. The root extracts are capable of curing whooping cough. It was used traditionally to cure sexual ailments, like infertility and gonorrhoea, to control menstrual discharge, and also as an aphrodisiac⁶. In traditional medicine, *C.ternatea* is used in treatment of various ailments like jaundice, migraine, sore throat, tumors, skin diseases, asthma, fever, urinary tract infections, constipation and indigestion and for central nervous system disorders. Its root extracts are capable of curing whooping cough. This plant was used widely to cure sexual ailments, like infertility and gonorrhoea and to control menstrual discharge. It also acts as an antioxidants⁷. Recent study showed that it has anti-helminthic⁸, anxiolytic, antidepressant, anti-convulsant⁹, anti-pyretic, anti-inflammatory and anti-stress activity¹⁰.

MATERIALS AND METHODS

(i) Collection and authentication of plant material

Fresh leaf of *Clitoria ternatea* was collected in the month of February from SKM Herbal Research Centre, Erode, Tamil Nadu. The plant was identified and authenticated by the taxonomic expert from the department of Botany, V.A.Chidambaranadar College, Tuticorin. A voucher specimen of the herbarium has been deposited at the same department.

(ii) Ash values and extractive values

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs¹¹. Equally important in the evaluation of crude drug, is the determination of ash value and acid insoluble ash value. The total ash is

particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matters such as metallic salts and/or silica¹².

Determination of total ash

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 1.0 g of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105°C for 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25°C. The crucible was allowed to cool in desiccators. The percentage of ash with reference to the air dried substance was then calculated.

Determination of water-soluble ash

The ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash and then the percentage of water soluble ash with reference to the air dried substance was calculated.

Determination of acid-insoluble ash

15 ml of water and 10 ml of hydrochloric acid were taken in the crucible along with the ash and it was covered with a watch glass. It was boiled for 10 minutes, filtered on an ash less filter paper, washed with hot water until the filtrate was neutral, ignited to dull redness, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried substance.

Determination of sulphated ash

1.0 gm of substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1.0 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25°C until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed.

The percentage of sulphated ash with reference to the air-dried substance was then calculated.

Determination of water soluble extractive

To 50 ml of water, 5.0 gm of the substance was added to a stoppered flask and allowed to stand for 10 minutes. 2.0 gm of Kieselghur was added, filtered and 5.0 ml of the filtrate was transferred to a tarred evaporating dish. Solvent was evaporated, dried for 2 hrs and the residue was weighed. Then the percentage of water – soluble extractive was calculated with reference to that of the air-dried substance.

Determination of ethanol soluble extractive

5.0 gm of the substance was macerated with 100 ml of ethanol in a closed flask for 24 hrs and agitated frequently for the first 6 hrs and allowed to stand for 18 hrs. It was filtered and 25 ml of the alcoholic extract was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and the percentage of ethanol – soluble extractive with reference to that of the air-dried substance was calculated.

(iii) Preparation of ethanol extract

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites in to the extracting solvent. The leaves of *C.ternatea* was washed with distilled water and separately dried under shadow for several days. The shade dried leaves were coarsely powdered by mechanical grinder. The dried powdered samples were extracted with 70% ethanol in a soxhlet extractor. Extraction process was continued until the colour of the final drop of the extracts became colourless. The extracts were concentrated in vacuum at 60°C using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50°C for 8 hours. The extracts so obtained, stored in airtight container for further studies.

(iv) Phytochemical analysis

Qualitative screening of ethanol extract of leaf of *C.ternatea* was performed for the identification of various classes of active chemical constituents like alkaloids, reducing sugars, flavonoids, steroids, glycosides,

proteins etc., using different methods^{13,14,15}. Total phenols, tannins and flavonoids were quantitatively measured according to the method^{16,17}. Vitamin C was estimated by the method¹⁸. Total carbohydrate and total protein were determined by the method^{19,20} respectively.

RESULTS

Ash values and extractive values

Dried coarsely powdered crude drug was used for the study of Ash values and extractive values. Results were shown in Table - 1.

Table - 1
Ash values and extractive values of the leaves of *C. ternatea*

S.No.	Physical evaluation	Values obtained (% w/w)
1.	Loss on drying	23.5
2.	Moisture content	11.52
3.	Ash values	
	Total ash	4.18
	Water soluble ash	1.31
	Acid insoluble ash	1.01
	Sulphated ash	6.97
4.	Extractive values	
	Water soluble extractive	20.41
	Alcohol soluble extractive	8.57

Preliminary phytochemical screening

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Analysis of various phytochemical constituents of ethanolic extract of leaf of *C.ternatea* was tabulated in Table - 2.

Table - 2
Preliminary phytochemical screening in ethanolic extract of leaves of *C.ternatea*

S.No.	Phytochemicals	Observation
1.	Alkaloids	+
2.	Flavonoids	+
3.	Free amino acids	+
4.	Glycosides	+
5.	Oils	-
6.	Phenols	+
7.	Proteins	+
8.	Reducing sugars	+
9.	Saponins	-
10.	Steroids	+
11.	Tannins	+
12.	Terpenoids	-

Note: (+) Present; (-) Absent

Quantitative estimation of phytochemicals and nutrients

The quantitative analysis of different phytochemicals and nutrient in ethanolic extract of *C.ternatea* was depicted in Table - 3.

Table - 3
Quantitative estimation of phytochemicals and nutrients in
ethanolic extract of leaves of *C.ternatea*

Parameters	Quantity present
Flavonoids (mg RE/g extract)	20.48 ± 0.96
Tannins (mg TAE/g extract)	78.75 ± 2.09
Total Phenols (mg TAE/g extract)	245.14 ± 6.97
Vitamin C (mg AAE/g extract)	118.83 ± 0.47
Total carbohydrate (mg glucose/g extract)	176.03 ± 1.19
Total protein (mg/g extract)	3110 ± 18.02

Values are means of three independent analysis of the extract ± standard deviation (n = 3).
 RE - Rutin Equivalents; TAE - Tannic Acid Equivalents;
 AAE – Ascorbic Acid Equivalents

DISCUSSION

Medicinal plants are the richest bio-resource for drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs²¹. The total ash value of leaf of *C.ternatea* is 4.18% respectively. The ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. The sample has more water soluble ash than acid insoluble ash. These ash values are generally considered as the index of the purity as well as identity of the drug. Extractive values are useful for the evaluation of phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the active constituents present in a crude drug. Phytochemical study of the leaf extract of *C.ternatea* showed that leaf comprised a wide range of active chemical constituents such as alkaloids, flavonoids, free amino acids, glycosides, phenols, proteins, reducing sugars, steroids and tannins while saponins and oils were absent. HPTLC analysis also confirmed the presence of alkaloids, flavonoids, steroids, glycosides and saponins in the studied plant. These tests are helpful in finding chemical constituents in the plant materials that may

lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound. The quantitative estimation of ethanolic extract of *C.ternatea* found to contain major phytoconstituent total phenols (245.14 ± 6.97 mg TAE/g) relatively high compared to tannins (78.75 ± 2.09 mg TAE/g) and flavonoids (20.48 ± 0.96 mg RE/g). Plant-derived substances have recently become a source of great interest owing to their versatile applications. Recent researches has shown that phenols contribute to the prevention of cardiovascular diseases, cancers, osteoporosis and antioxidant character with potential health and benefits^{22,23,24}. They are also known to have a role in the prevention of neurodegenerative diseases and diabetes mellitus²⁵. In plants, flavonoids serve as protectors against a wide variety of environmental stress while, in humans flavonoids appear to function as “biological response modifiers”. It has been demonstrated to have anti-inflammatory, anti-allergenic, anti-viral, anti-aging and anti-carcinogenic activity^{26,27,28}. Phenols, tannins and flavonoids which may act as antioxidant, anti-microbial, anti-diarrhoeal and anti-helmintic activity²⁹. *C.ternatea* also contains rich amounts of nutrients such as vitamin C (176.03 ± 1.19 mg AAE/g), total proteins (3110 ± 18.02 mg/g) and total carbohydrate (118.83 ±

0.47). Phytochemicals, working together with nutrients, may help to slow the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, diabetes mellitus, high blood pressure,

cataracts, osteoporosis and urinary tract infection³⁰. On the basis of the above results *C.ternatea* could serve as therapeutic agent for various ailments.

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