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# PHYSICOCHEMICAL AND PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF LEAVES OF *CLITORIA TERNATEA* LINN. (FABACEAE)

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## ABSTRACT

Plants have been known to relieve various diseases in Ayurveda. A large number of plants are claimed to possess the anti-cancer, antimicrobials, anti-diabetic and antibiotic properties in the traditional therapeutic systems and also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. *Clitoria ternatea* a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, dropsy, goitre, leprosy, mucous disorders etc., The leaf of *C.ternatea* was investigated for its physicochemical and phytochemical properties and screened for its active chemical ingredients. Ash values - total ash (4.18 % w/w), water soluble ash (98.69 % w/w) and acid insoluble ash (1.01 % w/w) was studied from dry weight of crude drug. For qualitative and quantitative phytochemical screening ethanol extract of *C.ternatea* was prepared and by using conventional identification tests different classes of secondary metabolites were identified. The presence of these secondary metabolites signifies *C.ternatea* as a source of therapeutic agent.

## INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. 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.

Shankpushpi (*Clitoria ternatea* Linn) is a perennial twining herbaceous plant, belonging to the Fabaceae family. It is distributed throughout tropical equatorial Asia and latter was distributed widely in South and Central America, East and West Indies, Bangladesh, China and India, where it has become naturalized[4]. It is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations[5]. In traditional medicine, *C.ternatea* is used in treatment of various ailments like jaundice, migraine, sore throat, tumors, eye infections, 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 aphrodisiac[6]. Recent study showed that it has antihelmintic[7], antistress, anxiolytic, antidepressant, anticonvulsant[8,9], antipyretic, anti-inflammatory and antistress activity[10].

## MATERIALS AND METHODS

### Collection and authentication of plant material

Fresh leaf of *Clitoria ternatea* was collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. The plant was identified and authenticated by the taxonomic expert from the department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

### Experimental Procedure

### Physico-chemical analysis

Shade dried coarse powder of *C.ternatea* was subjected to various physicochemical and phytochemical studies using method described by Ayurvedic Pharmacopeia of India[11].

### Ash values

Ash values are helpful in determining quality & purity of crude drug in powered form.

### 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 powered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105ºC for 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25ºC. 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 450ºC. 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.

**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 60ºC using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50ºC for 8 hours. The extracts so obtained, stored in air tight container for further studies.

**Phytochemical analysis**

Qualitative screening of ethanol extract of *C.ternatea* was performed for the identification of various classes of active chemical constituents like alkaloids, reducing sugars, flavonoids, glycosides, proteins, steroids etc., using different methods[12,13,14]. Total phenols, tannins and flavonoids were quantitatively measured according to the method[15,16]. Vitamin C was estimated by the method[17]. Total carbohydrate and total protein were determined by the method[18,19] respectively.

**RESULTS**

**Physico-chemical analysis**

Dried coarsely powdered crude drug was used for the study of physico-chemical analysis. Results were shown in **Table - 1.**

**TABLE – 1 PHYSICO-CHEMICAL CONSTANTS OF THE LEAVES OF *C. TERNATEA***

| Ash values | Values obtained percentage (% w/w) |
|---|---|
| Total ash | 4.18 |
| Water soluble ash | 98.69 |
| Water insoluble ash | 1.31 |
| Acid soluble ash | 98.99 |
| Acid insoluble ash | 1.01 |

**Qualitative phytochemical screening** Phytochemical parameters are mainly used in judging the purity and quality of the powder drug. Analysis of various phytochemical constituents of ethanolic extract of *C.ternatea* was tabulated in **Table - 2.**

**TABLE – 2 QUALITATIVE 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***

| S.No. | Parameters | Quantity present |
|---|---|---|
| 1. | Flavonoids (mg RE/g extract) | 20.48 ± 0.96 |
| 2. | Tannins (mg TAE/g extract) | 78.75 ± 2.09 |
| 3. | Total Phenols (mg TAE/g extract) | 245.14 ± 6.97 |
| 4. | Total carbohydrate (mg glucose/g extract) | 176.03 ± 1.19 |
| 5. | Total protein (mg/g extract) | 3110 ± 18.02 |
| 6. | Vitamin C (mg AAE/g extract) | 118.83 ± 0.47 |

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 drugs[20].Ash value of a drug gives an idea of the earthy matter or inorganic composition and other impurities present along with the drug. The ash values obtained from the plant tissue (physiological) as well as from extraneous matter (non-physiological). The determination of the physiological ash and non-physiological ash together is called the total ash determination. Total ash may vary within wide limits for specimen of genuine drugs due to the variable natural ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid – insoluble ash which represents most of the ash from the contaminating soil. Any significant deviation in the percentage of ash reported in this work may indicate adulteration or substitution of the 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. 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[21,22,23]. They are also known to have a role in the prevention of neurodegenerative diseases and diabetes mellitus[24]. 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[25,26,27]. Phenols, flavonoids and tannins which may act as antioxidant, antimicrobial, antithelmintic and antidiarrhoeal activity[28].

*C.ternatea* also contains rich amounts of nutrients such as total proteins (3110 ± 18.02 mg/g), total carbohydrate (118.83 ± 0.47) and vitamin C (176.03 ± 1.19 mg AAE/g). 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, osteoporesis, and urinary tract infection[29]. On the basis of the above results *C.ternatea* could serve as therapeutic agent for various ailments.

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