The present paper deals with phytochemical studies in *Clitoria ternatea* Linn. It is commonly known as 'butterfly pea' and 'shankhapushpi'. It is a traditional Ayurvedic medicinal plant belonging to the family Fabaceae. The plant extracts were subjected to phytochemical analysis for screening of medicinal constituents. Valuable data has been collected pertaining to the presence of various phytochemicals like Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols. Further, quantitative estimation of total Flavonoids, Saponins and Phenols was also carried out which has provided information regarding the medicinal potential of the plant.

**Key words:** *Clitoria ternatea*, phytochemical analysis, qualitative analysis, quantitative analysis.

*Clitoria ternatea* Linn. is an attractive perennial climber with conspicuous blue or white flowers. It belongs to the family Fabaceae and commonly known as “butterfly pea” and “shankhapuspi”. It is traditionally used to treat various ailments (Sivarajan and Balachandran 1994, Kokate 1999). The plant is native to south-east Asia and distributed in tropical Asia including India, the Philippines and Madagascar (Anonymous 1998). Roots, seeds and leaves of *C. ternatea* are commonly used in the Ayurvedic system of medicine. Extracts of this plant have been used as an ingredient in the Ayurvedic 'Medhya Rasayana' as a rejuvenating recipe used for treatment of neurological disorders and are considered to enhance the intellect (Sharma and Dash 1988). The whole plant and seed extracts are used for stomatitis, piles, sterility in females, hematemesis, insomnia, epilepsy, psychosis, leucorrhea and polyurea (Yoganarasimhan 2000). The roots are bitter, refrigerant, laxative, intellect-promoting, diuretic, anthelmintic, tonic and are useful in dementia, hemicrania, burning sensations, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy and as a cathartic (Nadkarni 1976). The root, stem and flower are also used for the treatment of snake bite and scorpion sting (Morris 1999). *C. ternatea* has been shown to possess number of pharmacological activities such as possessing anxiolytic, antidepressant, anticonvulsant, antistress (Jain et al. 2003), sedative (Kulkarni et al. 1988), antipyretic, anti-inflammatory, analgesic (Devi et al. 2003, Gomez and Kalamani 2003), Anthelmintic (Salhan et al. 2011) and anti-microbial activities (Kamilla et al. 2009). The extract of *C. ternatea* has been shown to improve learning ability, enhance memory, increase apical and basal dendritic branches, and increase acetylcholine content and acetyl cholinesterase activity in rats (Rai et al. 2001). The plant contains several secondary metabolites such as kaempferol and its glucoside–clitorin, taraxerol and a lactone aparajitin (Barik et al. 2007). Seeds contain - Sistosterol, hexacosanal, and anthoxanthin (Yoganarasimhan 2000).

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and industrial importance (Salhan et al. 2011). Phytochemical analysis of methanol extract of *Clitoria ternatea* roots confirmed the presence of tannins and resins and certain other constituents (Terahara et al. 1996, Uma 2009, Manalisha and Chandra 2011). The present study deals with the phytochemical analysis of different plant parts of *Clitoria ternatea* for the presence of Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols. Quantitative analysis of root extract for total Flavonoids, Saponins and Phenols and shoot, flower and seed extract for total flavonoids was also carried out.
### Table 1. Qualitative analysis of the plant extracts of *Clitoria ternatea* to screen for the presence of phytochemicals.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>Plant parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
</tr>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>2.</td>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Resins</td>
<td>+ve</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Saponins</td>
<td>-ve</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>-ve</td>
</tr>
<tr>
<td>8.</td>
<td>Phenols</td>
<td>-ve</td>
</tr>
</tbody>
</table>

+ Presence of the compound.
- Absence of the compound.

### Table 2. Quantitative analysis of the aqueous extracts of *Clitoria ternatea* for estimation of phytochemicals

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>Plant parts</th>
<th><em>Average Estimated value (mg/gm) (Mean ± S.E)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponins</td>
<td>Roots</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td>Root</td>
<td>45 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Root</td>
<td>110 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>22 ± 02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flower</td>
<td>42 ± 01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seed</td>
<td>19 ± 03</td>
</tr>
</tbody>
</table>

* The value is the average of studies conducted in triplicate.
MATERIALS AND METHODS

*Clitoria ternatea* Linn. plants were collected from Botanical garden, Department of Botany, Osmania University, Hyderabad. The plant parts namely leaves, roots, shoots, flowers and seeds were shade dried and powdered in a mechanical grinder for preparation of extract.

**Preparation of plant extracts**

The powdered plant parts were Soxhlet-extracted with methanol. The extract, on removal of solvent in vacuum, gave a dark greenish brown semisolid residue. The powdered material or the extracts of the plant parts mentioned above were used for the study.

**Qualitative analysis**

It comprised of tests for the presence of Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols.

**Test for Alkaloids**

About 0.5 gm of methanol extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Drangendorff’s and Mayer’s reagent. The treated solution was observed for precipitation of white or creamy colour.

**Test for Tannins:**

Five grams of the ground powder was extracted with 10 ml ammonical chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

**Test for Glycosides:**

About 0.5 gm of methanol extract was taken in a test tube and 1 ml glacial acetic acid containing traces of ferric chloride was added to it. To this solution, 1 ml concentrated sulphuric acid was added and observed for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

**Test for Resins:**

For the tests concerning the presence of Resins, 0.5 gm of methanol extract was taken in a test tube and 5 ml of distilled water was added to it and observed for turbidity which indicates the presence of Resins.

**Test for Steroids:**

About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.
Test for Saponins:
About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Flavonoids:
About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

Test for Phenols:
About 0.5 gm of extract was taken in a test tube, mixed with 100 ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

Quantitative analysis
Quantitative analysis of the root extract was carried out for total Flavonoids, Saponins and Phenols and the shoot, flower and seed extract for total flavonoids. The root extract was prepared as explained above.

Determination of total Flavonoids:
The Aluminium chloride colorimetric method (Chang et al. 2002) with some modifications was used to determine total Flavonoids content. The liquid extract was prepared (with mixing 0.5 gm of root/shoot/flower/seed extract in 100 ml of water) and 1.0 ml of this was mixed with 1.0 ml of methanol, 0.5 ml of aluminium chloride (1.2 %) and 0.5 ml of potassium acetate (0.1176 %). The mixture was allowed to stand for 30 min at room temperature. Later, the absorbance was measured at 415 nm in a spectrophotometer. Quercetin was used as standard; Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

Determination of Saponins:
The method of Obadoni and Ochuko (2001) was used for determination of Saponins. The root extract (20 gm) was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. It was heated over a hot water bath for 4 h 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 separator 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 and 60 ml of n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The content of Saponins was estimated as mg/gm of extracted compound.

Determination of Phenols
The method Gupta et al. (2010) was followed presently. To 5 gm of the root extract in a 250 ml beaker, 200 ml of 10 % acetic acid in ethanol was added, 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 precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue comprising of the phenols was dried, weighted and expressed as mg/gm of extracted compound.

RESULTS AND DISCUSSION
Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and industrial importance (Salhan et al. 2011). The present study contributes
valuable information of bioactive compounds present in *Clitoria ternatea*. Qualitative analysis of plant extract was carried out for Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols. Further, some plant extracts were quantitatively analyzed for Saponins, Flavonoids and Phenols.

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants and animals and some alkaloids have a bitter taste while many are toxic to other organisms (Gupta *et al*. 2010). In the present study, alkaloids are present in shoot, flower and seed extracts, which agrees with Uma (2009) and Chauhan *et al*. (2012) and absent in leaves and shoot extracts (Rao and Savitramma 2011). Tannins and Resins reported in the present study in shoots, leaves, flowers and seeds agrees with the findings of Rao and Savitramma (2011), Uma (2009), Manalisha and Chandra (2011). It was attributed that tannins contributed the property of astringency leading to faster healing of wounds and inflamed mucous membranes (Okwu and Josiah 2006). Whereas Terahara *et al*. (1996), Uma (2009) and Manalisha, and Chandra (2011) reported the presence of Tannins and Resins in the roots, we found that they are absent in roots. Glycoside compounds were present in *C. ternatea* leaf, shoot, flower and seed presently which agrees with Salhan *et al*. (2011). They were however absent in root extracts.

Presently, Steroids were present in leaf which confirms the report of Kamilla *et al*. (2009) and absent in shoot, flower and seed extracts which also confirms the report of Kamilla *et al*. (2009). Saponins were reported in the root of *C. ternatea* presently which agrees with Manalisha and Chandra (2011). Saponins were presently absent in shoot and seed extracts which agrees with Kamilla *et al*. (2009). Traditionally Saponins are extensively used as detergents, pesticides and molluscsicidcs, in addition to their industrial applications as foaming and surface active agents, they also have beneficial health effects (Shi *et al*. 2004). Similar to the present findings, Flavonoids were reported in root extract by Manalisha and Chandra (2011) and in shoot and flower extract by Uma (2009) and seed extract by Kamilla *et al*. (2009). Flavonoids have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic anti-tumour activity (Harbone and Williams 2000). Presently, Phenols were reported in the root extracts of *Clitoria ternatea* and agrees with Gupta *et al*. (2010). Primarily, phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics (Gupta *et al*. 2010). Bioactive polyphenols have attracted special attention because they protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing (Robards *et al*. 1999).

The root extracts were quantitatively analyzed for secondary metabolites like Flavonoids, Saponins, and Phenols. The shoot, flower and seed extracts were quantitatively analyzed for total Flavonoids (Table-2). A high percentage of Flavonoids were observed in the aqueous extract of roots presently (110±0.13 mg/100 gm), and a lower content in shoots, flowers and seeds. Optimum levels of Phenols (45±0.13 mg/100 gm) and Saponins (2.0±0.6 mg/100 gm) are also reported presently in the roots and these results are similar to those of Chauhan *et al*. (2012) and Kaisoon *et al*. (2011).

**CONCLUSION**

It is concluded that *Clitoria ternatea* is a plant with a variety of ethnic medicinal uses. The qualitative analysis of *Clitoria ternatea* shows the presence of bioactive compounds such as Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols.

The quantitative estimation of total Saponins, Flavonoids and Phenols in roots and of Flavonoids in shoots, flowers and seeds is also reported which is very important for the pharmaceutical industry. This is valuable information for preparation of drugs in
pharmaceutical industry and stress the need for more intensive research in this medicinal plant since the compounds play a great role in healthcare.

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