Phytochemical Screening And Ftir Analysis Of Clitoria Ternatea Leaves

Ch. N. Durga Maha Lakshmi*, B. Mahitha*, T. Madhavi* And N. John Sushma**
Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati-517502, A.P., India.

ABSTRACT:
Medicinal plants have served as a constant source of medicaments, which have a great efficacy and demand for the treatment of various diseases. One of the plants, that deserve attention is Clitoria ternatea because of its multipotent bioactive compounds. All parts of Clitoria ternatea roots, leaves and flowers have medicinal properties, leaves are used for hepatoprotective, cytotoxic, antifungal, antihyperglycemic, antihyperlipidemic, antimicrobial, antioxidant activity, etc. The present study was designed to investigate the preliminary phytochemical screening and FTIR analyses for the qualitative identification of bioactive compounds in Clitoria ternatea leaves extract. The phytochemical analyses showed presence of proteins, carbohydrates, glycosides, resins, alkaloids, steroids, tannins, and phenols. The FTIR spectra analyses confirmed the presence of different functional groups with a peak value of Phenols at 3389.57, Alkanes at 2925.41 and 2856.66, Primary amines at 1632.33, Aromatic amines at 1409.06, Carboxylic acids at 1057.61, Primary and Secondary Amines at 869. The presence of these photochemical of leaf extract revealed its medicinal values, Hence C. ternatea leaf methanic extract was concluded to be most effective and essential to discover bioactive natural products that may serve in the development of new pharmaceuticals for natural plant-based medicine.

Key Words: Clitoria ternatea, methanolic extract, phytochemical screening, FTIR spectra.

INTRODUCTION:
Plants are with wide range of medicinal values and has been used as herbal medicines. The history of herbal medicine is almost as old as human civilization. They are in great demand because of their great efficacy in treatment of various diseases without any side effects (Acharyya et al., 2011). Clitoria ternatea is commonly known as butterfly pea, belongs to Fabaceae family. It is a tropical plant, perennial twinning herb bearing blue or white flowers (Gomez and Kalamani, 2003). It is used in ayurvedic medicine because of its multipotent bioactive molecules. Many plant extracts medicinal values lies in some of it’s chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, glycosides and phenolic compounds (Ghasemzadeh et al., 2010).

In India Clitoria ternatea (CT) was traditionally used as medhya rasayan (brain tonic) for neurological disorders during ancient times. This plant extract has potential medicinal values such as anti-helminthic (Khadtakar et al., 2008), antipyretic, anti-inflammatory, antibacterial (Kamilla et al., 2009), analgesic (Devi et al., 2003), anxiolytic, antidepressant, anticonvulsant, sedative, hypoglycemic, anticancer properties (Jain et al., 2003, Sharma et al., 1990). All parts of clitoria ternatea have potential medicinal properties, the root’s are with cathartic, diuretics and has laxative effects, and it’s juice used for chronic bronchitis (Chopra et al., 1956), the roots has been evaluated for the medicinal values like anti-diarrhea. (Shyamkumar and Bhat Ishwar, 2012), anthihistamic, cholinergic activity, etc. The flowers of CT are used for collyrium, anti-inflammatory, analgesic, etc (Shyamkumar and Bhat Ishwar, 2012).

Clitoria ternatea leaves has been traditionally used to relieve joint pain, hectic fever and hepatopathy in Madagascar. The leaves are used for hepatoprotective activity (Jayachitra et al., 2012), and considered as a potential antihelmintic agent (Salhan et al., 2011). The leaves contains antifungal proteins and has been shown to be homologous to plant defenses (Rai et al., 2011). The medicinal values of leaves evaluated such as cytotoxic activity (Shahidur et al., 2006), antifungal, antimicrobial activity (Anand et al., 2011), antioxidant (Balachandar et al., 2013) and nephro-protective activity (Sarumathy et al., 2011), etc.

Fresh flowers of C. ternatea showed hypoglycemic and hypolipidemic effect (Abhishek et al., 2013). The aim of the present study was carried out to investigate the preliminary phytochemical screening and FTIR spectroscopic analysis of methanolic extract of C. ternatea leaves.

MATERIALS AND METHODS:

PLANT MATERIAL:
The Clitorea ternatea plant was collected from the local garden SPMVV, Tirupati and identified for authenticity. For the analysis, Fresh leaves of C. ternatea were collected and washed properly to remove all the dust debris and soil with double distilled water and dried under shade, dust-free condition for one week at room temperature. The leaves were then made powder in a mechanical grinder. The leaf powder was used for further study.

Chemicals Used:
Chemicals were obtained from Himedia, Merck, etc.

Extraction:
The leaf powder was macerated in 60% methanol for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman No.1). The residue will be further extracted using the same procedure. The extract was then filtered and rotary-evaporated at 40°C for...
Preliminary phytochemical analysis:

The methanolic extract of Clitoria ternatea L. leaves were subjected to different tests to identify the nature of bioactive chemical constituents present in the plant material. The crude extracts were screened qualitatively for the phytochemical constituents utilizing standard methods of analysis (Sofowora et al., 1993, Trease et al., 2002).

1) Test for Alkaloids:
5 gm of ground material was added to 10 ml of ammonical chloroform and 5ml of chloroform and the sample was extracted. The mixture was filtered and add few drops of 0.5 M sulphuric acid to the filtrate and shaken vigorously, Creamish white precipitate was observed for the presence of alkaloids.

2) Test for Carbohydrates:
Take diluted leaf extract in a test tube, to it add 2 ml each of Fehling-A and Fehling-B solutions and heated for few minutes and observed for the formation of brick red colour which indicates the presence of carbohydrates in the sample (Fehling’s test).

3) Test for Flavonoids:
To few ml of ethyl acetate add 0.5 g of the macerated sample was added and heated in boiling water. Then the mixture solution was filtered. 4 ml of filtrate was shaken with an ml of 1% aluminium chloride solution and observed for the formation of yellow colour in the presence of 1ml dilute ammonia solution which indicates the presence of flavanoids.

4) Test for Proteins:
1 ml of methanolic extract of C. ternatea was taken and added a few drops of nitric acid to the sides of the test tube very gently. Within few seconds the formation of yellow colour indicates the presence of proteins in the sample (Xanthoprotein test).

5) Test for Resins:
5 ml of distilled water was added to the 3 ml of the methanol extract of C. ternatea and observed for turbidity, which indicates the presence of resins in the present plant sample.

6) Test for Tannins:
   a) 2 g of the plant ground sample was added to 5 ml of 45% ethanol and boiled for 5 minutes. The mixture was cooled and filtrered. Then few drops of lead acetate solution was added to 1 ml of the filtrate. A gelatinous precipitate was observed which indicates the presence of tannins in the plant sample.

7) Test for Saponins:
To the 0.5 g of methanol extract, 5 ml of distilled water was added and the solution was shaken vigorously and observed for persistent froth. Then 3 drops of olive oil was mixed. The formation of an emulsion in the test sample was observed.

8) Test for Steroids:
To methanol extract, 2 ml of acetic anhydride and sulphuric acid were added gently by the side walls of the test tube and the colour change from violet or blue-green was observed, which indicates the presence of steroids.

9) Test for Phenols:
Few drops of methanol extract was mixed with distilled water and gently warmed. To this 2ml ferric chloride solution was added. The formation of green or blue colour indicates the presence of phenols in the leaf extract.

10) Test for Glycosides:
To 0.5ml of methanol extract add 1ml of glacial acetic acid with a trace amount of ferric chloride was added. To this solution 1ml of conc. sulphuric acid was added and in the presence of glycosides, formation of reddish brown colour ring at the junction of 2-layers was observed, upper layer turned in to bluish green colour.

FTIR-Spectroscopic analysis:
A small quantity of C. ternatea methanolic leaves extract was mixed with potassium bromide (kbr) and pellet was prepared and this pellet was analyzed by Bruker Alpha-T Model 109974 FTIR spectroscope. This was used to detect the characteristic peaks and their functional groups.

RESULTS:
The results of the preliminary phytochemical analysis of Clitoria ternatea methanolic leaf extract revealed the presence of different bioactive compounds qualitatively such as proteins, carbohydrates, resins, alkaloids, tannins, steroids, phenols, flavanoids and glycoproteins are shown in table-1. FTIR spectroscopic analyses was first reported on Clitoria ternatea, revealed the presence of different functional groups of the bioactive compounds present in the Clitoria ternatea methanolic leaves extract in the form of peaks. The functional groups were separated based on its peak ratio. The results of FTIR spectra analyses confirmed the presence of phenols with a peak at 3389.57 are corresponded to hydroxyl and CH stretching frequency respectively. The peak at 2925.41 and 2856.66 cm-1 assigned to the C-H stretching which means that some alkane compounds are present in Clitoria ternatea methanolic leaves extract. The peak at 1632.33 confirms Primary amines. The peak value at 1409.06 confirms Aromatic amines. The peak value at 1057.61 confirms Carboxylic acids, the peak value at 926.50 confirms Alkynes, and the peak value at 869.00 confirms Primary and Secondary Amines (figure 1 and table 2).

Table -1: The result of phytochemical analyses of clitoria ternatea methanolic leaves extract.

<table>
<thead>
<tr>
<th>SI no.</th>
<th>Phytochemicals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid</td>
<td>+ ve</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>+ ve</td>
</tr>
</tbody>
</table>
DISCUSSION:

Plants are important source of functional components for the development of new pharmaceutical agents. In the Phytochemical investigation of the methanolic extract of *Clitoria ternatea* revealed the presence of various phytochemicals such as proteins, carbohydrates, glycoside, resins, alkaloid, steroid, tannin, and phenols. The presence of steroidal compounds are important and interest in pharmacy because of their relationship with compounds as sex hormones (Okwu et al., 2001). previous studies described that plant phenols are highly effective free radical scavengers and act as antioxidants (Maijsuthisakul et al., 2007). In Unani and Ayurvedic system of medicine, phytomedicine have been used for the treatment of diseases. The phytochemicals screening serves as the initial step in predicting the types of potential active compounds (Suhumaran et al., 2011). The present studies revealed in the methanolic extract of Clitoria ternatea have higher contents of the phytochemical.

FTIR is one of the most widely used method to identify the chemical constituents and elucidate the compounds (Gopalakrishnan et al., 2012). FTIR allows infrared spectrum simultaneously providing speed and accuracy in measurements of whole range of biological specimens (Griffiths et al., 1986), and has been used as a requisite method to identify medicines in pharmacopoeia of many countries (Liu et al., 2006). When the methanolic extract of *Clitoria ternatea* run under IR region in the range of 400-4000 cm⁻¹ of FTIR Spectroscopy, the presence of different compounds was identified with a variation in the peaks ratio (Kalaiselvi et al., 2012). This was the first FTIR report on *Clitoria ternatea*. Based on the functional group analysis of *Clitoria ternatea* methanolic leaf extract showed the presence of phenolic componds and flavonoids. which can be isolated and further screened for different kinds of biological active compounds and their activities depending on their therapeutic uses.

ACKNOWLEDGMENT:

The authors are highly grateful to SERB-DST for providing financial assistance to this work. The authors are highly thankful to the DST-CURIE, Sri Padmavati Mahila Visvavidyalayam, Tirupati for providing FTIR spectroscope for our study.

REFERENCES :


