Evaluation of phytochemicals in the leaf extract of *Clitoria ternatea* Willd. through GC-MS analysis

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Abstract: *Clitoria ternatea* is a perennial herb of India which is reported to possess several therapeutic properties. It is also found in China, Philippines and Madagascar. It is a vigorous, persistent, herbaceous perennial legume. Most of the plant parts are reported to possess therapeutic properties. In the traditional system of medicine, *Clitoria ternatea* has been utilized for treatment of worm infestation, infertility, skin problems, tonsillitis, cough, asthma traditionally etc. In the present study, fifty percent hydromethanolic extract of leaf of *Clitoria ternatea* (CTE) was prepared and subjected to various biochemical qualitative tests and GC-MS analysis to detect the presence of various phytoconstituents in CTE. Biochemical tests confirmed the presence of various phytochemicals viz., saponins, resins, tannins, flavonoids, alkaloids, glycosides, etc. GC-MS analysis revealed the occurrence of thirty compounds in CTE. The main phyto-composition of *Clitoria ternatea* is predicted to be Butyl-2-methyl-propylphthalate (20.11%), Butyl-2-methyl-pentylphthalate (10.39%), Butyl-octyl-phthalate (11.29%), Diisonylonaphthalate (3.54%) etc., whereas, Butyl-2-ethyl-hexyl-phthalate was major phytoconstituents with 30.19% of total constituents. Thus it could be inferred that the therapeutic potential of CTE is because of different phytochemicals present in the extract prepared.

Keywords: *Clitoria ternatea* - GC-MS analysis - Phytochemicals.

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INTRODUCTION

*Clitoria ternatea* Willd. commonly known as butterfly-pea, blue-pea and cordofan-pea belong to the Fabaceae family. It is a perennial herb found in India, China, Philippines and Madagascar. It is widely found in the humid, low land tropics, occurring naturally as well as in cultivated form (Devi et al. 2003, Gupta et al. 2010). Varieties (white-flower and blue flower) of *C. ternatea* are found in India, China, Madagascar and Philippines. It is popularly called as “Shankpushpi” in India as the flowers of this plant resemble a conch shell (Kulkarni et al. 1988).

*Clitoria ternatea* is widely used as a nerve tonic since ancient time and is believed to promote memory and intelligence (Kulkarni et al. 1988). In Ayurvedic system of medicine, it has been used as a memory enhancer, nootropic, antistress, antidepressant, anticonvulsant, tranquilizing and sedative agent (Jain et al. 2003, Mukherjee et al. 2008). Several studies have been carried out to explore the medicinal properties likes anthelmintic, anti-hyperglycemic, anti-inflammatory, anti-diarrheal, antioxidant, hepatoprotective, Immunomodulatory, anti-histaminic; cholinergic activity of *C. ternatea* (Devi et al. 2003, Chauhan et al. 2012).
Leaves contain sitosterol, kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-neohesperidoside, kaempferol-3-O-rhamnosyl-(1,6)-glucoside, kaempferol-3-O-rhamnosyl-(1,6)-galactoside and kaempferol-3-O-rhamnosyl-(1,2)-O-chalmsnosyl-(1,2)-O-[rhamnosyl-(1,6)]-glucoside. Lactones aparajitin and clitorin from leaves were also reported. The leaves also contain an essential oil, colouring matter and mucilage (Tiwari & Gupta 1959, Rao et al. 2009, Shekhawat & Vijayvergia, 2010, Sarumathy et al. 2011). Keeping in view the mentioned facts, the present study was planned to explore phytochemicals in the fifty percent hydro-methanolic leaf extract of *C. ternatea* (CTE) through biochemical and GC-MS analyses.

**MATERIALS AND METHODS**

**Plant material**

The authentic plant material i.e., leaves of *C. ternatea* were obtained from the Medicinal Plant Research and Development Centre (MRDC), GBPUA&T, Pantnagar, Uttarakhand, India.

**Preparation of Extract of Clitoria ternatea (CTE)**

Leaves were washed properly, shade dried and ground into a fine powder and stored in sterile containers in a cool dry place till further use. Extraction was carried out by using solvents with different polarities. The hydromethanolic extract was prepared as described by Ukwuani et al. (2012). 50 gm of the powder was allowed to soak in 500 ml 50% methanol (v/v) for 48 hours under continuous agitation in a shaking incubator. The mixture was first filtered through muslin cloth, then through Whatmann filter paper No 1. The filtrate was then kept in the rotatory evaporator (45°C). Finally, the extract was obtained by drying the filtrate under hot circulating air at 40°C followed by lyophilization. The percent yield was calculated by dividing quantity of the plant extract obtained from dry leaves powder by 50. The prepared extract was kept at -20°C in air tight container till further use.

**Phytochemical Analyses of CTE**

Qualitative phytochemical tests for the identification of carbohydrates, resins, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides were carried out for 50% hydromethanolic extract of *C. ternatia* leaves (CTE) as per the methods described by Trease & Evans (1983), Harborne (1998), Sazada et al. (2009) and Thakur et al. (2018a, b).

**Characterization of CTE by GC-MS analysis**

The samples were analyzed at the commercial facility of GC-MS analysis available at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, with the following parameters as described earlier by Thakur et al. (2018a, b).

A. Sample preparation: 200 mg of the medicinal plant extract was dissolved in 2 ml of methanol and then filtered through a syringe filter (0.22µ). A finally prepared sample of each extract was loaded in GC-MS column.

B. GC-MS analysis: GC MS analysis was carried out by splitless injection of 1µl of the sample onto Shimadzu QP2010 GC-MS assembly fitted with a column, coupled with a mass detector. Following parameters were used during analysis of an extract of medicinal plants. Column Oven Temperature was set at 100.0°C, the pressure was 175.1 kPa with total Flow of 16.3 ml/min, column flow was 1.21 ml min⁻¹, linear velocity was 28.9 cm sec⁻¹ and purge flow was 3.0 ml/min. Mass detector was set with start time 6.00 min and end time 40.49 min. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and WELLY library as well as TOX Library. Various phytochemicals in the plant extract with the name of the compound along with its molecular weight and structure were determined.

**RESULTS**

**Percent Yield of CTE**

Total of 6.23 g of the hydromethanolic extract was prepared from 50 g of leaves of *Clitoria ternatea* with percent yield of 12.46%.

**Phytochemical analyses of CTE**

As per the biochemical tests conducted, CTE showed the presence of all the tested phytochemicals, viz. carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides (Table 1).

**GC-MS analysis of CTE**

The major phyto-composition of *C. ternatea* is predicted by comparison with Tox library and was found to be Butyl-2-methyl-propylphthalate (20.11%), Butyl-2-methylpropylphthalate (10.39%), Butyllocty-lphthalate
(11.29%), Diisononylphthalate (3.54%) etc., whereas, Butyl-2-ethyl-hexyl-phthalate was major phyto-
constituents with 30.19% of total constituents. Upon comparison with NIST and WELLY library, the major
phyto-constituent of *C. ternatea* was predicted to be 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
with 20.11% among total phyto-constituents (Fig. 1; Table 2, 3).

| S.No. | Phytoconstituents | CTE |
|-------|-------------------|-----|
| 1.    | Protein           | +   |
| 2.    | Carbohydrates     | +   |
| 3.    | Resins            | +   |
| 4.    | Tannins           | +   |
| 5.    | Saponins          | +   |
| 6.    | Flavonoids        | +   |
| 7.    | Alkaloids         | +   |
| 8.    | Steroids          | +   |
| 9.    | Phenols           | +   |
| 10.   | Glycosides        | +   |

**Table 1.** Phytochemicals present in the leaf extract of *Clitoria ternatea* Willd.

**Figure 1.** Chromatogram showing peaks for phytoconstituents in the leaf extract of *Clitoria ternatea* Willd.

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| PEAK | R TIME | AREA | AREA% | NAME | Formula | CAS No | Mol Wt. |
|------|--------|------|-------|------|---------|--------|--------|
| 1    | 10.508 | 31452| 0.21  | Lauric acid ME | C_{17}H_{35}O_{2} | 111-82-0 | 214    |
| 2    | 11.676 | 303199| 20.11 | Butyl-2-methylpropylphthalate | C_{16}H_{20}O_{4} | 17851-53-5 | 278    |
| 3    | 11.913 | 248936| 0.65  | Pentaedcanoic acid ME | C_{19}H_{16}O_{2} | 7132-64-1 | 256    |
| 4    | 12.025 | 156677| 10.39 | Butyl-2-methylpropylphthalate | C_{16}H_{20}O_{4} | 17851-53-5 | 278    |
| 5    | 12.151 | 145092| 0.96  | Decyloctylphthalate | C_{19}H_{18}O_{4} | 119-07-3 | 418    |
| 6    | 12.396 | 4551903| 30.19 | Butyl-2-ethylhexylphthalate | C_{16}H_{20}O_{4} | 85-69-8 | 334    |
| 7    | 12.529 | 1701712| 11.29 | Butylbutylphthalate | C_{16}H_{20}O_{4} | 84-78-6 | 334    |
| 8    | 12.668 | 364432| 0.24  | Disoctylphthalate | C_{22}H_{34}O_{4} | 27554-26-3 | 390    |
| 9    | 12.85 | 38739  | 0.26  | Amfetamineintermediate | C_{6}H_{2}NO_{2} | 705-60-2 | 163    |
| 10   | 12.942 | 178606| 0.18  | Decyltetradecylphthalate | C_{22}H_{34}O_{4} | 0-00-0 | 502    |
| 11   | 12.992 | 44006 | 0.29  | Isopropylbenzene | C_{6}H_{14} | 98-82-8 | 120    |
| 12   | 13.143 | 166239| 0.10  | Diethylphthalate | C_{12}H_{14}O_{4} | 84-66-2 | 222    |
| 13   | 13.233 | 65562 | 0.43  | 3-methylhexane | C_{6}H_{16} | 589-34-4 | 100    |
| 14   | 13.389 | 534008| 0.35  | Diisononylphthalate | C_{16}H_{20}O_{4} | 28553-12-0 | 418    |
| 15   | 13.881 | 235288| 0.56  | Decylhexylphthalate | C_{16}H_{20}O_{4} | 119-07-3 | 418    |
| 16   | 13.486 | 185302| 0.23  | Lignoceric acid ME | C_{22}H_{34}O_{4} | 2442-49-1 | 382    |
| 17   | 13.596 | 657030| 0.46  | Pcc | C_{12}H_{30}N_{2} | 3867-15-0 | 192    |
| 18   | 13.811 | 247004| 0.64  | Cyclotetradecane | C_{12}H_{28} | 295-17-0 | 196    |
| 19   | 13.968 | 491009| 0.26  | Decyltetradecylphthalate | C_{22}H_{34}O_{4} | 0-00-0 | 502    |
| 20   | 14.058 | 209979| 0.33  | Decyldodecylphthalate | C_{18}H_{30}O_{4} | 0-00-0 | 474    |
| 21   | 14.301 | 67108 | 0.45  | 2-methylpentane | C_{6}H_{14} | 107-83-5 | 86    |
| 22   | 14.738 | 177895| 0.18  | Diethylphthalate | C_{12}H_{14}O_{4} | 84-66-2 | 222    |
| 23   | 14.858 | 56097 | 0.37  | Decyldecylphthalate | C_{16}H_{20}O_{4} | -- | 474    |
| 24   | 18.156 | 90780 | 0.60  | Decyloctylphthalate | C_{26}H_{34}O_{4} | 119-07-3 | 418    |
DISCUSSION

Clitoria ternatea L. is a perennial twining herb with several medicinal properties. Various plant parts have different phytochemicals that are responsible for various pharmacological activities. The fatty acid content of Clitoria ternatea seeds includes palmitic, stearic, oleic, linoleic, and linolenic acids as well as a water soluble mucilage, delphinidin 3, 3’, 5’-triglucoside useful as a food dye, beta-sitosterol (Sinha 1960, Deb Nath & Chakravarti 1975, Joshi et al. 1981, Macedo & Xavier-Filho 1992, Husain & Devi 1998). Phytochemistry helps in standardizing the herbal preparations so as to get the optimal concentrations of known active constituents and in preserving their activities. Shekhwat & Vijayvergia (2010) studied the presence of metabolites in various plant parts of C. ternatea. Rai (2010) reported the presence of tannins, flavonoids and steroids in the ethanolic extract of C. ternatea that are known to be the reason for the antioxidative potential of the plants. The salient phytoconstituents present in C. ternatea are pentacetyltriterpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots showed the presence of tannins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone (Trease & Evans 1983). Leaves of C. ternatea are reported to contain beta-sitosterol, 3-rutinoside, 3-neohesperidoside, 3 monoglucoside, 3- o- rhamnosyl Glycoside, kaempferol- 3- o-rhamnosylband essential oils. The flower contains delphinidin-3-, 5-diglucoside, delphinidin-38- glucoside, and malvidin- 3B - glucoside, kaempherol, p-coumaricacid. Rootcontains ß-carotene, stigmast-4- ene- 3, 6, diene, taraxerol & taraxerone, starch, tannins & resins (Tiwari & Gupta 1959). The present study also reports the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides in CTE.

For many chronic and degenerative diseases, oxidative stress is considered one of the leading cause (Vadalapudi & Naid 2010). Petals of C. ternatea have been reported to exhibit potent anti-oxidant activity (Kankanon et al. 1999, Shan et al. 2005, Hinneburg et al. 2006). Aqueous extracts of petals showed stronger anti-oxidant activity in comparison to ethanolic extracts (Kamkaen & Wilkinson 2009). Aqueous leaf extracts of C. ternatea was subjected to various enzymatic and non-enzymatic antioxidative analyses to explore their antioxidant potential. In vitro antioxidant capacity was also determined using different assays such as Ferric reducing power assay (FRAP), Reducing activity assay, diphenypicrylhydrazyl (DPPH) assay and Hydroxyl radical scavenging activity. C. ternatea has shown significant antioxidative properties which were found to be comparable with standard antioxidants used in the study (Rao et al. 2009). Several workers reported its medicinal value such as anti-inflammatory (Devi et al. 2003), anti-oxidant (Sarumathy et al. 2011), immunomodulatory, hypoprotective (Daisy et al. 2004, Solanki & Jain 2011) etc.

Sarumathy et al. (2011) prepared an ethanolic extract of the aerial part of C. ternatea and subjected it to GC-MS analysis. Seven compounds were identified in this plant by GC-MS viz., n-hexadecanoic acid (48.77), 1-butanol, 3-methyl-acetate (30.27), propane, 1,1,3-trithoxy-( 3.92), Z, Z, Z-1, 4, 6, 9-nonadecatraene (4.60), undecanoic acid (2.80), 3-trifluoroacetoxy pentadecane (3.59) and 4-ethyl - 5-octyl- 2, 2- bis(trifluoromethyl) -cisc 1, 3 - dioxalane - (6.05) through coupled GC-mass spectroscopy. In the present study, a complex mixture of 24 different compounds was detected through GC-MS analysis in CTE. Butyl-2-methyl-propylphthalate (20.11%), Butyl-2-methylpropylphthalate (10.39%), Butyloxy-tpbthlate (11.29%), Diisononylphthalate (3.54%) and Butyl-2-ethyl-hexyl-phthalate (30.19%) were found to be the major compounds in CTE of total constituents. Thus it could be inferred from the present study that the presence of various phytochemicals as revealed through biochemical and GC-MS analyses may be responsible for antioxidative and medicinal potential of C. ternatea leaf extract. However, the advanced analysis is required to further harness the medicinal potential of C. ternatea.

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