



## Research article

## 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-methylpropylphthalate (10.39%), Butyloctyl-phthalate (11.29%), Diisononylphthalate (3.54%) etc., whereas, Butyl-2-ethyl-hexyl-phthalate was major phyto-constituents 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 nervine 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 like 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-chalmnosyl-(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. ternatea* 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<sup>-1</sup>, linear velocity was 28.9 cm sec<sup>-1</sup> and purge flow was 3.0 ml/min. Mass detector was set with start time 6.00 min and end time 40.49min. 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).

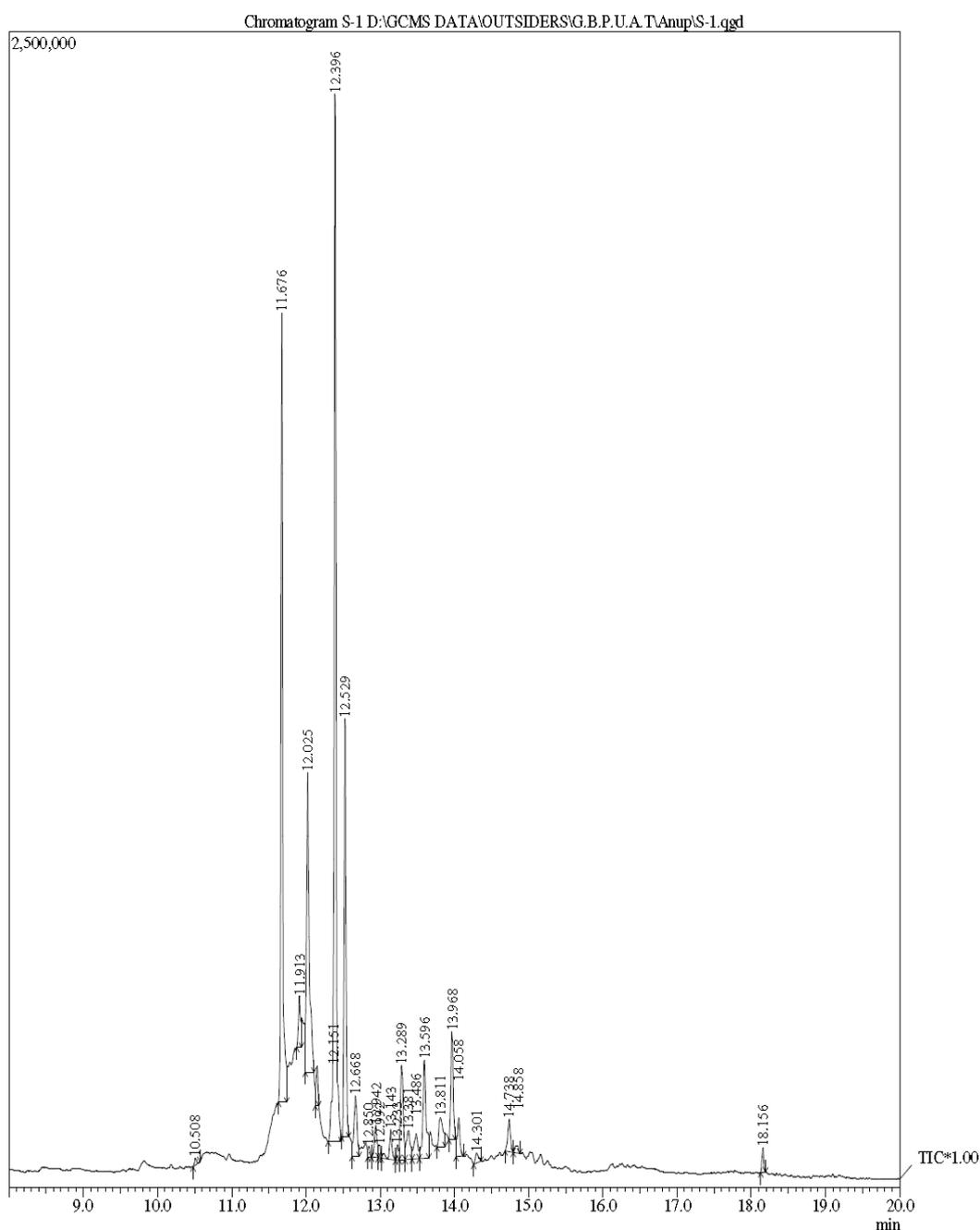
### *GC-MS analysis of CTE*

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%), Butyloctyl-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).

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

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



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

**Table 2.** Phyto-constituents present in the leaf extract of *Clitoria ternatea* after determining the retention time peaks with TOX (PERFUMERY and DRUG) Library. [CAS- Chemical Abstracts Service]

PEAK	R TIME	AREA	AREA%	NAME	Formula	CAS No	Mol. Wt.
1	10.508	31452	00.21	Lauric acid ME	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	111-82-0	214
2	11.676	3031299	20.11	Butyl-2-methylpropylphthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	17851-53-5	278
3	11.913	248936	01.65	Pentadecanoic acid ME	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	7132-64-1	256
4	12.025	1566771	10.39	Butyl-2-methylpropylphthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	17851-53-5	278
5	12.151	145092	00.96	Decyloctylphthalate	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	119-07-3	418
6	12.396	4551903	30.19	Butyl-2-ethylhexylphthalate	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	85-69-8	334
7	12.529	1701712	11.29	Butyloctylphthalate	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	84-78-6	334
8	12.668	364432	02.42	Diisooctylphthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	27554-26-3	390
9	12.85	38739	00.26	Amfetamineintermediate	C <sub>9</sub> H <sub>9</sub> NO <sub>2</sub>	705-60-2	163
10	12.942	178606	01.18	Decyltetradecylphthalate	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	0-00-0	502
11	12.992	44006	00.29	Isopropylbenzene	C <sub>9</sub> H <sub>12</sub>	98-82-8	120
12	13.143	166239	01.10	Diethylphthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	84-66-2	222
13	13.233	65562	00.43	3-methylhexane	C <sub>7</sub> H <sub>16</sub>	589-34-4	100
14	13.289	534008	03.54	Diisononylphthalate	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	28553-12-0	418
15	13.381	235288	01.56	Decyloctylphthalate	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	119-07-3	418
16	13.486	185302	01.23	Lignoceric acid ME	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub>	2442-49-1	382
17	13.596	657030	04.36	Pcc	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub>	3867-15-0	192
18	13.811	247004	01.64	Cyclotetradecane	C <sub>14</sub> H <sub>28</sub>	295-17-0	196
19	13.968	491009	03.26	Decyltetradecylphthalate	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	0-00-0	502
20	14.058	200979	01.33	Decyldodecylphthalate	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	0-00-0	474
21	14.301	67108	00.45	2-methylpentane	C <sub>6</sub> H <sub>14</sub>	107-83-5	86
22	14.738	177895	01.18	Diethylphthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	84-66-2	222
23	14.858	56097	00.37	Decyldodecylphthalate	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	--	474
24	18.156	90780	00.60	Decyloctylphthalate	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	119-07-3	418
		15077249	100.00				

**Table 3.** Phyto-constituents present in the leaf extract of *Clitoria ternatea* after determining the retention time peaks with NYST and WELLEY Library. [CAS- Chemical Abstracts Service]

PEAK	R TIME	AREA	AREA%	Name	Formula	CAS No	Mol. Wt.
1	10.508	31452	0.21	Dodecanoic Acid, Methyl Ester	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	111-82-0	214
2	11.676	3031299	20.11	1,2-Benzenedicarboxylic Acid, Bis (2-Methylpropyl) Ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-69-5	278
3	11.913	248936	1.65	Octadecanoic Acid, Methyl Ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	112-61-8	298
4	12.025	1566771	10.39	1,2-Benzenedicarboxylic Acid, Dibutyl Ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-74-2	278
5	12.151	145092	0.96	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-69-5	278
6	12.396	4551903	30.19	1,2-Benzenedicarboxylic Acid, Butyl 2-Ethylhexyl Ester12652777	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	85-69-8	334
7	12.529	1701712	11.29	1,2-Benzenedicarboxylic Acid, Butyl Decyl Ester	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>	89-19-0	362
8	12.668	364432	2.42	2-(((2-Ethylhexyl) Oxy) Carbonyl) Benzoic Acid	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	4376-20-9	278
9	12.85	38739	0.26	1-Penten-4-Yn-3-OL, 1-Chloro-3-Ethyl	C <sub>7</sub> H <sub>9</sub> ClO	113-18-8	144
10	12.942	178606	1.18	Di-Isopentylphthalate	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	0-00-0	306
11	12.992	44006	0.29	2-Propanone, 1,1-Dichloro-	C <sub>3</sub> H <sub>4</sub> Cl <sub>2</sub> O	513-88-2	126
12	13.143	166239	1.1	Phthalic Acid, 4-Cyanophenyl Nonyl Ester	C <sub>24</sub> H <sub>27</sub> NO <sub>4</sub>	0-00-0	393
13	13.233	65562	0.43	Phthalic Acid, Bis(7-Methyloctyl) Ester	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	20548-62-3	418
14	13.289	534008	3.54	1,2-Benzenedicarboxylic Acid, Mono (2-Ethylhexyl) Ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	4376-20-9	278
15	13.381	235288	1.56	1,2-Benzenedicarboxylic Acid, Diundecyl Ester	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	3648-20-2	474
16	13.486	185302	1.23	Hexanoic Acid, 2-Methyl126532777	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	4536-23-6	130
17	13.596	657030	4.36	1,2-Benzenedicarboxylic Acid, Dioctyl Ester	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	117-84-0	390
18	13.811	247004	1.64	9-Octadecenoic Acid (Z)	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	112-80-1	282
19	13.968	491009	3.26	1,2-Benzenedicarboxylic Acid, Dipentyl Ester	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	131-18-0	306
20	14.058	200979	1.33	Di-Hexylphthalate	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>		334
21	14.301	67108	0.45	Phthalic Acid, 4-Cyanophenyl Nonyl Ester	C <sub>24</sub> H <sub>27</sub> NO <sub>4</sub>		3087
22	14.738	177895	1.18	1,2-Benzenedicarboxylic Acid, Butyl Octyl Ester	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	84-78-6	334
23	14.858	56097	0.37	1,2-Benzenedicarboxylic Acid, Diundecyl Ester	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	3648-20-2	474
24	18.156	90780	0.6	Di-N-Octyl Phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	117-84-0	2832
		15077249	100				

## 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, Debnath & 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. Shekhawat & 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 pentacyclitriterpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots showed the presence of ternatins, 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-rhamnosyl and essential oils. The flower contains delphinidin-3, 5-diglucoside, delphinidin-3 $\beta$ - glucoside, and malvidin- 3 $\beta$  - glucoside, kaempferol, p-coumaric acid. Root contains  $\beta$ -carotene, stigmasterol- 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 (Vadlapudi & Naid 2010). Petals of *C. ternatea* have been reported to exhibit potent anti-oxidant activity (Kankonen *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, diphenylpicrylhydrazyl (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-triethoxy-( 3.92), Z, Z, Z-1, 4, 6, 9-nonadecatetraene (4.60), undecanoic acid (2.80), 3-trifluoroacetoxy pentadecane (3.59) and 4-ethyl - 5-octyl- 2, 2- bis(trifluoromethyl) - cis 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%), Butyloctyl-phthalate (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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