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Letter to the Editor

Phytochemical characterization and cancer cell line cytotoxicity of *Clitoria ternatea*

Sir,

Ayurveda, a prominent traditional system, is using seed, leaf and root of *Clitoria ternatea* for antidiabetic, anti-inflammatory, anti-depressant, cardiovascular disease, urinary problems, hepatopathy, ulcer; as local anesthesia and also to act against laryngeal, colon and breast carcinoma (Jain and Shukla, 2011). *C. ternatea* is an indigenous plant to the South East Asia. Its phyto-constituents having bioactivities are previously reported as alkaloids (ethyl D-galactopyranoside), triterpenoids like taraxerol and taraxerone from leaf (Singh and Tiwari, 2010); phenols like 3,5,7,4-tetrahydroxyflavone-3-rhamoglycoside, syringic, epicatechin, gallic acid; flavonoids like flavonol glycosides (rutin), kaempferol glycosides (kaempferol-3-neohesperidoside), p-coumaric, rosmarinic, chlorogenic, apigenin, caffeic, ferulic, cinnamic acids and hydroxycinnamic acid derivatives; phytosterol such as β-sitosterol and campesterol from seed are considered valuable. Likewise, the hydrophilic seed extract cumulatively having these compounds were evaluated to show 92.8% cytotoxic to Hep2 laryngeal carcinoma cell line at 250 µg (Hamedi et al., 2014).

The fresh, tender, healthy leaf and fruit of *C. ternatea* were screened for the anti-oxidant, antibacterial and cytotoxic efficiency by various solvent extractions. The collected sample were authenticated and provided by Dr. N. M. Ganesh Babu, Foundation of Revitalization of Local Health Traditions, Bangalore. The plant metabolites were extracted using five different polarity-based solvents by soxhlet method to derive all types of available compounds in these particular plant parts. In the phytochemicals investigation, the primary and secondary metabolites were recognized (Table I).

An UV analysis was carried out from 800-200 nm using

Table I

Identification of phytochemicals from *Clitoria ternatea* extracts by various standard tests

	Petroleum ether		Chloroform		Ethyl acetate		Methanol		Aqueous	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
Carbohydrate										
Fehling's test	+	+	+	+	+	+	+	+	+	+
Molisch's test	-	+	+	+	-	-	+	+	+	-
Protein										
Ninhydrin	-	+	-	-	-	+	-	+	-	+
Biuret	-	-	+	+	-	-	-	-	-	+
Phenols										
Ferric chloride	-	-	-	-	+	-	-	-	-	+
Lead acetate	-	+	-	-	-	-	-	+	+	+
Flavonoid	-	+	+	+	+	-	-	+	+	-
Glycoside										
Salkowski's test	+	+	-	-	+	+	-	-	-	-
Keller Kilani test	-	+	-	+	-	+	+	+	+	+
Steroid	-	-	+	-	-	+	-	+	+	+
Terpenoid	-	+	-	-	-	-	-	+	-	-
Alkaloid										
Mayer's	-	+	-	+	-	-	+	-	+	+
Wagner's	-	-	-	+	-	-	-	+	-	+
Hager's	+	-	-	+	-	-	-	+	+	+

"+" means present; "-" means absent

Jasco V-670 (Japan) spectrophotometer to detect a specific wavelength of the available phytochemicals (data not shown). The peaks in the subsequent ranges 300-450, 510, 540, 605 nm were seen. The compounds obtained at 330-420 nm are due to the oxidation of polyphenols like phenolic acids, flavonoids and their derivatives like flavones, flavonols, phenylpropenes and quinines. Generally, 510 nm belongs to anthocyanins, the more discussed anthocyanin from *Clitoria* flower is delphinidin (Andersen and Markham, 2005); peak of chlorophylls are developed at 600-660 nm.

Through gas chromatography-mass spectroscopy analysis, the following compounds were predicted (Table II) using their fragmented mass and retention time. Apart from that a compound 13-octadecenal (z), which had a earlier support from a plant *Cassia auriculata* in the same fabaceae family (Majumder and Paridhavi, 2013) was identified. Another compound lanosterol had prior report in the *Lablab purpureus* plant (Khare, 2008). An acyclic olefins compound, 1-pentadecene was also found in the leaf extract. Finally, an anti-cancer compound 2-methyl-z,z-3,13-octadecadienol was revealed (Hase et al., 2017).

In Fourier Transform-Infra Red analysis, a peak at 3277 cm^{-1} refers to be the presence of –NH or –OH stretching bond that corresponding alcohol and phenol group were noticed. A peak at 2922 cm^{-1} was assigned as alkane asymmetric C-H stretching vibration of methyl and methylene group. The peak 2852 cm^{-1} represented to sp2 and sp3 stretching of C-H bonds of aldehyde and methyl group was predicted (Easmin et al., 2017). The C –O valence vibration of primary alcohols and polysaccharide were observed at 1066 cm^{-1} (Mak et al., 2013). The frequency 1587 cm^{-1} was denoted to be aromatic C–C skeletal vibrations respectively (Bonde et al., 2012). The peak at 1049 was distinguished as C–N stretch, an aliphatic amines and C-N band was found at 1396 cm^{-1}.

A DPPH scavenging assay was demonstrated for fruit and leaf extract were found to be 65% and 12.3% and their IC_{50} value was 75 µg/mL for the fruit extract, in case of flower extract 195.5 µg/mL (Zakaria et al., 2018) and 480 µg/mL for leaf were mentioned before. From an antibacterial activity, the well diffusion method was performed and found to be 0.5 cm for fruit extract against a pathogen *E. coli* and 0.2 cm against *Staphylococcus aureus* at a concentration of 200 µg. In case of leaf extract, 0.6 cm bacterial growth inhibition were formed at 150 µg. By the positive control ampicillin, the bacterial sensitivity effect showed nearly 1.2 cm. A protein called finotin having antimicrobial property was isolated and reported formerly from the seed extract of *C. ternatea.*

The cytotoxic effect of methanolic fruit extract may be due to its enriched phytoconstituents were found to be 54 and 42% against the cervical cancer, HeLa. It was done at the concentration of 100 and 75 µg of extract by MTT colorimetric assay which can be used to measure the cell metabolic activity with the contribution of NADPH-dependent cellular oxidoreductases that reduces tetrazolium dye. A standard anti-cancer drug, cisplatin showed 84% cytotoxicity. As shown in the Figure 1A-D, a moderate anti-cancer activity was observed due to the change in their morphology that may lead to apoptosis. Similar results were reported previously and the formation of few formazan crystals might be due to the presence of lesser viable cells according to the drug dosages. The existing cyclic proteins like cyclotides from *C. ternatea* causes abnormal cells death by disrupting their cell membrane integrity i.e. membrane permeabilization. Cycloviolacin O2, the strongest cyclotide which was along with psyle cyclotides were proven to hold effective anticancer activity against doxorubicin-resistant MCF-7/ADR. The efficacy of doxorubicin was enhanced, when they were loaded along with that. Thus, they inhibit the proliferation of breast cancer cell lines (Sen et al., 2013).

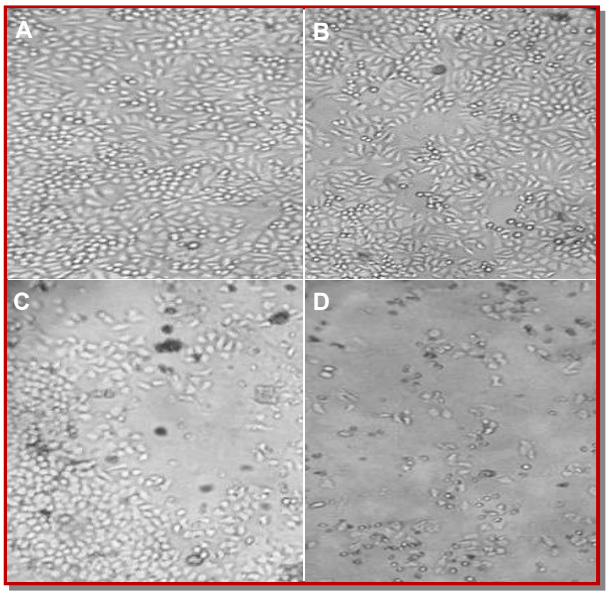
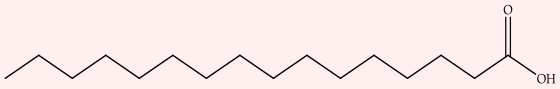
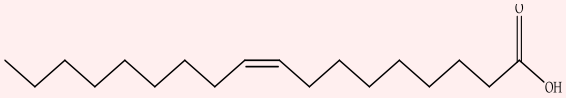
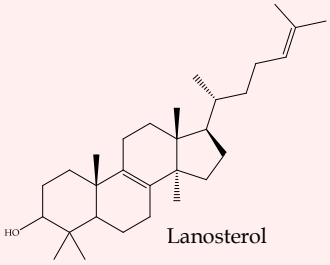
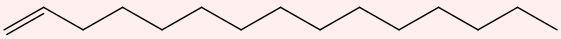
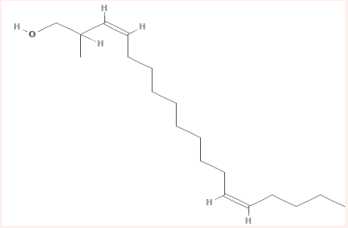
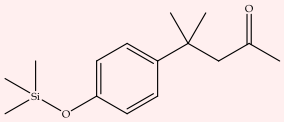
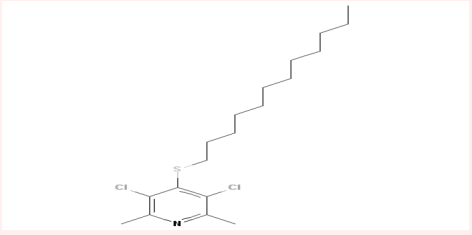


Figure 1: Human HeLa cervical carcinoma cell without treatment (A), at 75 µg (B), at 100 µg (C) of *Clitoria ternatea* extract and cisplatin-positive control (D)

This study explains that the *C. ternatea* extract having prospective cytotoxicity can be incorporated as an anticancer drug apart from its antibacterial and antioxidant property. To the best of our knowledge, there was no previous data stating the major bioactivities of fruit extract so far referring to the others attributes on remaining parts of *C. ternatea* elevates/highlights fruit extract selected as successful or powerful natural medicine. Additionally, the predicted compounds can

Table II

Identification of various compounds available in crude extract

Retention time (RT)	Peak area (%)	Fragmented mass (m/z)	Compound structure
18.0 and 20.1	31.1	185.3, 213.3, 227.3 and 256.4	 N-Hexadecanoic acid
19.7	20.0	123.3, 138.3, 207.2, 235.3 and 264.3	 13-Octadecenal (z)
19.9 and 21.2	10.7	281, 264, 207, 190, 135, 129 and 108	 Oleic acid
30.0	8.0	281.2, 313.4, 355.2 and 424.6	 Lanosterol
16.57	7.95	137.3, 138.3, 179.3 and 207.2	 L-Pentadecene
30.41	4.52	207	 2-Methyl-z,z-3,13-octadecadienol
29.6	6.2	281, 267, 207, 191, 176, 103	 Trimethyl[4-(2-methyl-4-oxo-2-phenoxy]silane
24.9	11.8	281, 207,163 and 133	 2,6-Lutidine 3,5-dichloro-4-dodecylthio

be isolated and further can be used as a lead molecule in cancer drug development as sometime the synergistic effect of the extract may not support or they prevent the toxicity towards cancer cells.

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References

Andersen OM, Markham KR. Flavonoids: Chemistry, biochemistry and applications. CRC Press, 2005.

Bonde SR, Rathod DP, Ingle AP, Ade RB, Gade AK, Rai MK. *Murraya koenigii*-mediated synthesis of silver nanoparticles and its activity against three human pathogenic bacteria. Nanosci Methods. 2012; 1: 25-36.

Easmin S, Sarker MZI, Ghafoor K, Ferdosh S, Jaffri J, Ali ME, Khatib A. Rapid investigation of α-glucosidase inhibitory activity of *Phaleria macrocarpa* extracts using FTIR-ATR based fingerprinting. J Food Drug Anal. 2017; 25: 306-15.

Hamedi A, Ghanbari A, Saeidi V, Razavipour R, Azari H. Effects of β-sitosterol oral administration on the proliferation and differentiation of neural stem cells. J Funct Foods. 2014; 8: 252-58.

Hase GJ, Deshmukh KK, Pokharkar RD, Gaje TR, Phatanagre ND. Phytochemical studies on *Nerium oleander* L. using GC-MS. Int J Pharmacog Phytochem Res. 2017; 9: 885-91.

Jain RA, Shukla SH. Pharmacognostic evaluation and phytochemical studies on stem of *Clitoria ternatea* Linn. Phcog J. 2011; 3: 62-66.

Khare, C. P. Indian medicinal plants: An illustrated dictionary. Springer Science and Business Media, 2008.

Majumder P, Paridhavi M. An ethno-phytochemical and pharmacological review on novel Indian medicinal plants used in herbal formulations. Int J Pharm Pharm Sci. 2013; 5: 74-83.

Mak Y W, Chuah L O, Ahmad R, Bhat R. Antioxidant and antibacterial activities of hibiscus (*Hibiscus rosa-sinensis* L.) and Cassia (*Senna bicapsularis* L.) flower extracts. J King Saud Univ Sci. 2013; 25: 275-82.

Sen Z, Zhan XK, Jing J, Yi Z, Wanqi Z. Chemosensitizing activities of cyclotides from *Clitoria ternatea* in paclitaxel-resistant lung cancer cells. Oncol Lett. 2013; 5: 641-44.

Singh J, Tiwari KN. High-frequency *in vitro* multiplication system for commercial propagation of pharmaceutically important *Clitoria ternatea* L: A valuable medicinal plant. Ind Crops Prod. 2010; 32: 534-38.

Zakaria NNA, Okello EJ, Howes MJ, Birch□Machin MA, Bowman A. *In vitro* protective effects of an aqueous extract of *Clitoria ternatea* L. flower against hydrogen peroxide□induced cytotoxicity and UV□induced mtDNA damage in human keratinocytes. Phytother Res. 2018; 2018.