HRLCMS ANALYSIS OF AQUEOUS FLOWER AND SEED EXTRACT OF CLITORIA TERNATEA AND ITS ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY AGAINST HELICOBACTER PYLORI

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ABSTRACT

Medicinal plants are the valuable sources of new bioactive compounds known as phytochemicals. Investigation on the plant based antibacterial compounds and the development of drugs against various infectious diseases with the goal of discovering new drugs is the order of research in scenario of nutritional disadvantages and pandemic disease outbreak. Furthermore, phytochemical research, based on the pharmacological information is generally deemed to be an effective approach for the discovery of new effective plant based secondary metabolites. In the present study, attempt has been made on the efficacy of aqueous flower and seed extracts of *Clitoria ternatea* against *Helicobacter pyroli*. The preliminary phytochemical analysis of aqueous flower and seed extract *C.ternatea* showed the presence of alkaloids, flavonoids, coumarins, triterpenoids, phenols, quinones, cardiac glycosides, tannins, and saponins. However, concerning the antibacterial activity exhibited by the aqueous flower and seed extracts of *C.ternatea* revealed that it was more obvious in the aqueous seed against *H.pyroli*at the highest concentration due to the presence of more numbers of secondary metabolites. The extracted compound were evaluated by DPPH and reducing power assay for their antioxidant properties. In that, when we compared to standard ascorbic acid, aqueous flower extract displayed 61.78% of DPPH and 27.77% of reducing power assay activity at the highest concentration of 200 µg/ml. Moreover, the HRLCMS analysis of aqueous flower extract of *C. ternatea* contained 24 compounds and the aqueous seed contains 29 compounds in both positive and negative modes.Based on pharmacological properties, the compounds such as Roxatidine acetate, Indoleacrylic acid, Kynurenic acid, Formononetin, 7-epi-jasmonic acid, Afrormosin, Eleganin, Sinensetin, Fortimicin AP and

Calpeptin etc. were present in both the extracts*C.ternatea*. This present study focused on the metabolites of aqueous flower and aqueous seed extracts of the plant *C. ternatea* against the *H.pylori*.

Key words: *Clitoria ternatea*, HR-LCMS, antioxidant, *Helicobater pylori*.

1. INTRODUCTION

Medicinal plants have been used for thousands of years to treat a variety of human diseases in India. Green plants are a significant source of numerous chemical compounds that have been used for thousands of years as medicines by human. It contains variety of chemical substances, including volatile oils, alkaloids, flavonoids, glycosides, phenols, resins, steroids, saponins and flavonoids, which are deposited in the plant's parts such as flowers, fruits, bark, leaves, roots and seeds (1).

Clitoria ternatea is a perennial herbaceous plant that is propagated from seeds. Some of the common names of *C. ternatea* plant include blue pea vine, Asian pigeon wings, butterfly pea and cordofan pea (2). Over Australia, Africa, tropical Asia, Central and South America and South-East Asia countries, these plant are used as a feed plant and to enhance native grassland in huge agriculture systems. *C. ternatea*, like other nitrogen-fixing legumes, has a symbiotic interaction with Rhizobium bacteria (3). Also*C. ternatea* plant has several medical benefits, including antidiabetics (4), antiproliferative (5), antibacterial (6 & 7), antihyperlipidemic (8) and anthelminthic properties (9). It also contains analgesic, anti-inflammatory and antipyretic properties (10).

Having been recognized as a potential herb, it is important to identify and quantify all secondary metabolites of *C. ternatea* in order to ensure the accuracy and repeatability of biological research into the risks and benefits of various medications. Currently, a leading method for detecting and recognizing pharmacologically active secondary metabolites is Liquid Chromatography with High Resolution Mass Spectrometry (LCHRMS) (11& 12). HR-LCMS study of *Casuarina equisetifolia* and *Annona squamosa* leaves methanol extracts reveales 9 and 6 main peaks, suggesting the presence of diverse phytochemical components. All these compounds were characterized and likely recognized upon comparison of the high resolution liquid chromatography and mass spectra of components with the primary library. The identified substances include Dihydromyricetin, Dihydrorobinetin, Rutin, Cosmosiin,

Barbituric acid, 5-ethyl-5-(2-hydroxyethyl), 2,2,9,9-tetramethyl-undecan-1,10-diol, Sinomenine, Dihydrodeoxystreptomycin, hexadecanedioc acid, Ethosuximide M5, Hydroxyanastrozole, 7- Desmethylpapaverine, Lyxosylamine, Isovaleric acid, Taurine, Minoxidil, 4-Trimethyl Ammoniobutanal, 6 betaNaltexol-3-glucuronide, Glucosylgalactosyl hydroxylysine (13). Using HRLCMS analysis, it was discovered that the methanolic extract of *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera* and *Gymnospora emarginata* contained therapeutically significant bioactive compounds like flavonoids, glycosides, alkaloids, coumarins, terpenoids and saponins (14).

Furthermore, *Helicobacter pylori* (*H.pylori*) is a spiral shaped gram negative bacterium that replicates in the gastric epithelial layer (15). There were approximately 4.4 billion people globally infected with *H.pylori* (16). This bacteria has been identified as a major cause of chronic gastric, peptic ulcers and primary gastric lymphoma and is classified as a category 1 carcinogenic agent by the International Association for Cancer Research (IARC) (17). *H.pylori* infection is significantly linked to duodenal and stomach ulcers (18). For the treatment of *H.pylori* infection, many pharmaceutical regimens have been investigated. The recommended standard treatment techniques include antibiotics (19 &20), proton-pump inhibitors (21 & 22), H2-blockers (23 & 24) and bismuth salts (25), which are generally used in dual, triple and quadruple-therapy regimens to eliminate *H.pylori* (19 & 26). Phytomedicine has shown to be an unexplored source of model compounds for the treatment of several ailments, including gastrointestinal (GI) issues (27).

Moreover, free radicals are continuously produced in the body throughout metabolism because they are necessary for a number of vital survival activities (28). A body with a poor defence system is unstable to combat these increased radicals, resulting in an imbalance, and this situation of more free radicals than antioxidants is referred to as oxidative stress. Recent research revealed that several synthetic antioxidants used in the food factory may be carcinogenic, raising concerns about their safety (29). As a result, there is a growing interest in natural antioxidants that may aid in the prevention of oxidative damage (30).

Interestingly, our study showed effectiveness specifically against *H.pylori* which is known to be a cancer causing pathogen. It’s important to continue investigating the antibacterial properties of this plant products and determine the mechanism of action and potential

therapeutic uses. And to the best of our knowledge we enrol that no other use this as massive to expertise this in core.

2. MATERIALS AND METHODS

2.1 Collection of plant materials: *Clitoria ternatea* flower (blue) and seed were collected from Manonmaniam Sundaranar University campus in Tirunelveli, Tamilnadu, India. The flower and seed were washed with distilled water and dried in shadowy place.

2.2 Extraction of flower and seed: 4 gramsof flower and seed powder were taken separately and mixed with 100 ml deionized water respectively and then being kept in a heating mantle for 10 – 15 minutes at 40°C. The mixture was filtered using No.1 Whatman filter paper and then filtratewas kept in the refrigerator until further use.

2.3 Qualitative analysis of phytochemicals: Preliminary qualitative analysis of phytochemical screening was performed using standard methods (31 & 32).

2.4 High Resolution Liquid Chromatography and Mass Spectrometry: HRLCMS analysis was performed on the aqueous flower and seed extracts. HRLCMS analysis of the sample was carried out at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. Agilent high resolution liquid chromatography and mass spectrometry model-G6550A with 0.01% mass resolution was used to create chemical fingerprints of selected medicinal plant extracts. The acquisition method was set to MS-minimum range-120 (M/Z) with a maximum range of 1200 dalton (M/Z) and a scanning rate of one spectrum per second. Gas chromatography was kept at 250°C with a gas flow rate of 13 psi/minute. The hip sampler model G4226A was utilized with an auxiliary speed of 100µl/minute, an ejection speed of 100 µl/minute, a flush out factor of 5 µl, and an injection volume of 5 µl for HRLCMS. For the 2 minutes of acquisition, the flow of solvent composition A: B was 95:5. For HRLCMS, solvent used were 100% water and Acetonitrile (100%).

2.5*In vitro* Antioxidant activity

2.5.1 DPPH free-radical scavenging activity: The radical scavenging capabilities of the aqueous flower and seed extracts was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) (33). The extract (1.6 mL) of different concentrations (25, 50, 75, 100 and 200µg/ml) were added to 2.4 mL of DPPH solution (0.5 mM) and vortexed. This mixture was

kept in dark for 30 minutes and the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

$$\text{DPPH scavenged (\%)} = ([A_{control} - A_{sample}]/A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of control reaction and A_{sample} is the absorbance in the presence of extracts.

2.5.2 Reducing power assay: 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v) are added to 1.0 mL of extract. The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 mL of Tri chloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 1 mL of ferric chloride (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample (34).

2.6 Determination of antibacterial activity by Agar well-diffusion method: Pureculture of *Helicobacter pylori,* was obtained from Rontgen Diagnostic Centre in Thanjavur. The antibacterial activity was determined using the agar well-diffusion method (35). Blood agar plates were swabbed by sterile cotton swabs with a 24 hour broth culture of the bacteria. Using a sterile cork borer, agar wells (5 mm diameter) were created in each of these plates. Samples of varying concentrations (25, 50, 75, 100 and 200 µg/ml) were introduced to the wells using sterilized dropping pipettes, and the plates were left for 1 hour to allow for pre-incubation diffusion. The plates were incubates upright at 37°C ± 2°C for 24 hours for bacterial pathogens. Results were recorded as the presence or absence of the inhibition zone. The diameters of the zones were measured using a measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity. Chloramphenicol (25mg/ml distilled water) is used as a standard.

3. RESULTS

3.1 Qualitative analysis of phytochemical: The Phytochemical screening of aqueous extracts of blue flower and seed of *C.ternatea* revealed the presence of terpenoids, glycoside, tannins and saponins in the flower; flavonoids, coumarins, phenols, quinones and tannins in aqueous seed extract (Table-1).

Table 1. Chemical constituents of *Clitoria ternatea* flower and seed extracts

S.no	Phytochemicals	Aqueous flower	Aqueous seed

1	Alkaloids	-	-
2	Flavonoids	-	+
3	Coumarin	-	+
4	Triterpenoids	+	-
5	Phenols	-	+
6	Quinones	-	+
7	Cardiac Glycosides	+	-
8	Tannins	+	+
9	Saponins	+	-

3.2 High resolution liquid chromatography and mass spectrometry (HR-LCMS) analysis: The high resolution-liquid chromatography-mass spectrometry analysis (HR-LCMS) of aqueous flower extract of *C.ternatea* contained 24 major compounds, in both positive mode and the negative modes (Figures 1 & 2). Then the compounds were identified based on their retention time, mass, molecular formula and structure as shown in Tables-2 & 3. These bioactive compounds had enormous amount pharmacological properties. The bioactive compoumds registered were: 2-(1,2,3,4-Tetrahydroxybutyl)-6-(2,3,4-trihyoxybutyl)pyrazine, d-Dethiobiotin, Roxatidine acetate, Leucyl-Valine, Indoleacrylic acid, 21-Hydroxy-5b-pregnane-3,11,20-trione, Isocarbostyril, Kynurenic acid, Fraxetin, Elastin, (±)7-epi Jasmonic Acid, 2-Hydroxychrysophanol, Garbogiol, (+)-Sophorol, Dihydrodeoxystreptomycin, 17-Hydroxylinolenic acid, Formononetin, 8,8-Diethoxy-2,6-dimethyl-2-octanol, C16 Sphinganine, Aspulvinone E, Phytosphingosine, N-(2R-Hydroxytricosanoyl)-2S-amino-1,3S,4R- octadecanetriol, Gulonic acid and Galactaric acid.

The high resolution-liquid chromatography-mass spectrometry analysis (HR-LCMS) of aqueous seed extract of *C.ternatea* contained 29 compounds in both positive mode and the negative modes (Figures 3 & 4) these compounds were identified. Based on their retention time, mass, molecular formula, and structure and shown in Table-4 & 5. The bioactive compounds recorded were: Halfordinol, L-isoleucyl-L-proline, 5-Hydroxy-L-tryptophan, L-phenylalanyl-L-proline, Isofebrifugine, Danazol, S-4-Hydroxymephenytoin, L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid, 4,6-Dihydroxyquinoline, Eleganin, Biotin sulfone, Sayanedine, Afrormosin, alpha,beta-Didehydrotryptophan, 5,6,2'-

Trimethoxyflavone, Oleandomycin 2'-O-phosphate, Sinensetin, Gibberellin A75, Butyl 3-O-caffeoylquinate, C16 Sphinganine, trans-p-Menthane-7,8- diol 8-glucoside, 2-Heptyl-3-hydroxy-quinolone, Norethindrone, Orphenadrine, Anacyclin, Alangicine, Lorcainide, Fortimicin AP and Calpeptin.

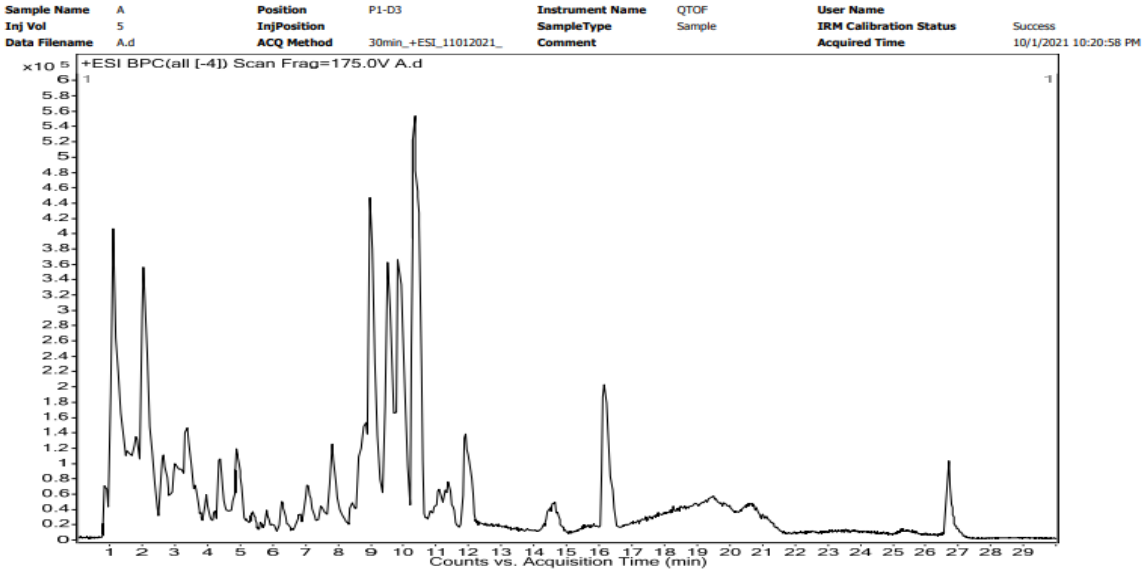
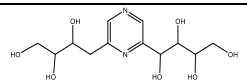
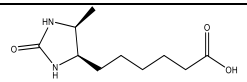
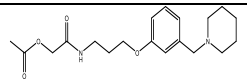
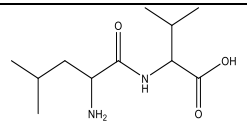
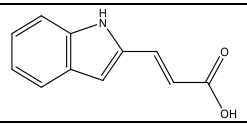
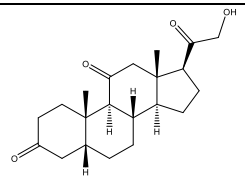
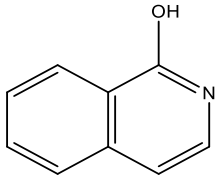
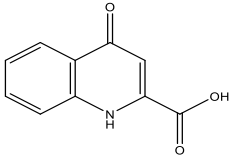
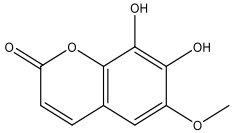
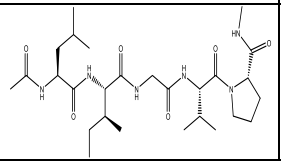


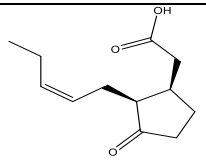
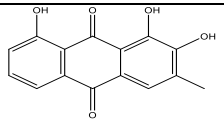
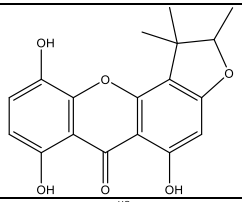
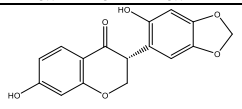
Fig.1 HRLCMS spectrogram of aqueous flower extract of *Clitoria ternatea* in positive mode.

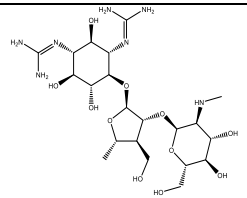
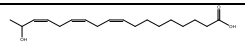
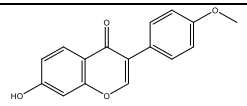
S.No	RT (min)	Compound name	IUPAC name	Formula	Structure	Mass	m/z	Db diff (ppm)	Medicinal uses

Table 2: Bioactive compounds identified in aqueous flower extract of *C. ternatea* by HRLCMS in + ve electron spray ionization mode.

1	1.145	2-(1,2,3,4-Tetrahydroxybutyl)-6-(2,3,4-trihydroxybutyl)pyrazine	1-[6-(2,3,4-trihydroxybutyl) pyrazin-2-yl]butane-1,2,3,4-tetrol	C12 H20 N2 O7		304.1268	305.134	0.79	
2	1.643	d-Dethiobiotin	6-[(4*R*,5*S*)-5-methyl-2-oxoimidazolidin-4-yl]hexanoic acid	C10 H18 N2 O3		214.1313	215.1386	2.13	
3	2.699	Roxatidine acetate	[2-oxo-2-[3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino]ethyl] acetate	C19 H28 N2 O4		348.2025	371.1919	6.85	Treatment in peptic ulcer and related disorder(36)
4	3.033	Leucyl-Valine	2-[(2-amino-4-methylpentanoyl)amino]-3-methylbutanoic acid	C11 H22 N2 O3		230.1626	231.1698	2.03	
5	3.371	Indoleacrylic acid	(*E*)-3-(1*H*-indol-3-yl)prop-2-enoic acid	C11 H9 N O2		187.0628	188.0701	2.67	Promote anti-inflammatory responses(37)

6	3.506	21-Hydroxy-5b-pregnane-3,11,20-trione	(5R,8S,9S,10S,13S,14S)-17-(2-hydroxyacetyl)-10,13-dimethyl-2,4,5,6,7,8,9,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,11-dione	C21 H30 O4		346.2121	369.2014	6.7	
7	3.685	Isocarbostyril	2H-isoquinolin-1-one	C9 H7 N O		145.0523	146.0596	3.36	Anti-tumor agent(38)
8	4.009	Kynurenic acid	4-oxo-1H-quinoline-2-carboxylic acid	C10 H7 N O3		189.0424	190.0495	0.93	Potential role in cognitive and memory impairments, Antioxidant activity, Antiulcerative (39, 40 & 41)
9	4.401	Fraxetin	7,8-dihydroxy-6-methoxychromen-2-one	C10 H8 O5		208.0367	209.044	1.92	Acted as a cancer suppressor in prostate cancer (42)
10	4.883	Elastin	(2S)-1-[(2S)-2-[[2-[[(2S,3S)-2-[[(2S)-2-acetamido-4-	C27 H48 N6 O6		552.3621	553.3699	2.67	In lung development(43)

			methylpentanoyl]amino]-3-methylpentanoyl]amino]acetyl]amino]-3-methylbutanoyl]-N-methylpyrrolidine-2-carboxamide						
11	8.45	(±)7-epi Jasmonic Acid	2-[(1*R*,2*S*)-3-oxo-2-[(*Z*)-pent-2-enyl]cyclopentyl]acetic acid	C12 H18 O3		210.1251	211.1324	2.29	Anti-cancer, anti-inflammatory(44)
12	8.555	2-Hydroxychrysophanol	1,2,8-trihydroxy-3-methylanthracene-9,10-dione	C15 H10 O5		270.0522	271.0594	2.48	Antitumor and antibacterial agent(45)
13	8.715	Garbogiol	5,7,10-trihydroxy-1,1,2-trimethyl-2*H*-furo[2,3-c]xanthen-6-one	C18 H16 O6		328.0941	329.1012	1.93	Inhibition of α-glycoside(46)
14	8.72	(+)-Sophorol	(3*R*)-7-hydroxy-3-(6-hydroxy-1,3-benzodioxol-5-yl)-2,3-dihydrochromen	C16 H12 O6		300.0627	301.07	2.16	

			-4-one						
15	9.003	Dihydrodeoxystreptomycin	2-[(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-3-(diaminomethylideneamino)-4-[(2*S*,3*R*,4*S*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-(methylamino)oxan-2-yl]oxy-4-(hydroxymethyl)-5-methyloxolan-2-yl]oxy-2,5,6-trihydroxycyclohexyl]guanidine	C21 H41 N7 O11		567.2879	568.2951	-2.68	Antibacterial compound shows inhibitory action on *Stevia rebaudiana* (47)
16	9.094	17-Hydroxylinolenic acid	(9*Z*,12*Z*,15*Z*)-17-hydroxyoctadeca-9,12,15-trienoic acid	C18 H30 O3		294.219	295.2261	1.84	
17	9.468	Formononetin	7-hydroxy-3-(4-methoxyphenyl) chromen-4-one	C16 H12 O4		268.0731	269.0804	1.64	Effective treatments for cancer(48)

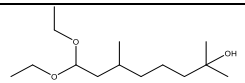
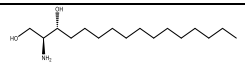
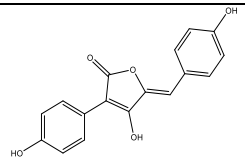
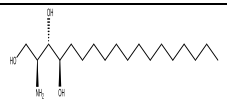
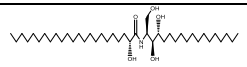
18	9.868	8,8-Diethoxy-2,6-dimethyl-2-octanol	8,8-diethoxy-2,6-dimethyloctan-2-ol	C14 H30 O3		246.2214	269.2106	-7.71	
19	10.305	C16 Sphinganine	(2*S*,3*R*)-2-aminohexadecane-1,3-diol	C16 H35 N O2		273.2665	274.2739	0.87	Posses antimicrobial activity(49)
20	10.945	Aspulvinone E	(5*Z*)-4-hydroxy-3-(4-hydroxyphenyl)-5-[(4-hydroxyphenyl) methylidene]furan-2-one	C17 H12 O5		296.0687	297.0761	-0.9	To develop novel antiinfluenza virus agents with high efficiency and low toxicity(50)
21.	11.925	Phytosphingosine	(2*S*,3*S*,4*R*)-2-aminooctadecane-1,3,4-triol	C18 H39 N O3		317.2922	318.2995	2.57	Antimicrobial activity(51)
22.	21.13	N-(2R-Hydroxytricosanoyl)-2S-amino-1,3S,4R-octadecanetriol	2-hydroxy-*N*-(1,3,4-trihydroxyoctadecan-2-yl)tricosanamide	C41 H83 N O5		669.6257	670.6333	2.09	

Table 3: Bioactive compounds identified in aqueous flower extract of *Clitoria ternatea* by HRLCMS in - ve electron spray ionization mode.

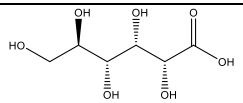
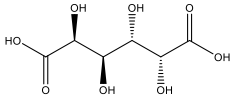
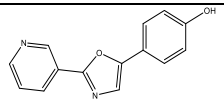
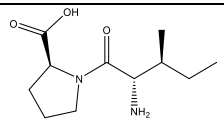
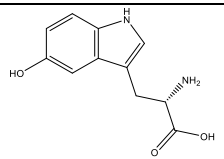
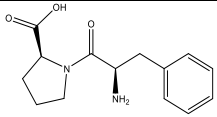
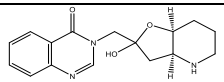
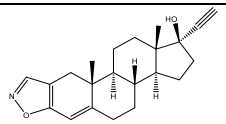
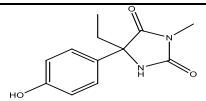
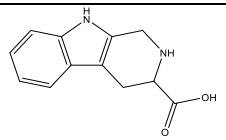
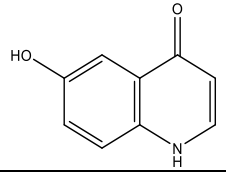
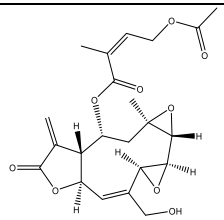
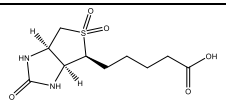
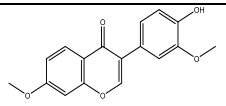
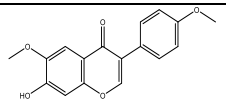
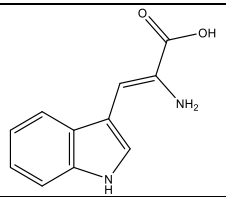
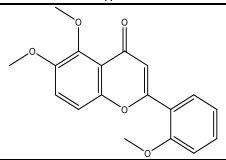
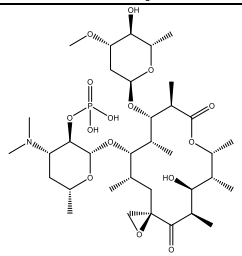
S.No	RT (min)	Compound name	IUPAC name	Formula	Structure	Mass	m/z	Db diff (ppm)	Medicinal uses
1.	1.046	Gulonic acid	(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanoic acid	C6 H12 O7		196.0604	195.0531	-10.6	Used as part of electrolyte salts to support cation-anion balance in solutions(52)
2.	1.116	Galactaric acid	(2*S*,3*R*,4*S*,5*R*)-2,3,4,5-tetrahydroxyhexanedioic acid	C6 H10 O8		210.0398	209.0325	-10.59	Used as a biomarker for diabetic nephropathy diagnosis(53)

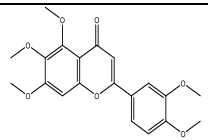
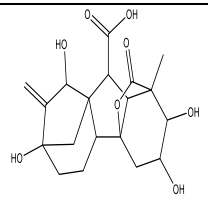
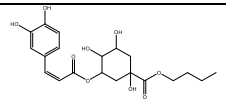
Table 4: Bioactive compounds identified in aqueous seed extract of *C. ternatea* by HRLCMS in +ve electron spray ionization mode.

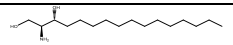
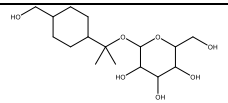
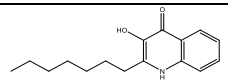
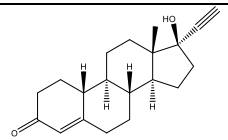
S.No	RT (min)	Compound name	IUPAC name	Formula	Structure	Mass	m/z	Db diff (ppm)	Medicinal uses
1	2.333	Halfordinol	4-(2-pyridin-3-yl-1,3-oxazol-5-yl)phenol	C14 H10 N2 O2		238.0739	239.0808	1.55	Good activity in lipolysis and anti-adipogenesis (54)
2	3.402	L-isoleucyl-L-proline	(2S)-1-[(2S,3S)-2-amino-3-methylpentanoyl]pyrrolidine-2-carboxylic acid	C11 H20 N2 O3		228.1471	229.1543	1.16	
3	6.357	5-Hydroxy-L-tryptophan	(2S)-2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid	C11 H12 N2 O3		220.0846	221.0918	1.04	Use as an antidepressant and to manage post-hypoxic myoclonus (55)
4	6.952	L-phenylalanyl-L-proline	(2S)-1-[(2R)-2-amino-3-phenylpropanoyl]pyrrolidine-2-carboxylic acid	C14 H18 N2 O3		262.1311	263.1384	2.38	
5	7.276	Isofebrifugine	3-[[(3aS,7aS)-2-hydroxy-3a,4,5,6,7,7a-hexahydro-3H-furo[3,2-b]pyridin-2-yl]methyl]quinazolin-4-one	C16 H19 N3 O3		301.1419	302.149	2.48	Possess good antimalarial activity (56)

6	7.456	Danazol	(1S,2R,13R,14S,17R,18S)-17-ethynyl-2,18-dimethyl-7-oxa-6-azapentacyclo[11.7.0.02,10.04,8.014,18]icosa-4(8),5,9-trien-17-ol	C22 H27 N O2		337.2017	360.1908	7.47	Used in the treatment of endometriosis and some benign breast disorders (57).
7	7.579	S-4-Hydroxymephenytoin	5-ethyl-5-(4-hydroxyphenyl)-3-methylimidazolidine-2,4-dione	C12 H14 N2 O3		234.1001	235.1074	1.39	
8	7.622	L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid	2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid	C12 H12 N2 O2		216.0896	217.0969	1.5	High anti-proliferative effect on human CRC cell line HCT-8, and it strongly induced the apoptosis of HCT-8 cells in a dose-dependent manner (58).
9	8.705	4,6-Dihydroxyquinoline	6-hydroxy-1H-quinolin-4-one	C9 H7 N O2		161.0472	162.0544	3.2	Act as biomarker to predict the probiotics effect in IBS (59).

10	9.377	Eleganin	[(1R,2R,4R,6R,7S,9S,10Z,12R)-10-(hydroxymethyl)-4-methyl-15-methylidene-14-oxo-5,8,13-trioxatetracyclo[10.3.0.04,6.07,9]pentadec-10-en-2-yl] (Z)-4-acetyloxy-2-methylbut-2-enoate	C22 H26 O9		434.1559	435.1632	4.02	Anti-proliferative activity (60)
11	9.59	Biotin sulfone	5-[(3aS,4S,6aR)-2,5,5-trioxo-1,3,3a,4,6,6a-hexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid	C10 H16 N2 O5 S		276.0772	277.0845	2.85	
12	9.818	Sayanedine	3-(4-hydroxy-3-methoxyphenyl)-7-methoxychromen-4-one	C17 H14 O5		298.0834	299.0907	2.39	
13	10.149	Afrormosin	7-hydroxy-6-methoxy-3-(4-methoxyphenyl)	C17 H14 O5		298.0834	299.0907	2.55	Anti-inflammatory properties (from stimulated human

			chromen-4-one						neutrophils) (61)
14	11.562	alpha,beta-Didehydrotryptophan	(Z)-2-amino-3-(1H-indol-3-yl)prop-2-enoic acid	C11 H10 N2 O2		202.0764	225.0657	-10.92	
15	11.852	5,6,2'-Trimethoxyflavone	5,6-dimethoxy-2-(2-methoxyphenyl)chromen-4-one	C18 H16 O5		312.0985	313.1058	4	
16	12.131	Oleandomycin 2'-O-phosphate	[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-2-[[(3*R*,5*S*,6*S*,7*R*,8*S*,9*R*,12*R*,13*R*,14*S*,15*R*)-14-hydroxy-8-[(2*R*,4*S*,5*S*,6*S*)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-6-yl]oxy]-6-	C35 H62 N O15 P		767.3877	790.3774	-2.54	

			methyloxan-3-yl] dihydrogen phosphate						
17	13.344	Sinensetin	2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxychromen-4-one	C20 H20 O7		372.1194	373.1267	4.01	Anti-inflammatory, antioxidant, antimicrobial, anti-obesity, anti-dementia and vasorelaxant activities. The studies provided some insights on its several mechanisms of action in cancer and other disease states (62).
18	14.024	Gibberellin A75	5,7,12,13-tetrahydroxy-11-methyl-6-methylidene-16-oxo-15-oxapentacyclo[9.3.2.15,8.01,10.02,8]heptadecane-9-carboxylic acid	C19 H24 O8		380.1479	403.1371	-1.98	
19	14.307	Butyl 3-O-caffeoylquinate	butyl 3-[(*Z*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclo	C20 H26 O9		410.1582	433.1474	-1.3	

			hexane-1-carboxylate						
20	14.573	C16 Sphinganine	(2S,3R)-2-aminohexadecane-1,3-diol	C16 H35 N O2		273.2659	274.2732	3.32	Biomarkers in Alzheimer disease(63) and liver disease(64)
21	14.831	trans-p-Menthane-7,8- diol 8-glucoside	2-(hydroxymethyl)-6-[2-[4-(hydroxymethyl)cyclohexyl]propan-2-yloxy]oxane-3,4,5-triol	C16 H30 O7		334.1979	357.187	3.61	
22	15.855	2-Heptyl-3-hydroxy-quinolone	2-heptyl-3-hydroxy-1H-quinolin-4-one	C16 H21 N O2		259.1567	260.164	2.03	Neuroprotective agent against glutamate-mediated HT22 cell death (65).
23	15.982	Norethindrone	(8R,9S,10R,13S,14S,17R)-17-ethynyl-17-hydroxy-13-methyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one	C20 H26 O2		298.1924	299.1997	3.1	Used for contraception, prevention of endometrial hyperplasia in hormone replacement therapy, and in the treatment of other hormone-mediated illnesses such as endometriosis (66).

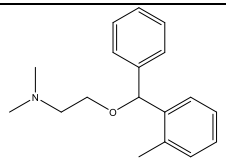
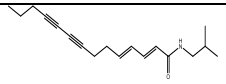
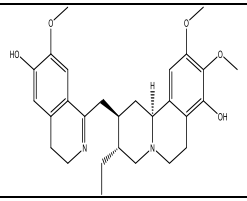
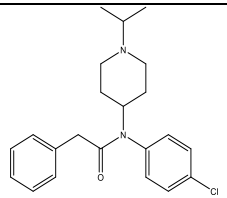
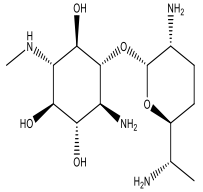
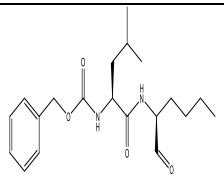
24	17.12	Orphenadrine	N,N-dimethyl-2-[(2-methylphenyl)-phenylmethoxy] ethanamine	C18 H23 N O		269.1773	270.1844	2.33	Adjunct to rest, physical therapy, and other measures for the relief of acute painful musculoskeletal conditions (67).
25	17.176	Anacyclin	(2E,4E)-N-(2-methylpropyl)tetradeca-2,4-dien-8,10-diynamide	C18 H25 N O		271.1928	272.2002	2.98	

Table 5: Bioactive compounds identified in aqueous seed extract of *C. ternatea* by HRLCMS in - ve electron spray ionization mode.

S.No	RT (min)	Compound name	IUPAC name	Formula	Structure	Mass	m/z	Db diff (ppm)	Medicinal uses
1.	14.704	Alangicine	(2*R*,3*R*,11*bS*)-3-ethyl-2-[(6-hydroxy-7-methoxy-3,4-dihydroisoquinoli	C28H36N2O5		480.2639	479.2567	-2.99	

			n-1-yl)methyl]-9,10-dimethoxy-2,3,4,6,7,11*b*-hexahydro-1*H*-benzo[a]quinolizin-8-ol						
2.	14.784	lorcainide	*N*-(4-chlorophenyl)-2-phenyl-*N*-(1-propan-2-ylpiperidin-4-yl)acetamide	C22H27ClN2O		370.181	369.1738	0.55	Antiarrhythmic agents (68).
3.	15.906	Fortimicin AP	(1*S*,2*S*,3*S*,4*R*,5*R*,6*S*)-6-amino-5-[(2*R*,3*R*,6*S*)-3-amino-6-[(1*S*)-1-aminoethyl]oxan-2-yl]oxy-3-(methylamino)cyclohexane-1,2,4-triol	C14H30N4O5		334.2196	333.2123	5.93	Antibacterial agent (69).
4.	24.987	Calpeptin	benzyl *N*-[(2*S*)-4-methyl-1-oxo-1-[[(2*S*)-1-oxohexan-2-yl]amino]pentan-2-yl]carbamate	C20H30N2O4		362.2196	421.2337	2.67	Treat to (SARS-CoV-2) infection, inhibit chronic inflammation, tissue damage and pulmonary fibrosis (70), suppresses the pancreatic cancer (71)

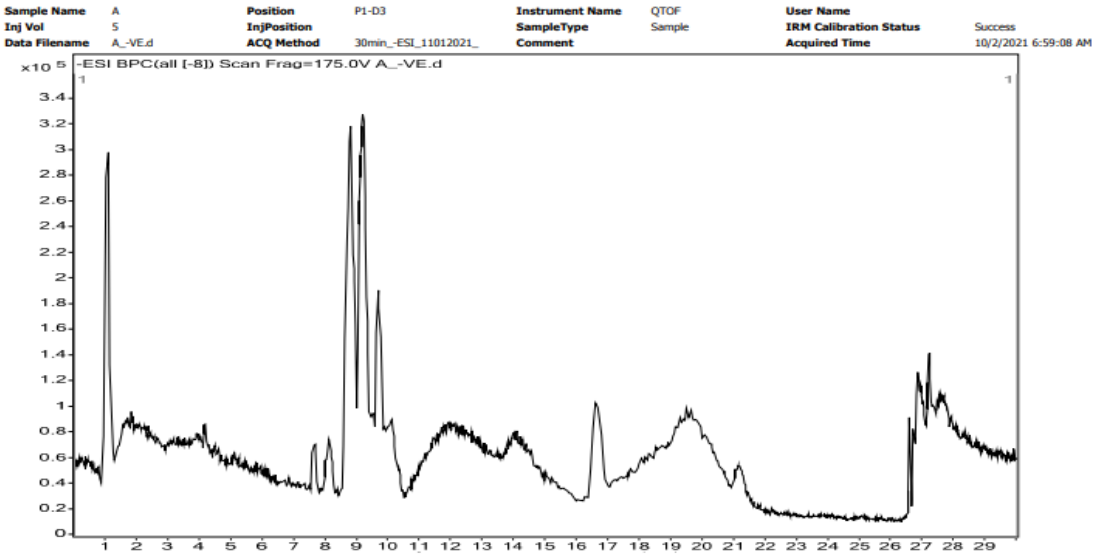


Fig.2. HRLCMSspectrogram of aqueous flower extract of *Clitoria ternatea* in negative mode.

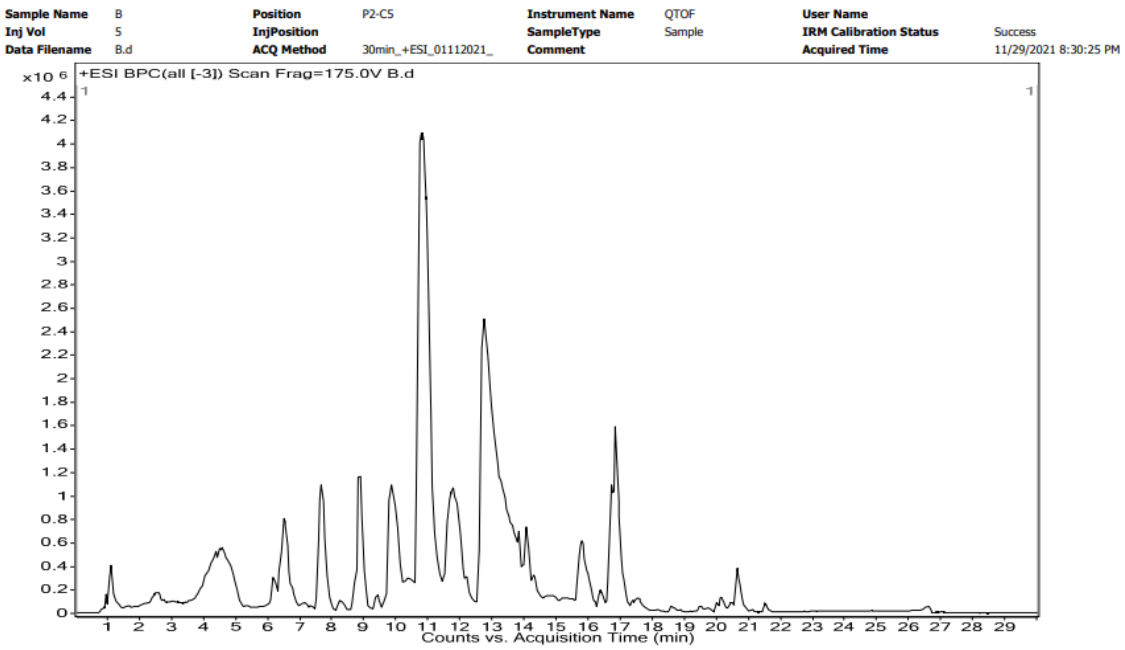


Fig.3. HRLCMSspectrogram of aqueous seed extract of *Clitoria ternatea* in positive mode.

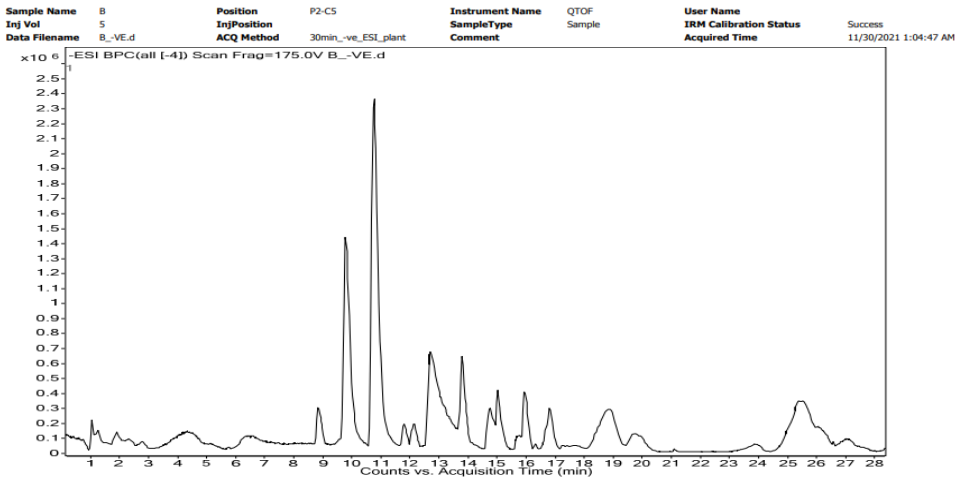


Fig.4. HRLCMS spectrogram of aqueous seed extract of *Clitoria ternatea* in negative mode.

3.3 Antioxidant Activity

3.3.1 DPPH free radical scavenging activity: DPPH is a stable free radical at ambient temperature, that can accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The absorbance of the DPPH radical was measured at 517nm to evaluate its reduction capacity. The reduction in the absorbance of DPPH radical is due to presence of antioxidant. Table-6 shows the DPPH free radical scavenging activity of *C. ternatea* aqueous flower and seed extract, with ascorbic acid as a standard. The percentage of DPPH radical scavenging activity of aqueous flower registered was low (32.22±0.8327) at 25 µg/ml concentration as against the higher value of 60.48±0.1212 recorded at 200 µg/ml. Likewise, the percentage DPPH radical scavenging activity of aqueous seed extract was 30.36±0.0252 in the lowest concentration of 25 µg/ml and it was 37.84±0.02 in the highest concentration of 200 µg/ml. However, the percentage DPPH radical scavenging activity of the standard ascorbic acid was 86.13±27.34027 in the lowest concentration of 25 µg/ml as against 97.90±45.66200 registered in the highest concentration of 200 µg/ml.

Table 6. *In vitro* antioxidant activity of DPPH radical scavenging activity

Concentration of extract (µg/ml)	Test samples / % Radical scavenging activity		
	Aqueous flower extract	Aqueous seed extract	Standard (Ascorbic acid)
25	32.22±0.8327	30.36±0.0252	86.13±27.34027
50	36.65±0.2762	32.54±0.0361	89.73±17.7678
75	37.97±0.06	32.74±0.0252	91.55±7.4015
100	44.41±0.0764	37.84±0.01	93.75±2.7937
200	60.48±0.1212	37.84±0.02	97.90±45.66200

Values are mean ± SD of three parallel measurements

3.3.2 Reducing power assay: The principle behind the reducing power assay method is that substances with reduction potential combine with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) and then interacts with ferric chloride to form ferric-ferrous complex in an absorbance at 700nm. The absorbance of reducing power in aqueous extract and standard showed linear increase with respect to increase in concentration. Table-7 shows the reducing power activity of aqueous flower and seed extract along with ascorbic acid as standard. The absorbance of aqueous flower extract showed linear increase and it ranged from 0.1014±0.00026 to 0.3066±0.0002. Similarly, for the aqueous seed extract it raised from 0.0761 ± 0.0001 to 0.1145 ± 0.00012. However, standard ascorbic acid showed the activity in the range of 0.0988±0 to 0.1254±0.0001 at the respective concentration of range from 25 µg/ml to 200 µg/ml.

Table 7. *In vitro* antioxidant activity of reducing power activity

Concentration of extract (µg/ml)	Test sample / Absorbance at 700 nm		
	Aqueous flower extract	Aqueous seed extract	Standard (Ascorbic acid)
25	0.1014±0.00026	0.0761±0.0001	0.4781±0.05569
50	0.1291±0.00021	0.0948±0.0003	0.5426±0.00015
75	0.12587±0.00021	0.079±0.0001	0.8393±0.00031
100	0.1734±0.00015	0.0892±0.00025	0.9478±0.00471

200	0.3066±0.00025	0.1145±0.00012	1.104±0.001

Values are mean ± SD of three parallel measurements

3.4 Antibacterial activity: The aqueous flower extract of *C. ternatea* registered a zone of inhibition of 0.50 mm at 75 µg/ml, 3.75 mm at 100 µg/ml and 6.30 mm at 200 µg/ml against *H.pylori*. Likewise, the aqueous seed extract had a zone of inhibition of 0.65 mm at 50 µg/ml, 2.80 mm at 75 µg/ml, 6.00 mm at 100 µg/ml and 10.25 mm at 200 µg/ml (Table-8 and Figure 5). Here, aqueous seed extract with concentration 200µg showed the maximum zone (10.25mm) at 200 µg/ml when compared with aqueous flower extract (6.30 mm). However, the standard Chloramphenicol exhibited the zone of 12.55mm in 30µl concentration. Futhermore, preliminary phytochemical analysis indicated the presence flavonoid, phenols, coumarins and quinones in aqueous seed extract; which were absent in aqueous flower extract. The maximum inhibitory activity exhibited in aqueous seed may be due to the presence of these secondary metabolites.

Table 8. Inhibition zone of aqueous flower and seed extract of *Clitoria ternatea*.

Form of extract	**Zone of inhibition(nm) Concentration of extract (µg/ml)**					**Standard drug (Chloramphenicol)**
	25	**50**	**75**	**100**	**200**	
Aqueous flower	Nil	Nil	0.50	3.75	6.30	12.55
Aqueous seed	Nil	0.65	2.80	6.00	10.25	12.60

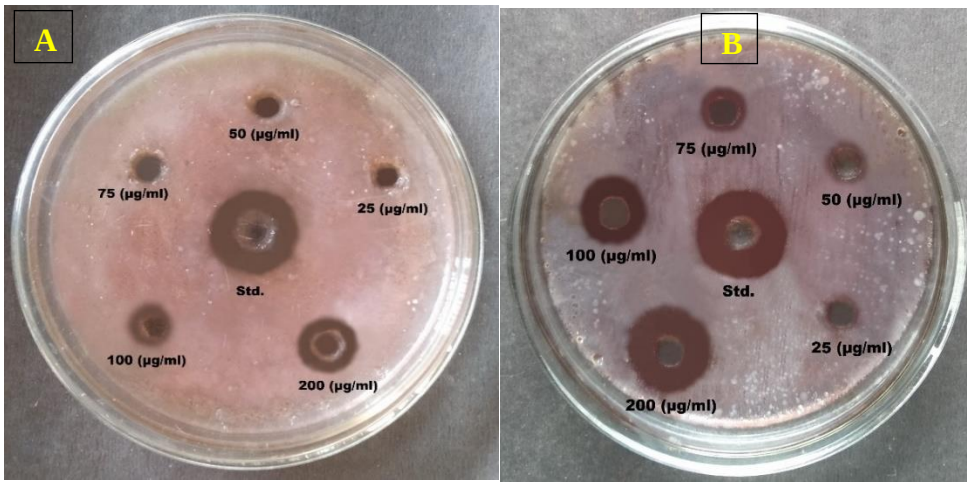


Fig 5. A) Antibacterialactivity of *C. ternatea* flower extract (aqueous), B) Antibacterial activity of *C. ternatea* seed extract (aqueous)

4. DISCUSSION: Plants have played an important role in drug discovery throughout history. Many drugs that are used today have their origins in plant compounds, such as morphine from the opium poppy, aspirin from willow bark, and taxol from the Pacific yew tree. In general, plants contain a vast array of chemical compounds, including alkaloids, terpenoids, flavonoids, and phenolic acids; many of which have pharmacological properties. Various compounds from the plants are isolated and purified to create novel drugs that are effective in treating tremendous diseases. In the present study, the metabolite profiling of aqueous flower and seed extracts of *Clitoria ternatea* was assessed by using HRLCMS. Attempt has also been made on antibacterial activity against *H.pylori* and *in vitro*antioxidant activity of test extracts.

HRLCMS analysis of aqueous flower and seed extract of *C.ternatea* indicated the presence of major compounds and they had potential pharmacological effects. Roxatidine acetate present in the aqueous flower extract of *C. ternatea* is used in the treatment of peptic ulcer and related disorder (36), Indoleacrylic acid promote anti-inflammatory responses (37), Isocarbostyril and Formononetin act as Anti-tumor agent (38 & 48), Kynurenic acid has a potential role in cognitive and memory impairments, antioxidant and antiulcerative activity (39,40 & 41), Fraxetin acted as a cancer suppressor in prostate cancer through inhibiting PLK4 expression thereby inactivating PI3K/Akt signaling (42), Elastin plays a vital role in lung development (43), (±)7-epi Jasmonic acid has shown an anti-cancer, anti-inflammatory (44), 2-Hydroxychrysophanol act as Antitumor and antibacterial agent (45), Dihydrodeoxystreptomycin is an Antibacterial compound showed inhibitory action on *Stevia rebaudiana* (47), C16 Sphinganine and Phytosphingosine with antimicrobial activity (49 & 51), Aspulvinone E is used to develop novel ant influenza virus agents with high efficiency and low toxicity (50). Likewise aqueous seed extract of *C.ternatea* contained bioactive compound with varied pharmaceutical activities; Accordingly, Halfordinol has good activity in lipolysis and anti-adipogenesis (54), 5-Hydroxy-L-tryptophan is used as an antidepressant and to manage post-hypoxic myoclonus.(55), Isofebrifugine showed good antimalarial activity (56), Danazol had application in the treatment of endometriosis and some benign breast disorders (57), L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid with high anti-

proliferative effect on human CRC cell line HCT-8, and it also strongly induced the apoptosis of HCT-8 cells in a dose-dependent manner(58). Furthermore, 4,6-Dihydroxyquinoline act as biomarker to predict the probiotics effect in IBS(59), Eleganin with Anti-proliferative activity(60), Afrormosin with anti-inflammatory properties (from stimulated human neutrophils)(61), Sinensetin with anti-inflammatory, antioxidant, antimicrobial, anti-obesity, anti-dementia and vasorelaxant activities (62), 2-Heptyl-3-hydroxy-quinolone with neuroprotective action against glutamate-mediated HT22 cell death(65), Norethindrone with an action on contraception, prevention of endometrial hyperplasia in hormone replacement therapy, and in the treatment of other hormone-mediated illnesses such as endometriosis(66), Lorcainide has antiarrhythmic agents(68), Calpeptin to treat to acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, inhibit chronic inflammation, tissue damage, pulmonary fibrosis (70) and also suppresses the pancreatic cancer (71).

Moreover, in pharmacokinetics antibacterial and antioxidant activities are two important properties that can play a role in preventing and treating various diseases. Bacterial resistance and bio-efficacy are two factors that may limit the effectiveness of natural compounds as treatments for bacterial infections. Therefore, further research is needed to determine the potential of natural compounds as treatments for diseases. By extension, it is important to consider the potential side effects and interactions of these compounds with other medications before using them as treatments for bacterial infections. The result of the present study showed, that compare to aqueous flower extract, aqueous seed extract exhibited better antimicrobial activity against *H.pylori*. Antibacterial compounds such as Sinensetin, Fortimicin AP, Calpeptin and Isofebrifugine, which were present in aqueous seed extract where responsible for antibacterial activity. *H.pylori* causes inflammation in the stomach; whereas, compound such as Afrormosin, Sinensetin, and Calpeptin has anti-inflammatory properties which were present in aqueous seed extract.

Further in pharmacology, DPPH and ferric-reducing power assays are used to measure the antioxidant activity of various compounds against various diseases. In practice, DPPH and ferric-reducing power assays can be useful tools for investigating the antioxidant activity of compounds various diseases. Consequently, aqueous extract showed better result in antioxidant activity when compared to aqueous seed extract. This may be due to the presence of compounds like Isocarbostyril, Kynurenic acid, Fraxetin, and (+)-Sophorol. In addition,

persistence is an important source to find new drugs and insights into drug development, and its role in drug discovery is likely to continue in the future.

5. CONCLUSION: The aqueous flower and seed extract of *C. ternatea* hadinnumerous amount of therapeutic compounds which was evidenced by HRLCMS analysis. These bioactive compounds played a vital role in antibacterial, antioxidant and antitumor activity. Extraction studies were involved in how much the solvent is environment-friendly and sourced in activities of antibacterial and antioxidants, which were the key parameters for further applications of the plant products. The aqueous seed extract of *C. ternatea* showed higher inhibitory activity against *H. pylori*. *In vitro* antioxidant studies such as DPPH and reducing power assay showed better results in aqueous flower extract when compared with aqueous seed extract. It inferred that presence of characteristics specific compounds either in aqueous flower extract or in aqueous seed extract is the driven force to exhibit antibacterial or antioxidant activities. Over all the present results provides the information that bioactive compound present in the extracts of *C. ternatea* had potential efficacy to cure gastric related disease which is caused by *H. pylori*. Furthermore, the *in vitro* antioxidant activities (DPPH and reducing power assay) of these extracts indicated their antitumor properties. Hence, it is present results through light on the possible use of aqueous flower and seed extracts of *C. ternatea* for the treatment of *H.pylori* infection both at *in vitro* and *in vivo* conditions.

ACKNOWLEDGEMENT: The corresponding author greatfully acknowledged UGC, New Delhi for the award of UGC-BSR-Faculty fellowship. The authors also acknowledged IIT, Bombay for HR-LCMS analysis of experimental extracts.

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