Uttar Pradesh Journal of Zoology

Volume 44, Issue 20, Page 39-68, 2023; Article no.UPJOZ.2785
ISSN: 0256-971X (P)

Bioactive Metabolites Profile of Methanol Flower and Seed Extracts of *Clitoria ternatea* (L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i203645

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar, Iraq.
Reviewers:
(1) Tita Ovidiu, University Lucian Blaga of Sibiu, Romania.
(2) Manal Hadi Ghaffoori Kanaan, Middle Technical University, Iraq.
(3) Aditi Munmun Sengupta, University of Calcutta, India.

Original Research Article

Received: 25/06/2023
Accepted: 31/08/2023
Published: 13/09/2023

ABSTRACT

Since ancient times, plants and plant products were used against numerous diseases. In this context, *Clitoria ternatea* (*C. ternatea*) was used for the various treatments of infectious diseases as a therapeutic role containing various phytochemical, antibacterial and antioxidant properties. The methanol flower and seed extracts of *C. ternatea* were analysed for antibacterial activity against *Helicobacter pylori* (*H. pylori*) using the agar well diffusion method. However, the probe of the antibacterial activity in both the methanol flower and methanol seed showed more or less the same zone of inhibition at 200 µg/ml. Furthermore, antioxidant properties were analysed by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity and reducing power assay. Results on the DPPH assay showed better results in the methanol flower (42.79±0.0819) extract than methanol seed extract (37.41±0.0265) 200µg/ml. Likewise, the reducing assay manifested in the extract of methanol flower (0.90737±0.00375) was supremacy. Moreover the High resolution liquid

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chromatography-mass spectrometry (HRLCMS) analysis of methanol flower and seed extract of *C. ternatea* contained 32 and 51 major bioactive compounds, respectively in positive and negative modes. In light of the study, the extracts of methanol flower and seed extract of *C. ternate*a are utilized in the mode of action against *H. pylori.* The methanolic flower and seed extracts authenticated the presence of extensive identified and unidentified phytochemicals in *C. ternatea* and through more light on the important bioactive compounds to be explored for medicinal applications in future research.

Keywords: Antioxidant; Clitoria ternatea; Helicobacter pylori; methanolic flower; seed extract.

1. INTRODUCTION

Infectious diseases are known as one of the most important leading causes of long- and short-term morbidity and mortality worldwide. They can cause illness due to pathogens or their toxic products that arise through transmission from an infected person. Infectious diseases occur very frequently in children and adults, when an infected person or contaminated animal object is introduced to a susceptible host [1]. Infectious diseases are responsible for an immune global burden system that impacts the world's economy [2]. Across the invasions, infectious diseases result from a combination of several agents (pathogens), hosts, and environmental factors. Besides the agents that may be living parasites (helminths or protozoans), fungi, bacteria, or non-living viruses or prions, the host will be exposed to one of these agents and also derive exposure output. Moreover, the agents and hosts interaction ordained a cascade of stages that include infection, disease, and recovery from death. Although infection will always cause a flourish within a host, it does not always result in disease. This is the unique characteristic of vast infections—odd exposure to certain infectious agents that have consequences for other individuals because an infected person can affect the source of infection. Since the pathogen is directly transmitted, it attacks the person. Although the prequel stage is required for each type of infection, can vary widely for a given type of infection depending on the agent host, and environmental factors may affect it [3]. Important prevention and control interventions for the target that is a susceptible host include both those that address the heed of plunk in the host. Treatment for infectious diseases include antibiotics, antiviral medications, or other drugs, depending on the specific infections. Despite the infectious diseases, *H. pylori* is a type of bacteria that infects the stomach lining and causes peptic ulcers, gastritis, and stomach cancer. Half of the world's population is being infected with this bacterium. *H.pylori* is gram-negative, microaerophile, and spiral bacillus [4]. It's about 3.5 µm long and above 0.5 µm in diameter, and it was originally assigned taxonomically to the genus Campylobacter [5]. It is now established that *H. pyroli* is a causative agent for duodenal ulcers [6, 7] and is predisposed to gastric ulcers [8]. Whether it may also be steep in a model of causing gastroesophageal, reflex disease (GELD), stomach cancer [9] and anaemia [10]. Since it is deemed that gastritis is a risk factor for peptic ulcers, lapse [11] is a condition that was innocuous for the treatment of the hasp and had successful eradication [12]. For the standard treatment, the takeover is which sublime combination of antibiotics has an acid-suppressing mechanism that allows it to work more effectively on the afflicted. On the other hand, the plants are abundantly used for their medicinal values and aesthetic qualities in the treatment of diseases, in which they play a vital role. Almost all plants are composed of leaves, stems, seeds, fruits, and roots [13], and this may produce the secondary metabolites for the various purposes of plant defence against the disease, which are highly immunomodulatory properties [14], making them useful in the prevention and treatment of infectious diseases and having versatile biological properties with the phytochemicals, antimicrobials, anticancer, and antioxidants [15]. Moreover, phytochemicals play an abundant role in the prevention and treatment of infectious diseases by proving and profoundly demonstrating the antimicrobial assay of various plant-based properties to assault infectious diseases. The phytochemical constituents exhibit protective prostatitis against stomach cancer like gastric cancer through several mechanisms, including inhabitation of cell proliferation [16], induction of apoptosis [17], autophagy [18], anti-angiogenesis [19], suppression of cell metastasis [20], modulation of gut microbiota [21] and inhabitation of *H. pyroli* [22]. Most of these plants are known to produce antimicrobial substances [22], which serve as plant defence mechanisms and resistance against abiotic and biotic stresses

and is deemed to be more important in medicines as antibiotics for disease-resistant infections. However, antibacterial agents play a crucial role in the treatment of human infectious diseases caused by bacteria. Other else Antioxidants are compounds that can neutralize free radicals and prevent oxidative damage to cell which oxidative stress instance a part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, cardiovascular and neurodegenerative diseases. The discovery of antioxidants from natural sources is salubrious to human health [23, 24] and as a matter of fact, antioxidants of natural origin have received deemed to be attention from the health and food industries in regard to identifying secondary metabolites. Antioxidants protect the body from radical damage by scavenging reactive oxygen species [25].

Furthermore, to *C. ternatea*, also called the butterfly pea flower, which is a plant that has been traditionally used in Ayurvedic medicine to treat various ailments, including stomach disorders. This plant also has great medicinal value, commonly grown as an ornamental plant, flowers are edible and known to be blue pea plants or flowers [26]. It was reported that, these flower extracts are found to have antimicrobial, antioxidant, antidiabetic, and inflammatory properties that are beneficial to human health. It has a role in treating the potential benefits of stomach disorders. They may help to reduce gastric ulcer formation and primitive-to-end stomach lining damage caused by the infection. Overall, it is a promising plant with a long history of use in traditional medicine. As with any natural remedy, it is important to consult with a healthcare professional before use and to use caution when combining it with other medications or supplements.

HR LCMS is a technique used to identify and quantify the chemical components of a sample. HR-LCMS is commonly used in phytochemical analysis to determine the chemical structure and identify the active compounds present in plants in short duration, which can then be used to develop new drugs and pharmaceuticals. Its high sensitivity, specificity, and resolution make it an essential tool in many areas of research and development. The aim of the work is to screen the biological lead molecules of methanol extracts of *C.ternatea* against *H.pylori* by measuring antioxidant and antibacterial activities that were expected to be resistance factors in further research.

2. MATERIALS AND METHODS

Sample Collection: *C. ternatea* flower (blue) and seed were collected from Manonmaniam Sundaranar University campus in Tirunelveli, Tamil Nadu, India. The flower and seed were washed thoroughly with distilled water and dried in shady place and make it as fine powder using blender.

Extraction of flower and seed: Here, soxhlet method was used for the extraction of flower and seed. The extraction was carried out by using the methanol as a solvent. 10 grams of flower and seed powder were taken separately and 300ml of methanol was used. The extraction process was done for 8 hours at temperature of 45°C- 50°C. The filtrate was kept in air tight container until further use.

High Resolution Liquid Chromatography and Mass Spectrometry: The extraction of methanol flower and seed of *C.ternatea* was used for fingering plant metabolites by using High Resolution Liquid Chromatography Mass Spectrometry. HRLCMS (model-G6550, Agilent resolution, USA) analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Bombay, Powai, Mumbai by 5µl of sample injection for 30 minutes. The temperature of gas was kept at 250°C and 100% water and Acetonitrile (100%) solvent were used.

Antibacterial activity: The microbial inhibitory activity of *H.pylori* methanolic flower and seed extract was performed by using agar well diffusion method [27]. *H.pylori* strain was obtained from Rontgen Diagnostic Centre in Thanjavur. Bacteria culture were swabbed in blood agar (Catalogue no. 70133- Sigma Aldrich) plates using sterile cotton swabs. Agar well sized in 5mm diameter were punched in each of these plates using sterile cork borer. Wells were filled with samples of methanol flower and seed extracts with different concentration (25, 50, 75, 100 and 200 µg/ml) and these plates were kept for one hour to allow for pre-incubation diffusion. The plates were kept for incubation upright at 37°C±2°C for 24 hours. The inhibitory zone around the wells were recorded and diameter were measured.

2.1 *In vitro* Antioxidant Activity

DPPH free-radical scavenging activity: The radical scavenging activities of methanolic flower

and seed extracts was carried out using DPPH (2, 2-diphenyl-1-picrylhydrazyl) [28]. Different concentrations (25, 50, 75, 100 and 200µg/ml) of plant extracts were added to 2.4 mL of DPPH solution (0.5 mM) and vortexed thoroughly. Ascorbic acid was used as standard. The reaction mixture was kept in dark condition for 30 minutes at the room temperature and the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the formula 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.

Reducing power method: Total reducing power assay was performed by following the method of Oyaizu [29]. 1ml of different concentrations (25, 50, 75, 100 and 200µg/ml) of extract was mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. This mixture was incubated at 50°C for 20 min and then 2.5ml of 10% Tri chloro acetic acid was added. This reaction mixture was centrifuged at 3000 rpm for 10 min and the upper layer of solution (2.5ml) was taken and mixed with 2.5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance was recorded at 700 nm against blank sample.

3. RESULTS

HRLCMS analysis: The HRLCMS analysis of methanolic flower and seed extracts were contained 32 and 51 major compounds, respectively in positive and negative ionization mode. Furthermore, these compounds confirmed by their retention time, mass, molecular formula, as shown in Tables 1-4. The chromatogram (Figs. 1-4) showed details the relative concentration of abundant compounds and the height of the peak specifies the concentrations of bioactive compounds. The bioactive compound found in methanolic flower extract were: N-Acrylylglycine methyl ester, Adenine, Hexyl 2-furoate, Quercetin, Kaempferol 4'-glucoside 7-rhamnoside, 6-C Galactosylluteolin, 6-Hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one, Morindone, (+)-Sophorol, Garbogiol, Formononetin, Betavulgarin, Celereoin, Afrormosin, Aspulvinone E, Picrotoxinin, Gyrocyanin, Bowdichione, 17beta-Hydroxyestr-5(10)-en-3-one, Phytosphingosine, 9Z-Octadecen-12-ynoic acid, 7-O-Acetylaustroinulin, Sclareol, Ganoderic acid, Goyaglycoside c, Fucosterol, Pheophytin a, 3-O-Methylcoumestrol, 7,9-Dimethyluric acid, Theophylline, Calpeptin and 14,19-Dihydroaspidospermatine. Likewise, the bioactive compounds present in methanol seed extracts were: Pirbuterol, Gentianadine, N-(Heptan-4- yl)benzo[d][1,3]dioxole-5-carboxamide, Isocarbostyril, 2-Carboxy-4-dodecanolide, 2-Hydroxy-6-oxo-octa-2,4-dienoate, cis-1,3,4,6,7,11b-Hexahydro-9-methoxy-2H- benzo[a]quinolizine-3- carboxylic acid, U 0521, Afrormosin, Eleganin, Oleandomycin 2'-O-phosphate, TR-Saponin B, Dihydrodeoxystreptomycin, Sayanedine, R1128C, C16 Sphinganine, Garbogiol, MG(18:2(9Z,12Z)/0:0/0:0)[rac], Ganoderic acid F, Ganosporelactone A, Imperialine, Glycerol triundecanoate, (E)-26,27-Dinorergosta-4,22-dien-3-one, Antimycin A1, DG(16:0/15:0/0:0), DG(18:2(9Z,12Z)/15:0/0:0), Schleicherastatin 6, Isomaltulose, Astragalin 7-rhamnoside, 7-Chloro-3,3',4',5,6,8- hexamethoxyflavone, Vestitone 7-glucoside, Apigenin 7,4'-dimethyl ether, Lopinavir, Laserpitin, Moracin P, Lathyrol, dolichyl D-xylosyl phosphates, L-Oleandrosyl-oleandolide, LysoPE(18:1(11Z)/0:0), Hovenine A, 3-Benzoyloxy-11-oxo-12-ursen- 28-oic acid, Myxalamid S, Linoleoyl Ethanolamide, 3beta-(1-Pyrrolidinyl)-5alpha- pregnane-11,20-dione, DG(16:1(9Z)/16:0/0:0), omega-hydroxy behenic acid, Azukisapogenol, DG(16:0/16:0/0:0) and Cohibin B.

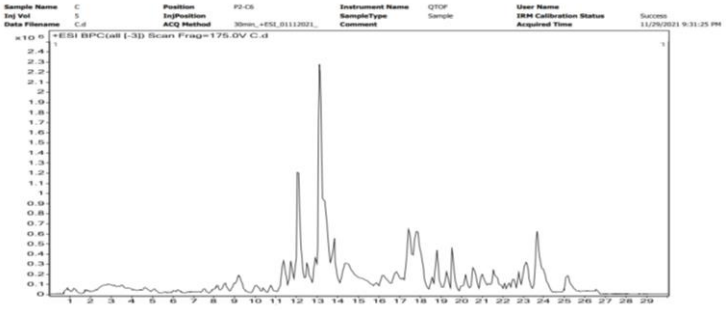


Fig. 1. HRLCMS of chromatogram of methanol flower extract *C. ternatea* in positive mode

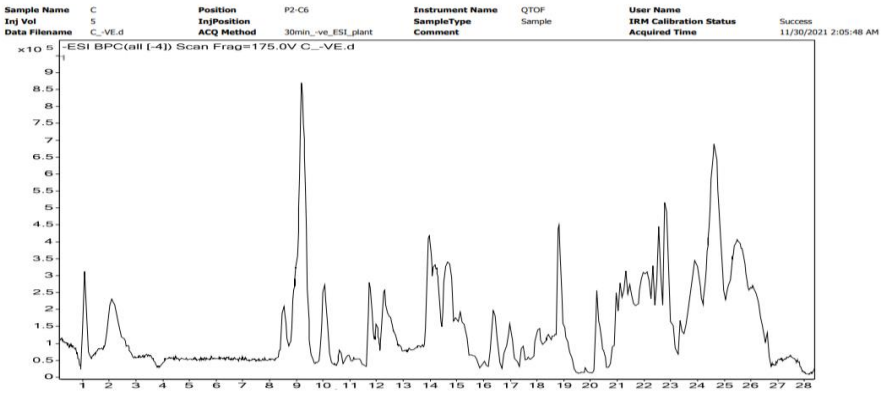


Fig. 2. HRLCMS of chromatogram of methanol flower extract of *C. ternatea* in negative mode

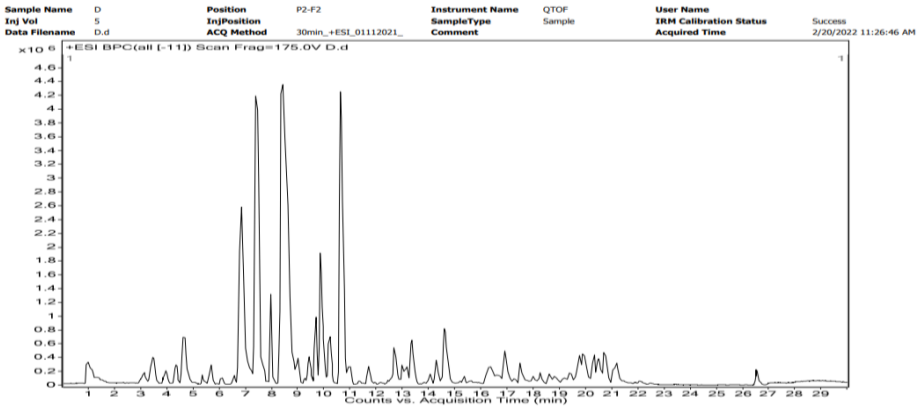


Fig. 3. HRLCMS of chromatogram of methanol seed extract of *C. ternatea* in positive mode

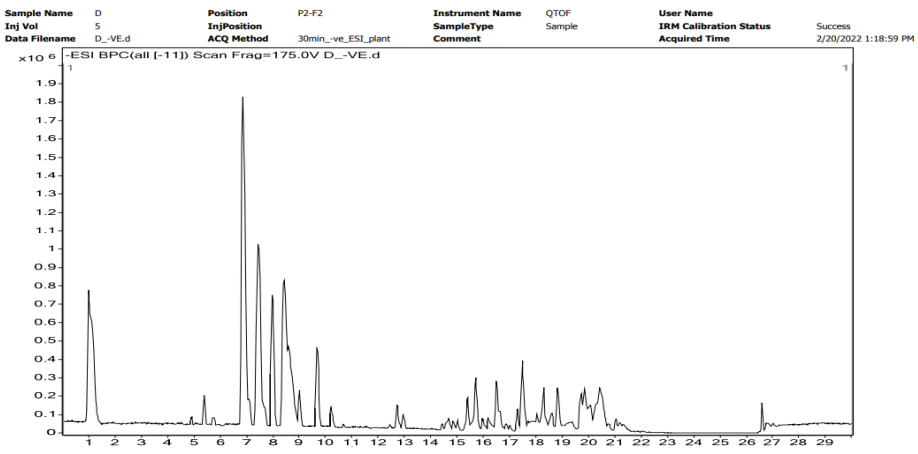
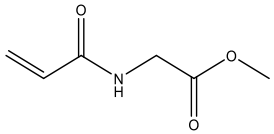
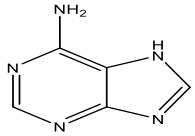
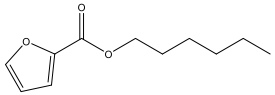
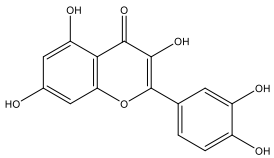
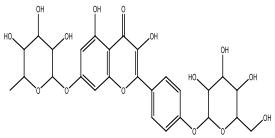
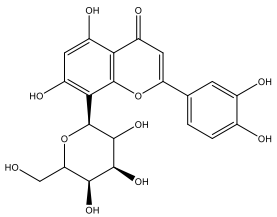
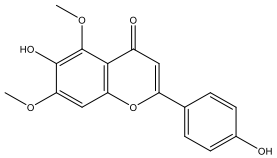
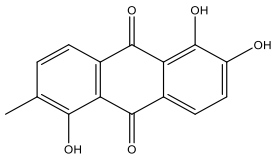
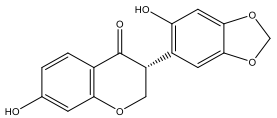
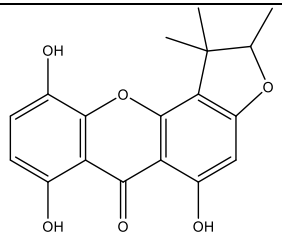
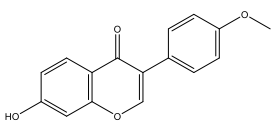
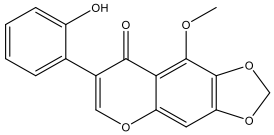
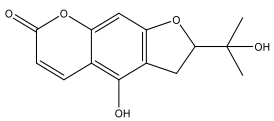
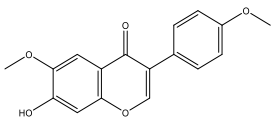


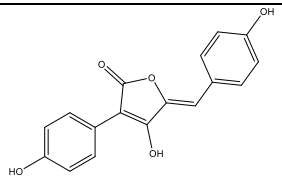
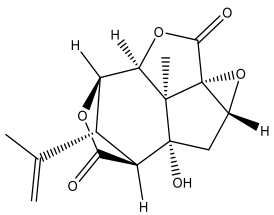
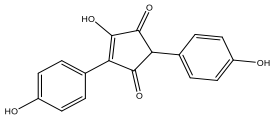
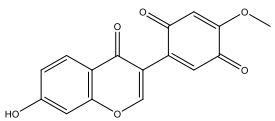
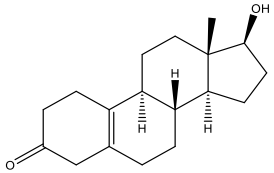
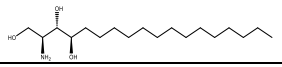
Fig. 4. HRLCMS of chromatogram of methanol seed extract of *C. ternatea* in negative mode

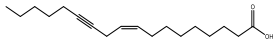
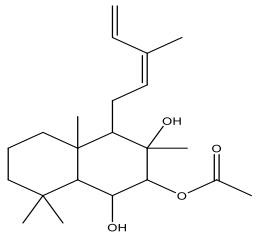
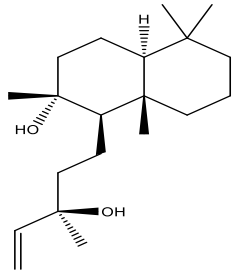
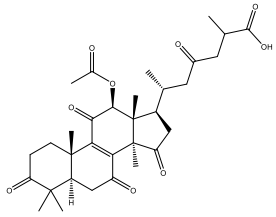
Table 1. Bioactive compounds identified in Methanol flower extract of *Clitoria ternatea* by High Resolution- Liquid Chromatography and Mass Spectrometry in + ve electron spray ionization mode

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
1	2.683	N-Acrylylglycine methyl ester	methyl 2-(prop-2-enolyamino) acetate	C6 H9 N O3		143.0579	144..0652	2.13	Used in drug delivery [30]
2	2.97	Adenine	7H-purin-6-amine	C5 H5 N5		135.0544	136.0616	0.72	Used in treatment for HIV, HBV, CMV and other virus-infected diseases.[31]
3	8.038	Hexyl 2-furoate	hexyl furan-2-carboxylate	C11 H16 O3		196.1092	197.1165	3.93	Flavouring agent[32]
4	8.349	Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	C15 H10 O7		302.0415	303.0488	3.75	Anticancer, cardiovascular protection, anti-inflammatory, antidiabetic, gastroprotection effects, anti-infective and inhibits gastric acid secretion and inhibits *Helicobacter pylori* infection [33]
5	8.976	Kaempferol 4'-glucoside 7-rhamnoside	3,5-dihydroxy-2-[4-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-7-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxychromen-4-one	C27 H30 O15		594.1554	303.0491	5.12	

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
6	9.0201	6-C Galactosylluteolin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2*S*,4*R*,5*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	C21 H20 O11		448.0984	449.1056	4.93	Therapeutic approach for coronavirus disease (COVID-19) [34]
7	11.161	6-Hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one	6-hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxychromen-4-one	C17 H14 O6		314.0784	315.0854	1.98	Antimicrobial acivitiy [35]
8	11.739	Morindone	1,2,5-trihydroxy-6-methylanthracene-9,10-dione	C15 H10 O5		271.0-595	271.0594	2.55	To treat a variety of health problems including, high blood pressure, arthritis, ulcers, depression, sprains, menstrual cramps, pain relief, inflammation, burns, fever, food poisoning, intestinal worms, and joint problems [36]
9	11.903	(+)-Sophorol	(3*R*)-7-hydroxy-3-(6-hydroxy-1,3-benzodioxol-5-yl)-2,3-dihydrochromen-4-one	C16 H12 O6		301.0699	301.0699	2.68	
10	12.253	Garbogiol	5,7,10-trihydroxy-1,1,2-trimethyl-2*H*-furo[2,3-c]xanthen-6-one	C18 H16 O6		328.0968	329.101	3.24	Inhibition of α-glucosid [37]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
									
11	12.61	Formononetin	7-hydroxy-3-(4-methoxyphenyl)chromen-4-one	C16 H12 O4		268.0727	269.0799	3.39	Used in treatment for cancer [38]
12	12.955	Betavulgarin	7-(2-hydroxyphenyl)-9-methoxy-[1,3]dioxolo[4,5-g]chromen-8-one	C17 H12 O6		312.0622	313.0695	3.73	Anticancer agent against breast cancer [39]
13	13.297	Celereoin	4-hydroxy-2-(2-hydroxypropan-2-yl)-2,3-dihydrofuro[3,2-g]chromen-7-one	C14 H14 O5		262.0844	263.0916	-1.32	
14	13.494	Afrormosin	7-hydroxy-6-methoxy-3-(4-methoxyphenyl)chromen-4-one	C17 H14 O5		298.0832	299.0905	3.18	Anti-inflammatory properties (from stimulated human neutrophils) [40]
15	13.782	Aspulvinone E	(5*Z*)-4-hydroxy-3-(4-hydroxyphenyl)-5-[(4-hydroxyphenyl)methylidene]furan-2-one	C17 H12 O5		296.0678	297.0751	2.31	To develop novel antiinfluenza virus agents with high

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
									efficiency and low toxicity [41]
16	14.063	Picrotoxinin	(1*R*,3*R*,5*S*,8*S*,9*R*,12*S*,13*R*,14*R*)-1-hydroxy-13-methyl-14-prop-1-en-2-yl-4,7,10-trioxapentacyclo[6.4.1.1^{9,12}.0^{3,5}.0^{5,13}]tetradecane-6,11-dione	C15 H16 O6		292.0958	315.085	-3.82	Used as a central nervous system stimulant, antidote, convulsant, and GABA (gamma aminobutyric acid) antagonist [42]
17	14.064	Gyrocyanin	4-hydroxy-2,5-bis(4-hydroxyphenyl)cyclopent-4-ene-1,3-dione	C17 H12 O5		296.0674	297.0741	3.59	
18	15.918	Bowdichione	2-(7-hydroxy-4-oxochromen-3-yl)-5-methoxycyclohexa-2,5-diene-1,4-dione	C16 H10 O6		298.0469	299.0543	2.77	Anti-inflammatory activity [43]
19	16	17beta-Hydroxyestr-5(10)-en-3-one	(8*R*,9*S*,13*S*,14*S*,17*S*)-17-hydroxy-13-methyl-2,4,6,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-one	C18 H26 O2		274.1927	275.2	2.15	
20	16.637	Phytosphingosine	(2*S*,3*S*,4*R*)-2-aminooctadecane-1,3,4-triol	C18 H39 N O3		317.292	318.2991	3.61	Antimicrobial activity [44]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
21	16.877	9Z-Octadecen-12-ynoic acid	(*Z*)-octadec-9-en-12-ynoic acid	C18 H30 O2		278.2238	279..2311	2.6	
22	19.305	7-O-Acetylaustroinulin	[1,3-dihydroxy-3,4*a*,8,8-tetramethyl-4-[(2*Z*)-3-methylpenta-2,4-dienyl]-2,4,5,6,7,8*a*-hexahydro-1*H*-naphthalen-2-yl] acetate	C22 H36 O4		364.2602	365.2671	3.23	
23	19.553	Sclareol	(1*R*,2*R*,4a*S*,8a*S*)-1-[(3*R*)-3-hydroxy-3-methylpent-4-enyl]-2,5,5,8*a*-tetramethyl-3,4,4*a*,6,7,8-hexahydro-1*H*-naphthalen-2-ol	C20 H36 O2		308.2703	309.2776	3.92	Reduced swelling in the paws and lower histological arthritic scores, shows that sclareol potentially mitigates collagen-induced arthritis [45]
24	20.961	Ganoderic acid F	(6*R*)-6-[(5*R*,10*S*,12*S*,13*R*,14*R*,17*R*)-12-acetyloxy-4,4,10,13,14-pentamethyl-3,7,11,15-tetraoxo-2,5,6,12,16,17-hexahydro-1*H*-cyclopenta[a]phenanthren-17-yl]-2-methyl-4-oxoheptanoic acid	C32 H42 O9		570.2838	593.2731	-1.62	Inhibits the growth of cancer cells, anti-angiogenic and displays significant cytotoxicity against cancer cells [46]
25	21.579	Goyaglycoside c	(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(hydroxymethyl)-6-[[(1*R*,4*S*,5*S*,8*R*,9*R*,12*S*,13*S*,16*S*)-19-methoxy-8-[(*E*,2*R*)-6-methoxy-6-methylhept-4-en-2-yl]-5,9,17,17-tetramethyl-18-	C38 H62 O9		662.4434	663.4504	-6.08	Used as a bitter stomachic, a laxative, an antidiabetic, and an

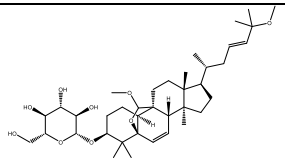
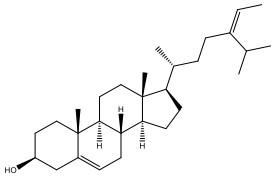
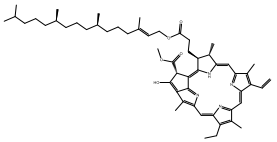
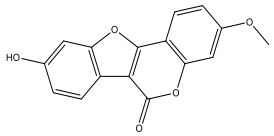
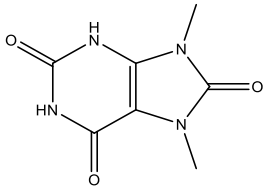
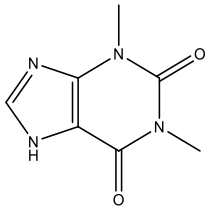
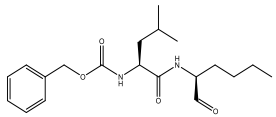
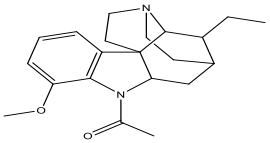
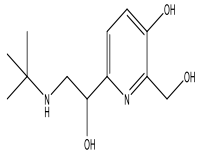
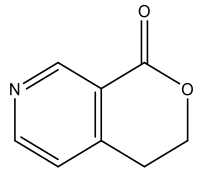
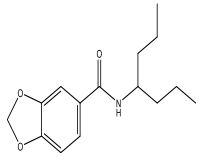
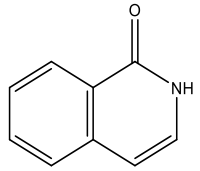
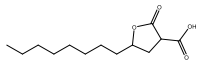
S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
			oxapentacyclo[10.5.2.0^{1,13}.0^{4,12}.0^{5,9}]nonadec-2-en-16-yl]oxy]oxane-3,4,5-triol						anthelmintic for children [47]
26	21.844	Fucosterol	(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-[(*Z*,2*R*)-5-propan-2-ylhept-5-en-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol	C29 H48 O		412.3686	413.3759	4.56	Help to reduce blood cholesterol , blood vessel thrombosis preventive and butyrylcholinesterase inhibitory activities [48]
27	24.105	Pheophytin a	methyl (3*R*,21*S*,22*S*)-16-ethenyl-11-ethyl-4-hydroxy-12,17,21,26-tetramethyl-22-[3-oxo-3-[(*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enoxy]propyl]-7,23,24,25-tetrazahexacyclo[18.2.1.1^{5,8}.1^{10,13}.1^{15,18}.0^{2,6}]hexacosa-1,4,6,8(26),9,11,13(25),14,16,18(24),19-undecaene-3-carboxylate	C55 H74 N4 O5		870.5629	871.5703	3.52	Anti-inflammatory activity [49]
28	12.049	3-O-Methylcoumestrol	9-hydroxy-3-methoxy-[1]benzofuro[3,2-c]chromen-6-one	C16 H10 O5		282.0521	283.0591	2.47	

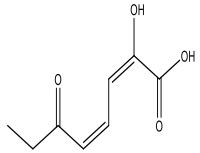
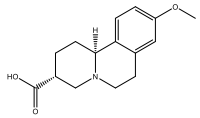
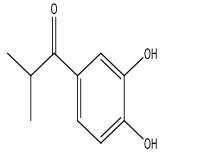
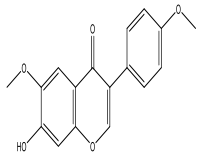
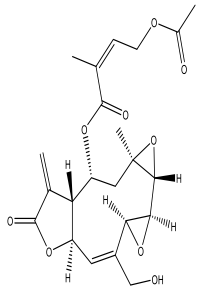
Table 2. Bioactive compounds identified in Methanol flower extract of *Clitoria ternatea* by High Resolution- Liquid Chromatography and Mass Spectrometry in - ve electron spray ionization mode

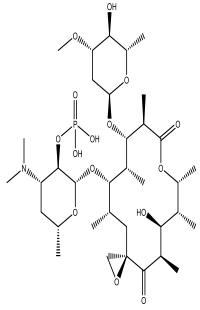
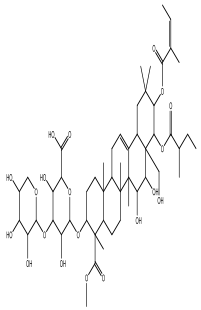
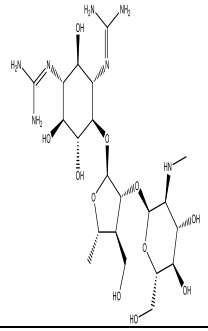
S.NO	RT	COMPOUND NAME	IUPAC NAME	FORUMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
1	1.829	7,9-Dimethyluric acid	7,9-dimethyl-3H-purine-2,6,8-trione	C7 H8 N4 O3		196.0606	195.0533	-4.92	
2	2.035	Theophylline	1,3-dimethyl-7*H*-purine-2,6-dione	C7 H8 N4 O2		180.0655	179.0581	-4.2	Treatment for asthma, chronic obstructive lung diseases,infant apnea [50]
3	25.447	Calpeptin	benzyl *N*-[(2*S*)-4-methyl-1-oxo-1-[[(2*S*)-1-oxohexan-2-yl]amino]pentan-2-yl]carbamate	C20 H30 N2 O4		362.2193	421.2335	3.48	Suppresses the pancreactic cancer [73] and Treat to acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, inhibit chronic inflammation, tissue damage and pulmonary fibrosis [51]
4	26.59	14,19-Dihydroaspidospermatine	1-(18-ethyl-6-methoxy-8,14-diazapentacyclo[9.5.2.$0^{1,9}$.$0^{2,7}$.$0^{14,17}$]octadeca-2(7),3,5-trien-8-yl)ethanone	C21 H28 N2 O2		340.2129	339.206	6.34	

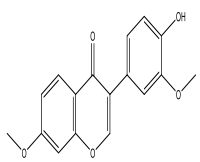
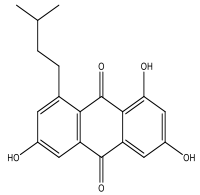
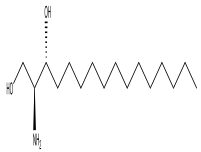
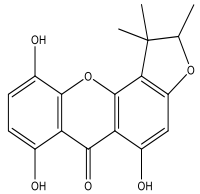
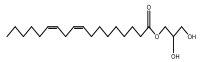
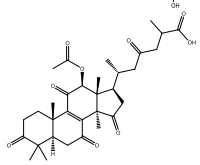
Note: RT- Retention Time; IUPAC name- International Union of Pure and Applied Chemistry; m/z- mass / charge *number*

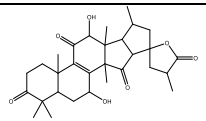
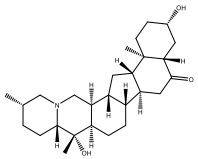
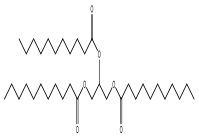
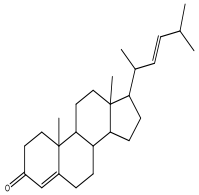
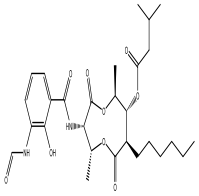
Table 3. Bioactive compounds identified in Methanol seed extract of *Clitoria ternatea* by High Resolution- Liquid Chromatography and Mass Spectrometry in + ve electron spray ionization mode

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
1	1.493	Pirbuterol	6-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)pyridin-3-ol	C12 H20 N2 O3		240.1466	241.1538	3.2	Used for the treatment of asthma [52]
2	3.041	Gentianadine	3,4-dihydropyrano[3,4-c]pyridin-1-one	C8 H7 N O2		149.0472	150.0543	3.12	Anti-inflammatory and muscular relaxant actions [53]
3	3.363	N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	*N*-heptan-4-yl-1,3-benzodioxole-5-carboxamide	C15 H21 N O3		263.1514	264.1588	2.87	Used in food and beverage applications [54]
4	3.487	Isocarbostyril	2H-isoquinolin-1-one	C9 H7 N O		145.0522	146.0595	3.78	Anti-tumor agent [55]
5	3.659	2-Carboxy-4-dodecanolide	5-octyl-2-oxooxolane-3-carboxylic acid	C13 H22 O4		242.1506	265.1398	5.16	

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
6	3.926	2-Hydroxy-6-oxo-octa-2,4-dienoate	(2*E*,4*Z*)-2-hydroxy-6-oxoocta-2,4-dienoic acid	C8 H10 O4		170.0576	171.0648	1.88	
7	4.614	cis-1,3,4,6,7,11b-Hexahydro-9-methoxy-2H-benzo[a]quinolizine-3-carboxylic acid	(3*R*,11*bS*)-9-methoxy-2,3,4,6,7,11*b*-hexahydro-1*H*-benzo[a]quinolizine-3-carboxylic acid	C15 H19 N O3		261.1362	264.1588	0.96	
8	4.898	U 0521	1-(3,4-dihydroxyphenyl)-2-methylpropan-1-one	C10 H12 O3		180.0781	262.1433	2.82	Has potential in the treatment of Parkinson's disease [56]
9	5.723	Afrormosin	7-hydroxy-6-methoxy-3-(4-methoxyphenyl)chromen-4-one	C17 H14 O5		298.0834	299.0907	2.58	Anti- inflammatory activity [40]
10	6.591	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		434.1558	435.1632	4.29	Anti-prolferative activity [57]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
11	7.69	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-methyloxan-3-yl] dihydrogen phosphate	C35 H62 N O15 P		767.388	790.377	-3	
12	8.476	TR-Saponin B	6-[[7,8-dihydroxy-8*a*-(hydroxymethyl)-4-methoxycarbonyl-4,6*a*,6*b*,11,11,14*b*-hexamethyl-9-(2-methylbutanoyloxy)-10-[(*Z*)-2-methylbut-2-enoyl]oxy-1,2,3,4*a*,5,6,7,8,9,10,12,12*a*,14,14*a*-tetradecahydropicen-3-yl]oxy]-3,5-dihydroxy-4-(3,4,5-trihydroxyoxan-2-yl)oxyoxane-2-carboxylic acid	C52 H80 O20		1024.5218	1047.5112	2.41	
13	8.954	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		567.2871	568.2945	-1.24	Antibacterial compound shows inhibitory action on *Stevia rebaudiana* [58]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
14	9.553	Sayanedine	3-(4-hydroxy-3-methoxyphenyl)-7-methoxychromen-4-one	C17 H14 O5		298.0835	299.0908	2.18	
15	9.806	R1128C	1,3,6-trihydroxy-8-(3-methylbutyl)anthracene-9,10-dione	C19 H18 O5		326.1144	327.1216	3.1	Used in estrogen-receptor positive breast cancer [59]
16	10.649	C16 Sphinganine	(2*S*,3*R*)-2-aminohexadecane-1,3-diol	C16 H35 N O2		273.2661	274.2733	2.56	
17	13.23	Garbogiol	5,7,10-trihydroxy-1,1,2-trimethyl-2*H*-furo[2,3-c]xanthen-6-one	C18 H16 O6		328.0937	329.101	3.06	Inhibition of α-glucosid [37]
18	14.693	MG(18:2(9Z,12Z)/0:0/0:0)[rac]	2,3-dihydroxypropyl (9Z,12Z)-octadeca-9,12-dienoate	C21 H38 O4		354.2759	355.2837	3.21	Inhibition of bacterial spores [60]
19	17.61	Ganoderic acid F	(6*R*)-6-[(5*R*,10*S*,12*S*,13*R*,14*R*,17*R*)-12-acetyloxy-4,4,10,13,14-pentamethyl-3,7,11,15-tetraoxo-2,5,6,12,16,17-hexahydro-1*H*-cyclopenta[a]phenanthren-17-yl]-2-methyl-4-oxoheptanoic acid	C32 H42 O9		570.2837	593.2731	-1.44	Inhibits the growth of cancer cells, anti-angiogenic and act as cytotoxicity against cancer cells [61]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
20	18.007	Ganosporelactone A	10',20'-dihydroxy-2',3,7',9',13',17',17'-heptamethylspiro[oxolane-5,5'-pentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icos-1(12)-ene]-2,3',11',16'-tetrone	C30 H40 O7		512.2786	535.2677	-2.34	
21	18.309	Imperialine	(1*R*,2*S*,6*S*,9*S*,10*S*,11*R*,14*S*,15*S*,18*S*,20*S*,23*R*,24*S*)-10,20-dihydroxy-6,10,23-trimethyl-4-azahexacyclo[12.11.0.0^{2,11}.0^{4,9}.0^{15,24}.0^{18,23}]pentacosan-17-one	C27 H43 N O3		429.3228	430.33	3.4	Treatment of inflammatory disease [62]
22	18.685	Glycerol triundecanoate	2,3-di(undecanoyloxy)propyl undecanoate	C36 H68 O6		596.4987	597.506	4.92	Used to maintain liver glycogen [63]
23	19.397	(E)-26,27-Dinorergosta-4,22-dien-3-one	10,13-dimethyl-17-[(E)-5-methylhex-3-en-2-yl]-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one	C26 H40 O		368.3085	391.2977	-1.68	
24	19.466	Antimycin A1	[(2*R*,3*S*,6*S*,7*R*,8*R*)-3-[(3-formamido-2-hydroxybenzoyl)amino]-8-hexyl-2,6-dimethyl-4,9-dioxo-1,5-dioxonan-7-yl] 3-methylbutanoate	C28 H40 N2 O9		548.2765	549.2838	-5.66	Anti-angiogenic agent, in hibitor of mitochondrial electron transport system, and depletion of mitochondria [64]

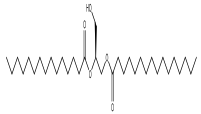
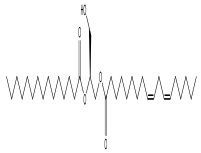
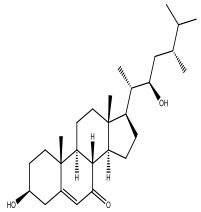
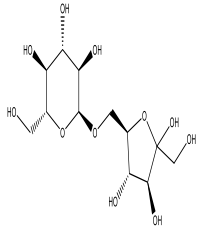
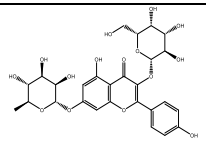
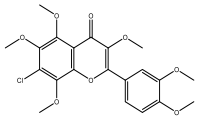
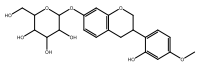
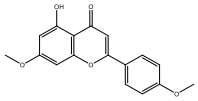
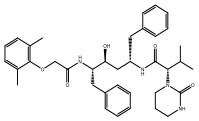
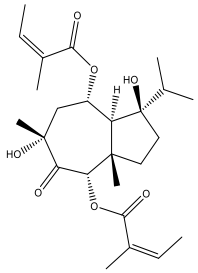
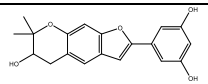
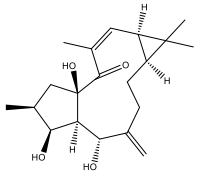
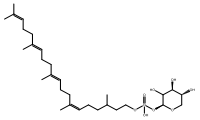
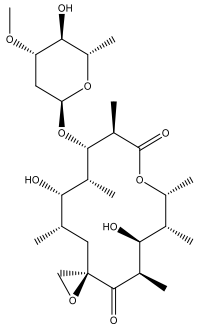
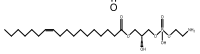
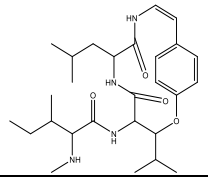
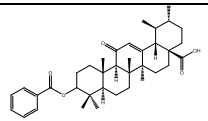
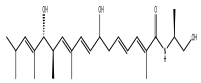
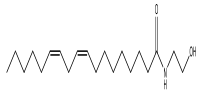
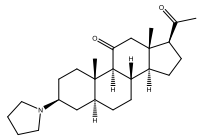
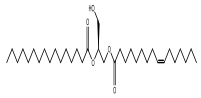
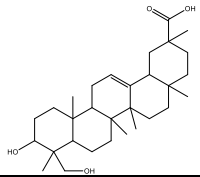
S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
25	19.704	DG(16:0/15:0/0:0)	[(2S)-3-hydroxy-2-pentadecanoyloxypropyl] hexadecanoate	C34 H66 O5		554.4914	577.4805	-0.59	
26	20.641	DG(18:2(9Z,12Z)/15:0/0:0)	[(2S)-3-hydroxy-2-pentadecanoyloxypropyl] (9Z,12Z)-octadeca-9,12-dienoate	C36 H66 O5		578.4886	579.496	4.13	
27	21.272	Schleicherastatin 6	(3S,8S,9S,10R,13R,14S,17R)-3-hydroxy-17-[(2S,3R,5R)-3-hydroxy-5,6-dimethylheptan-2-yl]-10,13-dimethyl-1,2,3,4,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-7-one	C28 H46 O3		430.3426	431.3498	4.97	

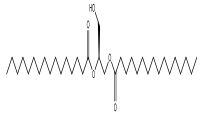
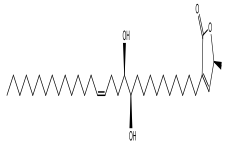
Table 4. Bioactive compounds identified in Methanol seed extract of *Clitoria ternatea* by High Resolution- Liquid Chromatography and Mass Spectrometry in - ve electron spray ionization mode

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
1	1.04	Isomaltulose	(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-[[(2*R*,3*S*,4*S*)-3,4,5-trihydroxy-5-(hydroxymethyl)oxolan-2-yl]methoxy]oxane-3,4,5-triol	C12 H22 O11		342.1156	387.1139	1.73	Can be used as alternative sweetners [65]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
2	5.675	Astragalin 7-rhamnoside	5-hydroxy-2-(4-hydroxyphenyl)-3-[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-7-[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one	C27 H30 O15		594.1582	593.1512	0.41	
3	5.759	7-Chloro-3,3',4',5,6,8-hexamethoxyflavone	7-chloro-2-(3,4-dimethoxyphenyl)-3,5,6,8-tetramethoxychromen-4-one	C21 H21 Cl O8		436.0925	495.1064	0.04	
4	6.545	Vestitone 7-glucoside	2-[[3-(2-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-chromen-7-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol	C22 H26 O9		434.1574	433.15	0.74	
5	6.854	Apigenin 7,4'-dimethyl ether	5-hydroxy-7-methoxy-2-(4-methoxyphenyl)chromen-4-one	C17 H14 O5		298.085	297.0777	-3.02	Antioxidant activity, antidiabetic activity and antiobesity potential [66]
6	8.431	Lopinavir	(2S)-N-[(2S,4S,5S)-5-[[2-(2,6-dimethylphenoxy)acetyl]amino]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide	C37 H48 N4 O5		628.3612	687.3755	2.02	Used in combination with ritonavir to treat human immunodeficiency virus (HIV) infection [67]
7	8.715	Laserpitin	[(3R,3aS,4S,6R,8S,8aS)-3,6-dihydroxy-6,8a-dimethyl-8-[(Z)-2-methylbut-2-enoyl]oxy-7-oxo-3-propan-2-yl-1,2,3a,4,5,8-hexahydroazulen-4-yl] (Z)-2-methylbut-2-enoate	C25 H38 O7		450.2635	495.2616	-3.98	Improves serum lipoprotein metabolism by elevation of HDL levels and inhibition of hepatic cholesterol synthesis [68]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
8	10.273	Moracin P	5-(6-hydroxy-7,7-dimethyl-5,6-dihydrofuro[3,2-g]chromen-2-yl)benzene-1,3-diol	C19 H18 O5		326.1158	325.1085	-1.15	For the development of novel anti-inflammatory drugs [69]
9	10.657	Lathyrol	(1R,3Z,5R,7S,11R,12R,13S,14S)-1,11,13-trihydroxy-3,6,6,14-tetramethyl-10-methylidenetricyclo[10.3.0.05,7]pentadec-3-en-2-one	C20 H30 O4		334.2154	333.2082	-2.92	Treatment of lung cancer [70]
10	14.403	Dolichyl D-xylosyl phosphates	[(6Z,10E,14E)-3,7,11,15,19-pentamethylicosa-6,10,14,18-tetraenyl] [(2S,4S,5R)-3,4,5-trihydroxyoxan-2-yl] hydrogen phosphate	C30 H53 O8 P		572.347	571.3399	1.34	
11	14.645	L-Oleandrosyl-oleandolide	(3R,5S,6S,7R,8S,9R,12R,13R,14S,15R)-6,14-dihydroxy-8-[(2R,4S,5S,6S)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-1,11-dioxaspiro[2.13]hexadecane-10,16-dione	C27 H46 O10		530.3098	529.3027	-1.28	
12	14.685	LysoPE(18:1(11Z)/0:0)	[(2R)-3-[2-aminoethoxy(hydroxy)phosphoryl]oxy-2-hydroxypropyl] (Z)-octadec-11-enoate	C23 H46 N O7 P		479.3008	478.2941	0.78	
13	15.383	Hovenine A	3-methyl-2-(methylamino)-N-[(10Z)-7-(2-methylpropyl)-5,8-dioxo-3-propan-2-yl-2-oxa-6,9-diazabicyclo[10.2.2]hexadeca-1(14),10,12,15-tetraen-4-yl]pentanamide	C27 H42 N4 O4		486.3197	531.3181	1.79	

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
14	15.469	3-Benzoyloxy-11-oxo-12-ursen-28-oic acid	10-benzoyloxy-1,2,6a,6b,9,9,12a-heptamethyl-13-oxo-1,2,3,4,5,6,6a,7,8,8a,10,11,12,14b-tetradecahydropicene-4a-carboxylic acid	C37 H50 O5		574.3624	573.3554	5.9	
15	16.268	Myxalamid S	(2E,4E,8E,10E,12R,13R,14E)-7,13-dihydroxy-N-[(2S)-1-hydroxypropan-2-yl]-2,10,12,14,16-pentamethylheptadeca-2,4,8,10,14-pentaenamide	C25 H41 N O4		419.3036	418.2965	-0.13	
16	16.444	Linoleoyl Ethanolamide	(9Z,12Z)-N-(2-hydroxyethyl)octadeca-9,12-dienamide	C20 H37 N O2		323.2827	382.2965	-0.81	Anti-inflammatory effects of LE were examined using *in vitro* cell culture and *in vivo* animal experiments [71]
17	16.651	3beta-(1-Pyrrolidinyl)-5alpha-pregnane-11,20-dione	(3S,5S,8S,9S,10S,13S,14S,17S)-17-acetyl-10,13-dimethyl-3-pyrrolidin-1-yl-1,2,3,4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-11-one	C25 H39 N O2		385.2986	444.3126	-1.37	
18	17.921	DG(16:1(9Z)/16:0/0:0)	[(2S)-1-[(Z)-hexadec-9-enoyl]oxy-3-hydroxypropan-2-yl] hexadecanoate	C35 H66 O5		566.4936	611.4919	-4.5	
19	18.393	omega-hydroxy behenic acid	22-hydroxydocosanoic acid	C22 H44 O3		356.3301	355.3229	-2.9	
20	18.545	Azukisapogenol	10-hydroxy-9-(hydroxymethyl)-2,4*a*,6*a*,6*b*,9,12*a*-hexamethyl-1,3,4,5,6,6*a*,7,8,8*a*,10,11,12,13,14*b*-tetradecahydropicene-2-carboxylic acid	C30 H48 O4		472.3568	471.3496	-3.28	Used for the treatment of inflammation, fever, and bleeding [72]

S.NO	RT	COMPOUND NAME	IUPAC NAME	FORMULA	STRUCTURE	MASS	m/z	DB DIFF (ppm)	MEDICINAL USES
21	18.791	DG(16:0/16:0/0:0)	[(2S)-2-hexadecanoyloxy-3-hydroxypropyl] hexadecanoate	C35 H68 O5		568.5094	613.5079	-4.86	
22	19.681	Cohibin B	(2*S*)-4-[(*Z*,11*R*,12*R*)-11,12-dihydroxytriacont-15-enyl]-2-methyl-2*H*-furan-5-one	C35 H64 O4		548.4838	593.482	-6.08	

Note: RT- Retention Time; IUPAC name- International Union of Pure and Applied Chemistry; m/z- mass / charge number.

Antibacterial activity against *H. pylori*: The antibacterial suspectible test for methanol flower and seed extracts with different concentration of 25, 50, 75, 100, 200 µg/ml were done by agar well diffusion assay against *H.pylori*. Both the methanol flower and seed extracts did not form zone at the minimum concentration of 25 µg/ml. However,as the concentration of the extracts increased, the diameter of zone of inhibition also enlarged. Consequently, methanol flower and seed extracts exhibited 9.55 mm and 9.80 mm zone of inhibition in diameter at the maximum concentration 200 µg/ml. The standard Chloramphenicol registered 12 mm zone in diameter. To evaluate the inhibitory effect of *H.pylori*, the diameter of zone of inhibition (Figs. 5 and 6) was measured and expressed in millimeter (Table 5). Methanol flower and seed extracts exerted more or less same zones of inhibition at 200µg/ml.

Table 5. Agar well diffusion assay of methanol flower and seed extract of *C. ternatea* against *H. pylori*

Name of the sample	Zone of inhibition(mm) / Concentrations of sample (µg/ml)					Standard (Chloramphenicol)
	25	50	75	100	200	(30 µl)
Methanol flower	Nil	0.55	1.75	5.40	9.55	12.60
Methanol seed	Nil	0.25	2.50	5.75	9.80	12.60

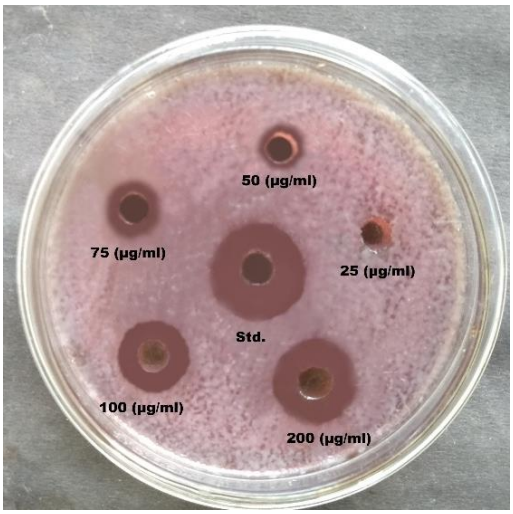


Fig. 5. Antibacterial activity of methanol flower extract

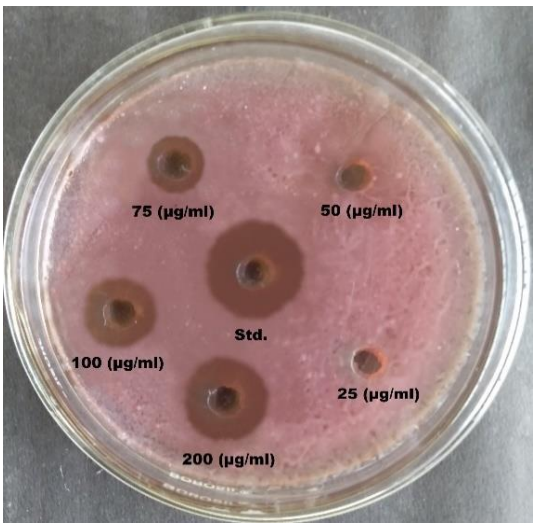


Fig. 6. Antibacterial activity of methanol seed extract

Table 6. Dpph radical scavenging activity of methanol flower and seed extract

Concentration of sample (µg/ml)	Radical scavenging activity (%)		
	Methanol flower extract	Methanol seed extract	Standard (Ascorbic acid)
25	33.72±0.0379	30.71±0.0252	86.13±27.34027
50	35.08±0.0503	31.87±0	89.73±17.7678
75	35.99±0.0153	32.01±0.01	91.55±7.4015
100	37.86±0.0289	35.28±0.1212	93.75±2.7937
200	42.79±0.0819	37.41±0.0265	97.90±45.66200

Values are mean±SD of three parallel measurements

Table 7. Reducing power assay of methanol flower and seed extract

Concentration of sample (µg/ml)	Absorbance at 700 nm		
	Methanol flower extract	Methanol seed extract	Standard (Ascorbic acid)
25	0.143±0.000265	0.0988±0	0.4781±0.05569
50	0.4147±0	0.1098±0	0.5426±0.00015
75	0.43027±0.00029	0.1161±0.00021	0.8393±0.00031
100	0.7329±0.00110	0.1228±0.00015	0.9478±0.00471
200	0.90737±0.00375	0.1254±0.0001	1.104±0.001

Values are mean±SD of three parallel measurements

3.1 Antioxidant Activity

Effect of methanol flower and seed extracts of *C. ternatea* on DPPH radical scavenging activity: Table 6 showed the percentage of radical scavenging activity of methanol flower and seed extracts and standard ascorbic acid as a function of concentrations (25, 50, 75, 100, 200 µg/ml). In low concentration (25µg/ml) of methanol flower and seed extracts had 33.72% and 30.71% DPPH radical scavenging activities. However, at the highest concentration (200µg/ml) of methanol flower and seed extracts, the DPPH radical scavenging activity recorded were: 42.79% and 37.41% respectively. At this high concentration (200 µg/ml), the DPPH radical scavenging activity of methanol flower extract was relatively with (42.79%), when compared with methanol seed extract (37.41%). Furthermore, when compared with standard ascorbic acid methanol flower extract showed better results.

Effect of methanol flower and seed extracts of *C. ternatea* on reducing power assay: The Table 7 showed the reducing power of the methanolic extracts and standard. The results showed a linear increase with the increase in the concentration of sample and standard. At low concentration (25µg/ml) of methanol flower and seed extracts and ascorbic acid the absorbance recorded were: 0.143, 0.0988 and 0.4781 at 700nm respectively. However, at high concentration (200µg/ml) the absorbance registered were: 0.90737, 0.1254 and 1.104, respectively. It indicated that at the high concentration (200 µg/ml) the reducing power assay values registered for ascorbic acid (1.104) and methanol flower extract (0.90737) were more or less similar, these values were greater than methanol seed extract.

4. DISCUSSION

In recent days, plants play a vital role in producing the bioactive novel drug compound. The phytochemicals which were present in the plants exhibits enormous medicinal properties, which is used in Unani and Ayurveda for the treatment of disease. This present study, was focused on the identification of secondary metabolites of methanol flower and seed extract of *C. ternatea* using HRLCMS and assessing their antibacterial (*H. pylori*) and antioxidant activities (DPPH and Reducing power assay).

HRLCMS analysis of methanol flower and seed extract of *C.ternatea* showed the presence of more numbers of bioactive compounds which were had different pharmacological activities. Bioactive compounds present in methanol flower extract of *C.ternatea* such as Adenine is used in the treatment of HIV, HBV, CMV and other virus –infected diseases [31], Quercetin is to treat anticancer, cardiovascular protection, anti-inflammatory, antidiabetic, gastroprotection effects, anti-infective and inhibits gastric acid secretion and inhibits *Helicobacter pylori* infection [33], 6-C Galactosylluteolin had employed in therapeutic treatment for coronavirus disease (COVID-19) [34], 6-Hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one and Phytosphingosine has shown Antimicrobial activity [35 & 44], Morindone is used to treat a variety of health issues including, high blood pressure, arthritis, ulcers, depression, menstrual

cramps, pain relief, inflammation, burns, fever, food poisoning, intestinal worms, and joint problems [36], Formononetin has effective treatment for cancer [38], Garbogiol is used in Inhibition of α-glucosid [37], Betavulgarin has emerged as Anticancer agent against breast cancer [39], Afrormosin, Bowdichione and Pheophytin a had Anti-inflammatory properties [40, 43 & 49], Aspulvinone E, is a marine metabolite used to develop novel antiinfluenza virus agents with high efficiency and low toxicity [41], Ganoderic acid F inhibits the growth of cancer cells, anti-angiogenic and displays significant cytotoxicity against cancer cells [46], Goyaglycoside c is used as a bitter stomachic, laxative, is used as an antidiabetic, and anthelmintic for children [47], Fucosterol help to reduce blood cholesterol, blood vessel thrombosis preventive and butyrylcholinesterase inhibitory activities [48], Theophylline were used in the treatment of asthma, chronic obstructive lung diseases [50], Calpeptin suppresses the pancreatic cancer [73] and also to treat acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, inhibit chronic inflammation, tissue damage and pulmonary fibrosis [51].

Bioactive compound which were reported the methanol seed extract of *C.ternatea* such as Pirbuterol is used for the treatment of asthma [52], Gentianadine is employed to treat anti-inflammatory and muscular relaxant actions [53], Isocarbostyril act as Anti-tumor agent [55], U 0521 has potential in the treatment of Parkinson's disease [56], Afrormosin, Imperialine and Linoleoyl Ethanolamide have showed anti-inflammatory activity [40,62 &71], Eleganin has anti-proliferative activity [57], Dihydrodeoxystreptomycin is an antibacterial compound showed inhibitory action on *Stevia rebaudiana* [58], Garbogiol showed inhibition of α-glycoside [37], MG(18:2(9Z,12Z)/0:0/0:0)[rac] showed inhibition of bacterial spores (60), Ganoderic acid F inhibits the growth of cancer cells, anti-angiogenic and displays significant cytotoxicity against cancer cells [61], Apigenin 7,4'-dimethyl ether had antioxidant activity, antidiabetic activity and antiobesity potential [66], Lopinavir used in combination with Ritonavir to treat human immunodeficiency virus (HIV) infection [67], Laserpitin improves serum lipoprotein metabolism by elevation of HDL levels and inhibition of hepatic cholesterol synthesis [68], Moracin P has the potential for the development of novel anti-inflammatory drugs [69], Lathyrol is used in the treatment of lung cancer [70] and Azukisapogenol used for the treatment of inflammation, fever, and bleeding [72].

It was reported that *H. pylori* is a type of bacteria that can impinge on the stomach and cause a variety of diseases with gastrointestinal symptoms, including ulcers and gastritis. *H. pylori* infection affects 50% of the world population [74]. WHO has classified *H. pylori* is classified as Class I carcinogen by WHO and it is reported that when the combination of certain antibiotic can kill this bacteria [75]. However, combination of antibiotics may causes side effects, these may lead the researchers to move on to the natural sources especially plant based sources. Nostro et al., [76] investigated the antibacterial activity of 17 plant materials against *H. pylori*. In their study, *Cynara scolymus L.* and *Zingiber officinalis* showed anti- *H.pylori* activity at lowest concentration (0.6 mg/ml). Recent study reported that crude extract of *Monteverdia ilicifolia* with acetone: water (MIC 64 µg/ml) showed better activity against *H.pylori* [78]. *H.pylori* is to cause inflammation in the stomach (gastritis) which may lead to cause cancer. Traditionally, two antibiotics and a proton pump inhibitor are used to eradicate the *H.pylori*. Unfortunately, these treatment take excessive amount of drug and cause side effects and expensive. The present study results showed that methanol flower and seed extract of *C.ternatea* had shown a better zone of inhibition at 200µg/ml with the diameter of 9.55 mm and 9.80 mm respectively. This may be attributed to the presence of vital bioactive compounds such as 6-Hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one and Phytosphingosine which were reported to have antimicrobial activity [35 & 44], Afrormosin, Bowdichione and Pheophytin with anti-inflammatory properties [40, 43 & 49], Quercetin which has shown anti-inflammatory properties, and also potentially inhibits *Helicobacter pylori* infection [33] in the methanol flower extract. Furthermore, the methanol seed extract also had major bioactive compounds such as Imperialine, Linoleoyl Ethanolamide and Gentianadine with anti- inflammatory activity [62, 71 & 53], Dihydrodeoxystreptomycin reported to be an antibacterial compound showed inhibitory action on *Stevia rebaudiana* [58], Azukisapogenol were used for the treatment of inflammation, fever, and bleeding [72]. Bioactive compounds from both methanol and seed extract of *C.ternatea* have shown an anti-inflammatory properties and antimicrobial activity too and it may lead to identification of novel drug against *H.pylori*.

Furthermore, antioxidants are nutrient as well as enzymes that plays a vital role in the disease management. In general, there are various methods for determination of antioxidant activities. Here, we applied DPPH and reducing power assay. The result of present study evidenced that *C.ternatea* methanol flower extract had better radical scavenging activity when compared to methanol seed extract at 200µg/ml (Table 6). The decrease in the absorbance is due to the presence of unique potential antioxidant in the plant extracts which causes reaction between free radicals and antioxidant. When an antioxidant reacts with DPPH it forms to DPPHH. This is due to the presence of lower amount of hydrogen, and it lower absorbance than DPPH, which may leads to decolourization [77].

In reducing power assay estimation, methanol flower extract showed better result than the methanol seed extract at 200µg/ml. At 200µg/ml of methanol flower extract the reducing power assay (absorbance) recorded was 0.90737±0.00375 (Table 7), which was comparable with the values recorded for the standard ascorbic acid. This is due to the principle that when antioxidant substance react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) and also react with ferric chloride to form ferric-ferrous complex which has the absorbance at 700nm. This may be attributed the presence of flavonoid compounds like Quercetin, Kaempferol 4'-glucoside 7- rhamnoside, and 6-C-Galactosylluteolin; and isoflavonoids like 3-O-Methylcoumestrol, (+)-Sophorol, Formononetin, Afrormosin; Phenol like Gyrocyanin; Amines like Phytosphingosine in methanol flower extract of *C.ternatea*. All these metabolites, which were present in methanol flower extract have promoted its potent antioxidant activity.

5. CONCLUSION

Clitiora ternatea is a universal plant known as an edible flower with medicinal and ornamental value and as a natural remedy for various health issues. The present results inferred that methanol seeds and flower extracts showed obvious antibacterial activity against *H.pylori.* These extracts contained a range of bioactive compounds, which contributed to their antibacterial properties. However, the methanol flower extract registered better with antioxidant activity that resists the noxious unstable free radical molecules that can damage cells and contribute to the development of chronic diseases, compared to methanol seed extract. This study implied that both extracts had potential biomedical applications.

ACKNOWLEDGEMENT

The second author Dr.A.Palavesam great fully acknowledge the UGC- New Delhi for the award of UGC-BSR-Faculty fellowship (UGC-F.No.5-1-151 (2020)). The authors also acknowledged IIT, Bombay for HR-LCMS analysis of experimental extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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