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QUALITATIVE EVALUATION OF THE ANTIMICROBIAL, ANTIOXIDANT, AND MEDICINALLY IMPORTANT PHYTOCHEMICAL CONSTITUENTS OF THE ETHANOLIC EXTRACTS OF THE LEAVES OF *GLIRICIDIA SEPIUM* (JACQ.) WALP.

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ABSTRACT

The leaves of *Gliricidia sepium* (Jacq.) Walp. have been reported to have ethnomedicinal uses such as wound healing, skin itching or dermatitis, and others. This study was therefore conducted to evaluate its biological properties, whether ethanolic leaf extract of *G. sepium* (Jacq.) Walp. has antimicrobial and antioxidant activities, and whether these are reflected in the presence of antimicrobial and antioxidant phytochemicals in the extract. Antimicrobial properties using the agar well-diffusion method showed inhibition against bacterial organism *Bacillus subtilis* and fungus *Candida albicans*. The antioxidant activity evaluated by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) photometric assay showed that *G. sepium* (Jacq.) Walp. is a medium antioxidant with an IC_{50} of 144 ppm. Phytochemicals present in the extract showed the presence of flavonoids, saponins, steroids, and tannins, which are known as antimicrobial and antioxidant agents. Evaluation of the bioactive compounds in the extract using Gas Chromatography-Mass Spectrometry identified twenty-two (22) possible bioactive compounds. Of these, ten (10) were found to be known antimicrobials, while eight (8) compounds were found to be known antioxidants based on published literature. This study has shown support to the ethnomedicinal uses of the leaves of *G. sepium* (Jacq.) Walp. as an antimicrobial and antioxidant plant.

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Introduction

Medicinal properties of plants always have interested people since ancient times. Different cultures around the world have a variety of medicinal traditions that include the use of plants and other plant products in curing different diseases [1]. A report of the World Health Organization tells that 80% of the world population mainly rely on traditional therapies which involve the use of plant extracts or their active substances [2]. Plant products remain the principal source of pharmaceutical drugs and agents used in traditional medicine [3]. With about half a million plants around the world, medicinal plants have a promising future [4]. Medicinal plants are considered as a rich resource of ingredients which can be used in drug development including pharmacopoeial, non- pharmacopoeial, or synthetic drugs [5]. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants and secondary metabolites, of which at least 12,000 have been isolated; a number estimated to be less than 10% of the total [4]. Through the Department of Science and Technology (DOST), the Philippine government is now addressing the need to assess scientifically the use of medicinal plants in the country [6].

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In the Philippine setting, the use of herbal medicine has been a part of its rich culture and tradition. Even during the early times, Filipinos prepared medicines to treat some illnesses out of the plants available in their backyards. An example of these plants is Kakawate (*G. sepium* (Jacq.) Walp) which has folkloric uses such as wound healing, skin itching, or dermatitis [7]. Gliricidia, a native of the seasonally dry Pacific Coast of Central America, has long been cultivated and is naturalized in tropical Mexico, Central America, and northern South America. It was also introduced to the Caribbean and later to West Africa. The Spaniards took it to the Philippines in the early 1600s. From Trinidad, it was taken to Sri Lanka in the 1800s; and reached other Asian countries, including Indonesia (about 1900), Malaysia, Thailand, and India [8]. In Mexico, *G. sepium* (Jacq.) Walp is used as shade for cocoa and coffee plantations, and this for reason, it is called 'Madrecacao' (mother of cocoa). It is also used as a poison for rodents and in fact, the Latin name Gliricidia means rodent poison [9].

Entire parts of this plant- leaves, barks, roots, etc. have been reported to have ethno-medicinal properties [2]. It has been used as a folk remedy for alopecia, boils, bruises, burns, colds, cough, debility, eruptions, erysipelas, fever, fractures, gangrene, headache, itch, prickly heat, rheumatism, skin tumors, ulcers, urticarial, and wounds [10]. In Panama, the decoction of *G. sepium* (Jacq.) Walp leaves is used in urticaria, rash and also in burns and erysipelas. In Guatemala and Costa Rica, bark decoction is used against bacterial and protozoal infections [9]. The traditional use of branches and leaves of *G. sepium* is against pruritic ailments, fever, and is frequently used plant for skin infections [11]. This is served as the basis for the antimicrobial evaluation in this study.

Gas Chromatography-Mass Spectroscopy, a hyphenated system is a very compatible technique, and the most commonly used technique for the identification and quantification purposes [12]. It is one of the best techniques to identify the bioactive constituents of long-chain branched chain hydrocarbons, alcohols, acids, ester, etc. [13]. Understanding these biological properties of *G. sepium* (Jacq.) Walp may help in a clearer understanding of the biological basis of the efficacy of the plant in the treatment of health-related problems. To explore the medicinal importance of the leaf ethanolic extract of *G. sepium* (Jacq.) Walp, this was screened primarily for the phytochemicals present in it and was analyzed using GC-MS.

Fig. 1. *Gliricidia sepium* (Jacq.) Walp

Materials and Methods

The leaves of *Gliricidia sepium* (Jacq.) Walp. were collected and air dried for one week and ground into powder (Fig. 2). Two hundred fifty grams (250.0 grams) of powdered leaves were soaked in one and a half liters (1500 ml) of ethanol for one week in one of the laboratories in College of Science and Mathematics at Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines. Since most of the polar compounds are easily eluted using ethanol, it was used as a solvent in the extraction and used for antimicrobial activity evaluation [14]. The supernatant was filtered using Whatman filter paper No. 1. A rotary evaporator was used at 45°C to concentrate the filtrate. The obtained viscous crude extract was stored in storage vials for antimicrobial and antioxidant activities, phytochemical screening, DPPH assay, and GC-MS analysis. The ethanolic leaf extracts were screened for the presence of phytochemicals and antioxidant activity at the Department of Chemistry in MSU-IIT (Mindanao State University-Iligan Institute of Technology), Iligan City, Philippines and was qualitatively analyzed using GC-MS at the Analytical Services Laboratory of the Ateneo de Davao University-Chemistry Department in Davao City, Philippines. The antimicrobial assay was done at the Microbiological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman.

Fig. 2. The leaves of *G. sepium* (Jacq.) Walp. used in the study.

For the antimicrobial properties of the ethanolic extract, agar disk diffusion test was used against the selected test microorganisms. These were the Gram-negative bacteria *Klebsiella pneumoniae* UPCC 1360 and *Salmonella typhimurium* UPCC 1368, the Gram-positive bacteria *Bacillus subtilis* UPCC 1295 and *Staphylococcus aureus* UPCC 1143, and the fungi *Candida albicans* UPCC 2168 and *Aspergillus niger* UPCC 4219. The agar disk diffusion test was used for its ability to detect the antimicrobial activity of the isolates [15]. The qualitative method of the zone of inhibition was used to measure the susceptibility of the bacteria towards the standard antibiotic [16] and the extracts since it offers many advantages being simple, inexpensive, has the ability to test enormous numbers of microorganisms and antimicrobial agents, and the simplicity to interpret results provided [17]. Chloramphenicol was used as the positive control to compare with the extracts for the gram-negative and gram-positive bacteria which is said to have properties that diffuse efficiently in the body and does not ionize at physiological [18]. Chloramphenicol is an antibiotic for the treatment of serious and systemic infections [19]. Canesten solution (with 1% Clotrimazole) was used as the positive control to compare the response of the extracts with the fungi. Clotrimazole is an antifungal medication sold under the brand name Canesten. It shows an antifungal activity by targeting the biosynthesis of ergosterol, allowing the inhibition of fungal growth [20].

Free radical scavenging activity of root ethanolic extracts of *G. sepium* (Jacq.) Walp plant was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH). DPPH radical scavenging assay is a simple and accurate test used for measuring the ability of different compounds to act as free radical scavengers as well as to evaluate the antioxidant activity of medicinal plants [21].

For phytochemical screening, the powdered leaf ethanolic extract of *G. sepium* (Jacq.) Walp. was subjected to qualitative evaluation for the presence of alkaloids, anthraquinones, cyanogenic glycosides, flavonoids saponins, steroids, and tannins which were recorded using a 3-point scale [+ turbid, ++ moderate and +++ heavy] in scoring based on the Handbook of Philippine Medicinal Plants [22]

The GC-MS analysis was performed following the protocol of Chipiti *et al*. (2015) [23], with modifications to identify the compounds present in the ethanolic extract of *G. sepium* (Jacq.) Walp. leaves. Gas-chromatography mass-spectrometry is an analytical method used to facilitate, identify, and quantify several different metabolites present in a plant extract which results in comprehensive coverage of primary metabolic pathways [24].

Results and Discussion

Ethnomedicinal Uses

The folkloric uses of *G. sepium* (Jacq.) Walp. were determined through findings based on literature. Entire parts of this tree-leaves, barks, roots, etc. have been reported to have ethnomedicinal properties [2]. Other reported uses are shown in Table 1.

Table 1. Ethnomedicinal Uses of the Gliricidia sepium (Jacq.) Walp.

Country	Traditional Preparation	Parts Used	Medical Uses
Panama	Decoction	Leaves	Urticaria, rash, burns, erysipelas [9]
Costa Rica	Decoction	Bark	Bacterial and Protozoal Infection [9]
Guatemala	Decoction	Bark	Bacterial and Protozoal Infection [9]
Philippines	Poultice	Leaves	Wound healing, skin itching or dermatitis [7]
Philippines	Decoction and Poultice	Branches and Leaves	Pruritic ailments, fever, skin infections [11]
Columbia	Decoction	Leaves	Pruritic ailments, fever, body ache

In vitro Antimicrobial Assay

The *in vitro* antimicrobial activities of the ethanolic crude extract of *G. sepium* (Jacq.) Walp. against Gram-positive and Gram-negative bacteria, as well as fungi assessed using agar well diffusion method is shown in Figure 3 and Table 2. Results showed that the extract has inhibitory effects against some of the test microorganisms but not as efficient when compared to the standard antibiotic, chloramphenicol, and Clotrimazole.

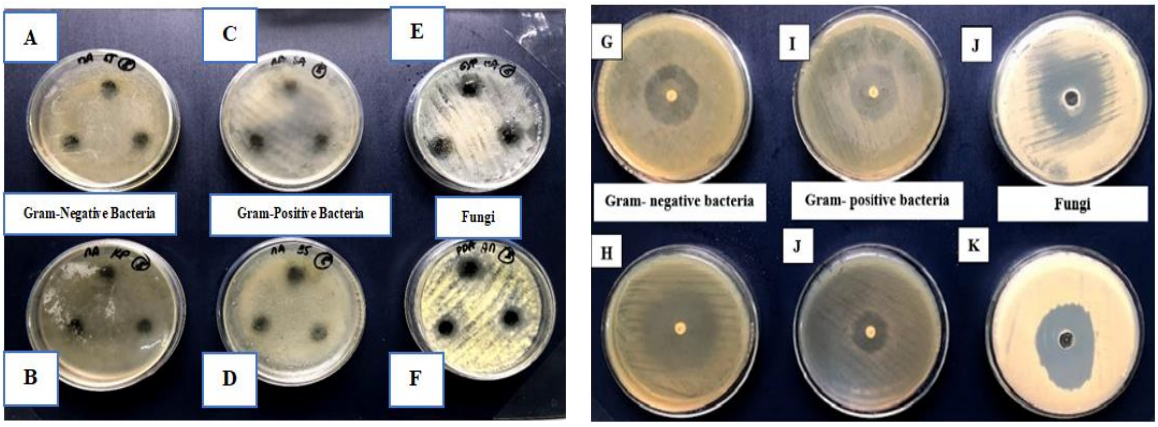

Figure 3. The Antibacterial Indices of Gram-negative bacteria: *S. typhimurium* (**A**) and *K. pneumoniae* (**B**); Gram-positive bacteria: *S. aureus* (**C**) and *B. subtilis* (**D**); and Fungi: *C. albicans* (**E**) and *A. niger* (**F**); positive control Chloramphenicol used in Gram-negative bacteria (**G&H**) and Gram-negative bacteria (**I&J**); Canesten solution in Fungi (**K&L**) using agar well diffusion method in three replicates of the *G. sepium* (Jacq.) Walp. *leaf* ethanolic extract.

Table 2. Computed average of Inhibition Zone (mm) and Antimicrobial Index (AI) from the test organisms in the G. sepium (Jacq.) Walp. leaf ethanolic extract

Test Organisms	Sample	Clearing Zone, mm			AI
		1	2	3	
[1]*S. typhimurium*	Madre de cacao (*G. sepium)*	-[a]	-	-	0
	Chloramphenicol disc[b]	30			4.0
[2]*k. pneumoniae*	Madre de cacao (*G. sepium)*	-	-	-	0
	Chloramphe nicol disc	38			5.3
[3]*S. aureus*	Madre de cacao (*G. sepium)*	-	-	-	0
	Chloramphenicol disc	33			4.5
[4]*B. subtilis*	Madre de cacao (*G. sepium)*	13	13	13	0.3
	Chloramphenicol disc	20			2.3
[5]*C. albicans*	Madre de cacao (*G. sepium)*	13	13	13	0.3
	Canesten solution[c] 100 µL	35			2.2
[6]*A. niger*	Madre de cacao (*G. sepium)*	-	-	-	0
	Canesten solution 100 µL	42			3.2

The result showed antimicrobial activity against the Gram-positive bacterium *B. subtilis* with an antimicrobial index of 0.3mm, and it is concurrent with earlier studies of *G. sepium* (Jacq.) Walp leaf ethanolic extract, which also reported anti-bacterial activity [25]. Other than *B. subtilis*, the leaf extract also inhibited the growth of the *C. albicans* with an antimicrobial index of 0.3mm. However, it did not show antimicrobial activity against the Gram-negative bacteria *S. typhimurium* and *K. pneumoniae*, the Gram-positive bacteria *S. aureus*, and fungus *A. niger* (Fig. 3, Table 2). But, in the study of Akharaiyi *et al.* (2012), the ethanolic leaf extract of *G. sepium* inhibited the growth of *S. typhimurium, K. pneumoniae, S. aureus* and other clinical bacteria like *Pseudomonas aeruginosa, S. marcescens, E. coli, B. cereus, Proteus mirabilis*, and *Enterococcus feacium*. It exhibited antibacterial potency on all the organisms with an inhibitory halo of 6 to 17 mm in diameter. The differences in the results could be due to variations in the variety and the ecology of the plant used.

However, even the results have shown slow inhibition to the 2 out of the six bacterial and fungal microorganisms; it is essential to note that the Madre de cacao has inhibitory actions. This may explain its use against infections such as *B. subtilis*, an aerobic spore-forming rod bacteria that can cause endocarditis, meningitis, osteomyelitis [26], and the fungus *C. albicans*, an opportunistic pathogenic yeast that overgrowth of these fungi can cause problems in the skin, oral cavity, the gastrointestinal tract, and the reproductive tract [27]. These may explain the earlier reported use of the coconut root against infections. The root extract's antimicrobial activities may provide a scientific basis for its use in traditional medicine.

In Vitro Antioxidant Assay

Table 3 shows the result of the antioxidant activity evaluation of the *G. sepium* (Jacq.) Walp. leaf ethanolic extract. The ethanolic extracts have shown to be a potential source for antioxidants with an IC_{50} value of 144ppm. IC_{50} was used to determine the antioxidant capacity of the sample compared to standard. The sample that had IC50 < 50 ppm, was a very strong antioxidant, 50- 100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50}>150 ppm [28]. Based on the result, it can be inferred that *G. sepium* (Jacq.) Walp. is a medium antioxidant.

Table 3. Antioxidant properties of the leaf ethanolic extract of G. sepium (Jacq.) Walp.

Extract Concentration (ppm)	**Percent Inhibition (%)**
Control	0
5	1.12
10	13.48
20	15.11
30	15.61
50	23.85
100	38.08
200	65.67
300	**81.52**
IC_{50}=**144 ppm**	

Phytochemical Screening

The result of the phytochemical screening of *Gliricidia sepium* (Jacq.) Walp, presented in Table 4, confirmed the presence of various metabolites in the leaf extract. The ethanolic extract of *G. sepium* (Jacq.) Walp exhibited the absence of alkaloids, anthraquinones and cyanogenic glycosides and the presence of flavonoids, saponins, steroids, and tannins. Flavonoids [29], steroids [30], and saponins [31] are good antioxidant agents. The results are concurrent with an earlier study of Akharaiyi *et al*. (2014) which also reported the presence of Flavonoids, saponins, steroids, tannins, phenols, and terpenes and absence of glycosides in the leaf ethanolic extract of *G. sepium* (Jacq.) Walp. But in the present study, alkaloids are absent while they were present in the previous study.

Table 4. Result of phytochemical screening of the leaf ethanolic extract of *G. sepium (*Jacq.) Walp.

Alkaloids	Anthraquinones	Glycosides	Flavonoids	Saponins	Steroids	Tannins
-	-	-	++	++	+++	+++

(+) indicates present: +turbid, ++moderate, +++ heavy; **(-)** indicates absent

The absence of cyanogenic glycosides and anthraquinones in *G. sepium* (Jacq.) Walp ethanolic leaf extract may indicate no or less toxic effect as these compounds, anthraquinones and its derivatives may cause nausea, vomiting, abdominal cramps and diarrhea with both therapeutic dose and overdose [32] while cyanogenic glycosides may cause food poisoning resulting in gastric irritation and damage [33]. Glycosides in certain cases have toxic effects [34] and though plant glycosides are not normally toxic when ingested orally, they are known to inhibit chloride transport in the stomach [35]. Exposure to cyanide from the accidental or intentional intake of cyanogenic glycosides may result in acute intoxications, characterized by growth retardation and neurological symptoms from tissue damage in the central nervous system (CNS) [36]. Since these two phytochemicals were absent in the extract, it showed that the plant is safe and can be used for medical purposes and traditional preparation such as drink orally for treating diseases.

The phytochemical screening showed that the ethanolic leaf extract of *G. sepium* (Jacq.) Walp has a moderate presence of flavonoids, which are synthesized by plants in response to microbial infection. In vitro, it was found out that flavonoids are antimicrobial against a wide array of microorganisms [37], effective as hepatoprotective, anti-inflammatory, and anticancer [29] and possess anti-aging, antidiabetic and cardioprotective activities [38], the source for anti-allergy, cytotoxicity, osteogenic activity, and estrogenic activity [39]. Known antimicrobial mechanisms associated with flavonoids may explain the effectiveness of its antimicrobial activity of these compounds from the crude extract [40]. This is said to be due to their ability to bind with the bacterial cell wall, which allows the inhibition of the microbial growth. The presence of flavonoids, in *G. sepium* leaves can be a potential source to treat bacterial infections, cardiovascular diseases, and inflammation that correlates to the ethnomedicinal uses of the plant. They are also found to be a good source of antioxidant activities as they can prevent injury caused by free radicals through direct scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes and inhibition of oxidases [41] similar to that of vitamins C in fruits [42].

Moderate presence of saponins was also observed in the extracts. Saponins are known to have the following properties: an inhibitory effect against inflammation, it helps in hypercholesterolemia, has antibiotic properties [37], has antiviral activities, have been used as anti-protozoan, anti-carcinogenic agents [43, 44], it helps in treating typhoid, hemorrhoids, impetigo and

malaria [45, 46] and they have anti-fungal and anti-yeast properties [47]. They also have been noted to have antimicrobial properties, prevent mold formation, and insect attack on plants [48]. This information may explain the ethnomedicinal uses of *G.* sepium leaves to treat bacterial and protozoal infections.

The phytochemical screening of the Ethanolic leaf extract of *G. sepium* (Jacq.) Walp. also showed a heavy presence of tannins. Tannins are said to improve the immune system and are considered to have a wide array of anti-infective effects [49], also known to have general antimicrobial and antioxidant activities [50], antifungal and anti-diarrheal activities [46, 51-53], antiparasitic effects [54], anti-inflammatory, and antihemorrhoidal properties [55]. Tannins were found to be effective antimicrobials [56] specifically against *Staphylococcus aureus, Shigella boydii, Shigella flexneri, Escherichia coli*, and *Pseudomonas aeruginosa* [57], especially in wound healing by its ability to form a protective layer over the exposed tissue keeping the wound from getting more infected [58]. This information may explain the ethnomedicinal uses of *G.* sepium leaves to treat wounds and skin inflammation.

There was also a heavy presence of steroids in the phytochemical screening of the *G. sepium* (Jacq.) Walp. leaves. Steroids have been reported to have antibacterial and analgesic [59], anti-inflammatory, anti-cancer, cytotoxic, and antiproliferative properties [60], and are effective against rheumatoid arthritis [61].

Gas Chromatography-Mass Spectrometry Analysis

The results of the bioactive compound using GCMS showed that a total of ten compounds had antimicrobial properties (Table 4, Figs. 4 and 5). These bioactive compounds include n- tridecane [62, 63], n- hexadecane [64-66], Dodecane, 4,6-dimethyl- [64], n- eicosane [67], heneicosane [68], Neophytadiene [69, 70], 3,7,11,15- Tetramethyl-2-hexadecen-1-of [71, 72], lupeol acetate [73], phytol [74, 75], hexadecanoic acid, and ethyl ester [74]. The exact mechanisms which these active components of the plant extract contribute to the antibacterial activity is not yet known but could be due to the hydrophobic activity of the membrane which enables thermo-partition of the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and allowing them to be become more permeable [75]. The results however, still indicate that the leaves of *G. sepium (*Jacq.) Walp. can be a great potential source for antimicrobials [76-78].

Table 5. Bioactive compounds qualitatively identified from the leaf ethanolic extracts of *G. sepium (*Jacq.) Walp. using GC-MS analysis

	Name of Compound	**Formula**	**SI[a]**	**Mol. Wt.[b]**	**Reported Biological Properties**
1	n-Tridecane	$C_{13}H_{28}$	97	184	**Antibacterial** [62, 63] **Antioxidant** [63] **Antiproliferative against HeLa cells** [61]
2	2H-1-Benzopyran-2-one, 3,4-dihydro-	$C_9H_8O_2$	97	148	No activity reported[c]
3	Hexadecane	$C_{16}H_{34}$	95	226	**Antibacterial** [63-66] **Antifungal** [65, 79, 80] **Antimicrobial** [81] **Antioxidant** [66, 79]
4	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	91	198	**Antibacterial** [63]
5	2,4-Di-t-butylphenol	$C_{14}H_{22}O$	82	206	**Antioxidant** [82]
6	Fumaric acid, ethyl isobutyl ester	$C_{10}H_{16}O_4$	79	200	No activity reported
7	Hexadecane	$C_{16}H_{34}$	96	226	**Antibacterial** [63-66] **Antifungal** [65, 79, 80] **Antimicrobial** [81] **Antioxidant** [66, 79]
8	Heneicosane	$C_{21}H_{44}$	91	296	**Antibacterial** [83] **Anti-inflammatory** [84] **Antimicrobial** [68] **Antioxidant** [84, 85]
9	Undecanal, 2-methyl-	$C_{12}H_{24}O$	85	184	No activity reported[c]
10	Octadecanal	$C_{18}H_{36}O$	89	268	No activity reported[c]
11	Hexadecane	$C_{16}H_{34}$	94	226	**Antibacterial** [63-66] **Antifungal** [65, 79, 80] **Antimicrobial** [81] **Antioxidant** [66, 79]
12	Neophytadiene	$C_{20}H_{38}$	93	278	**Anticancer agent** [86] **Antifungal** [87]

					Anti-inflammatory [88] **Antimicrobial** [69,70] **Antioxidant** [89]
13	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	89	268	No activity reported[c]
14	Neophytadiene	$C_{20}H_{38}$	89	278	**Anticancer agent** [86] **Antifungal** [87] **Anti-inflammatory** [88] **Antimicrobial** [69,70] **Antioxidant** [89]
15	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	88	296	**Antimicrobial** [71, 72] **Anti-diuretic/Anticancer** [90] **Antioxidant** [90]
16	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	72	306	No activity reported[c]
17	Eicosane	$C_{20}H_{42}$	88	282	**Antibacterial Cytotoxic effects** [91] **Anti- corrosive agents** [92] **Anti-fungal** [67] **Antioxidant** [93] **Antitumor activity** [91, 93]
18	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	93	284	**Antibacterial** [38, 76] **Antioxidant** [38]
19	Heneicosane	$C_{21}H_{44}$	90	296	**Antibacterial** [83] **Anti-inflammatory** [84] **Antimicrobial** [68] **Antioxidant** [84, 85]
20	Phytol	$C_{20}H_{40}O$	96	296	**Anticancer** [74, 75] **Anti-inflammatory properties** [74, 75, 88] **Antimicrobial** [74, 75]
21	cis,cis,cis- 7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	88	234	No activity reported[c]
22	Lupeol acetate	$C_{32}H_{52}O_2$	85	468	**Anti-snake venom, antimicrobial, anti-inflammatory, antioxidant** [73]

[a]Similarity Index; [b]Molecular weight; [c]needs further study

Evaluation of the results from the GCMS analysis showed the presence of eight bioactive compounds known to have antioxidant activities. These bioactive compounds include n- tridecane [62], n- hexadecane [66, 79], n- eicosane [67], heneicosane [84, 85], Neophytadiene [89] lupeol acetate [73], hexadecanoic acid, ethyl ester [38] and the polyphenolic compounds 2,4-Di-tert-butylphenol [82] and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol [90]. Polyphenols are argued to act as strong antioxidants as they can prevent oxidative damage and reduce inflammation [94].

It can be seen from this study that the leaf extract from *G. sepium* (Jacq.) Walp. have phytochemicals that are a good source of an antioxidant such as saponins [95], tannins [96], flavonoids and phenolic acids that are said to be good sources for antioxidants enabling the reduction of oxidative stress and allow the protection from the degenerative disease [97]. Thus, the folkloric uses of the *G. sepium* (Jacq.) Walp. leaves as a treatment for selected diseases have a biological and biochemical basis, as shown by the results of the study.

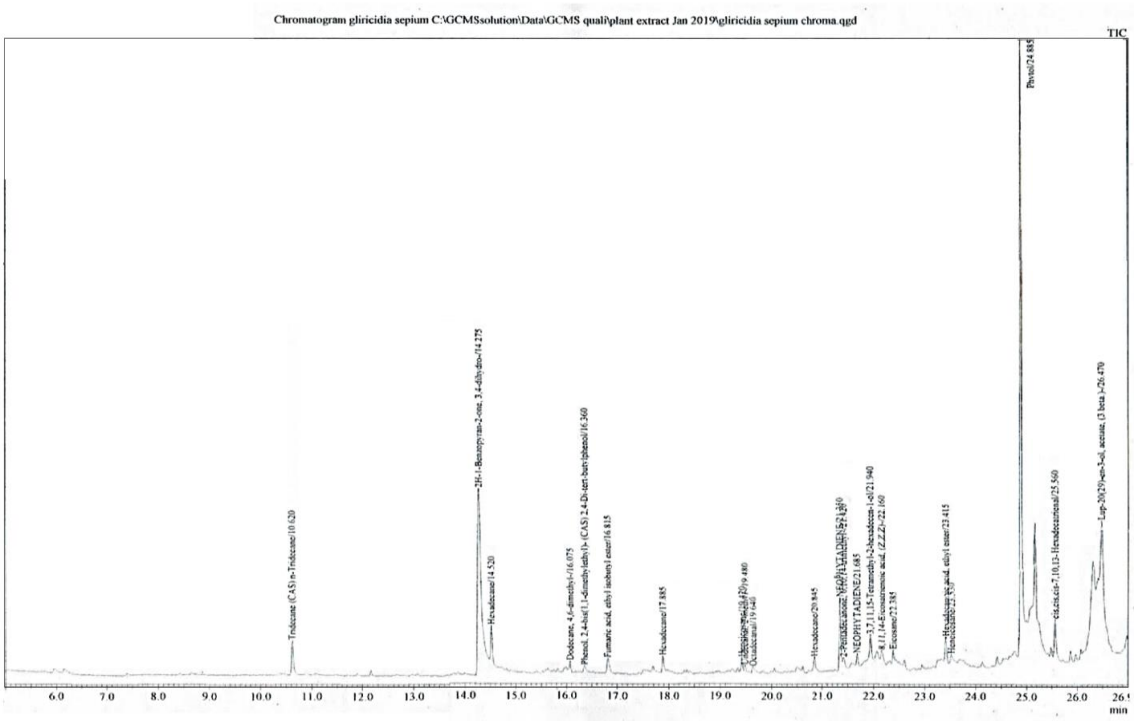

Figure 5. The mass peak of the bioactive compounds against its retention time

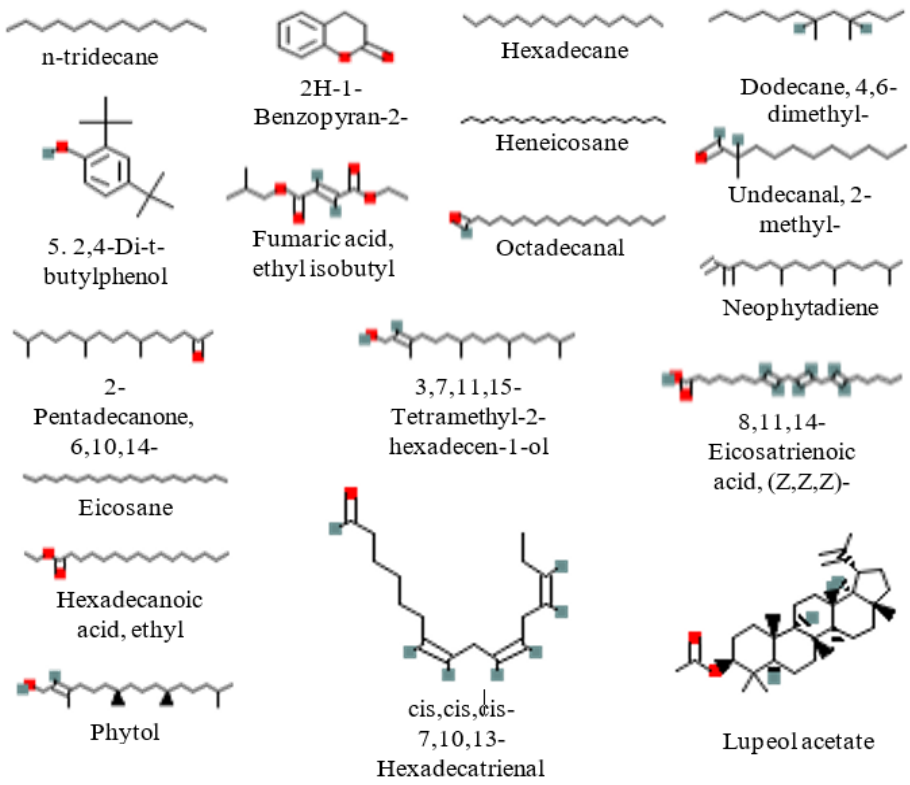

Figure 6. The compounds identified in the leaves of *G. sepium* (Jacq.) Walp.

SUMMARY/CONCLUSION

The results of the study have shown the ethnomedicinal importance of *G. sepium* based on its antimicrobial and antioxidant properties and the presence of phytochemical compounds that are of biomedical importance in the ethanolic extract. The GC-MS analysis revealed that out of the identified twenty-two (22) bioactive compounds identified, ten were antimicrobials while eight were known antioxidants. The study, therefore, shows that the folkloric use of the leaf extract to treat some health concerns may have a scientific basis.

References

1. Morilla, L.J., Sumaya, N.H., Rivero, H., & Madamda, M.R.S. (2014). Medicinal Plant of the Subanens in Dumingag, Zamboanga del Sur, Philippines. International Conference on Food, Biological, and Medical Sciences (FBMS-2014).
2. Kumar, N.S. & Simon, N. (2016). In vitro antibacterial activity and phytochemical analysis of Gliricidia sepium (L.) leaf extracts. Journal of Pharmacognosy and Phytochemistry, 5(2): 131-133.
3. Mahalingam, R., Ambikapathy, V., Panneerselvam, A. & Prince, L. (2011). Biological activities of some medicinal plants against Setophaeria rostrata causing seedling blight disease in sugarcane. Asian Journal of Plant Science and Research, 1(2): 92-95.
4. Akharaiyi, F.C., Boboye, B. and Adetuyi, F.C. (2012). Antibacterial, Phytochemical and Antioxidant Activities of the Leaf Extracts of Gliricidia sepium and Spathodea campanulata. World Applied Sciences Journal 16 (4): 523-530, ISSN 1818-4952.
5. National Health Portal (2016). Introduction and Importance of Medicinal Plants and Herbs. Retrieved from https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and herbs_mtl.
6. Elias, N.U., Nuñeza, O.M, & Uy, M.M. (2014, October) Evaluating the Potential Cytotoxic Activity of Acmella grandiflora Flower and Whole Plant using Brine Shrimp Lethality Test. International Research Journal of Biological Sciences, Vol. 3(10), 90-92, ISSN 2278-3202.
7. Gacusan, L.G., (2007). Antibacterial Activity of Kakawate [Gliricidia Sepium (Jacq.) Walp.] Leaves against Wound Pathogens. Central Luzon State University. Agris: International Information System for the Agricultural Science and Technology.
8. Wiersum, K.F. & Nitis, I.M. (2016). Gliricidia sepium (PROSEA). Plant Resources of South-East Asia. Retrieved from https://uses.plantnet-project.org/en/Gliricidia_sepium_(PROSEA)
9. Jose, B. & Reddy, L.J. (2010). Evaluation of Antibacterial Activity of the Leaf and Flower Essential Oils of Gliricidia Sepium from South India. International Journal of Applied Pharmaceutics, 2(2): 2(3):177-179.
10. Filipino Herbs Healing Wonder (2009). Kakawate – Scientific name: Gliricidia sepium (Jacq.) Steud. Healing Wonders of Philippine Medicinal Plants. Retrieved from http://www.filipinoherbshealingwonders.filipinovegetarianrecipe.com/kakaw.htm
11. Tatengco, O.G., and Jacinto, S.D. (2015). Cytotoxic activity of crude extracts and fractions from Premna odorata (Blanco), Artocarpus camansi (Blanco) and Gliricidia sepium (Jacq.) against selected human cancer cell lines. Asian Pacific Journal of Tropical Biomedicine 5(12):1037-1041.
12. Thomas, E., Aneesh T. P, Thomas, D.G., & Anandan, R. (2013). GC-MS Analysis Phytochemical Compounds Present In The Rhizomes Of Nervilia Aragona Gaud. Asian Journal of Pharmaceutical Clinical Research, 6(3): 68-74.
13. Nishaa, S., Vishnupriya, M., Sasikumar, JM, and Gopalakrishnan, VK (2013). Phytochemical Screening and GC-MS Analysis of Ethanolic Extract of Rhizomes of Maranta arundinacea L. Research Journal of Pharmaceutical, Biological, and Chemical Sciences. 4(2):
14. Azwanida, N. N. (2015). A review of the extraction methods uses in medicinal plants, principle, strength, and limitation. Med. Aromat. Plants, 4(3): 3-8.
15. Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. In Manual of Clinical Microbiology, Eleventh Edition (pp. 1253-1273). American Society of Microbiology.
16. Bhargav, H. S., Shastri, S. D., Poornav, S. P., Darshan, K. M., & Nayak, M. M. (2016). Measurement of the Zone of Inhibition of an Antibiotic. In 2016 IEEE 6th International Conference on Advanced Computing (IACC), (pp. 409-414). IEEE
17. Balouiri, M., Sadiki, M. & Koraichilbnsouda, S. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. Volume 6, Issue 2, April 2016, Pages 71-79
18. Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS microbiology reviews, 28(5): 519-542.
19. Tian, F., Wang, C., Tang, M., Li, J., Cheng, X., Zhang, S., Ji, D., Huang, Y. and Li, H. (2016). The antibiotic chloramphenicol may be an effective new agent for inhibiting the growth of multiple myeloma. Oncotarget, 7(32), p.51934.
20. Crowley, P. D., & Gallagher, H. C. (2014). Clotrimazole as a pharmaceutical: past, present, and future. Journal of applied microbiology, 117(3), 611-617.
21. Marinova, G., & Batchvarov, V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science, 17(1):11-24.
22. De Padua, L.S., Lugod, G.C., and Pancho J.V. (2005). Handbook of Philippine Medicinal Plants. University of the Philippines, Los Baños 1981; 1-4: 66.
23. Chipiti T, Ibrahim MA, Koorbanally NA, and Islam MS (2015). In-vitro antioxidant activity and GC-MS analysis of the ethanol and aqueous extracts of Cissus cornifolia [baker] splanch [vitaceae]parts. Acta Poloniae Pharmaceutica Drug Research, 72(1):119-127.

24. Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography-mass spectrometry-based metabolite profiling in plants. Nature protocols, 1(1), 387.
25. Nazli R., Sohail T., Nawab, B. and Yaqeen, Z. (2011). Antimicrobial Property of Gliricidia sepium Plant Extract. Pakistan J Agric. Res. 24(1-4): 2011.
26. Turnbull, P., Kramer, J., & Melling, J. (1990). Bacillus In: Topley and Wilson Principles of Bacteriology. Virology and Immunity 8th ed Edward Arnold, London, 185-210.
27. Nobile, C. J., & Johnson, A. D. (2015). Candida albicans biofilms and human disease. Annual Review of Microbiology, 69: 71-92.
28. Kusmardiyani, S., Novita, G., & Fidrianny, I. (2016). Antioxidant Activities from various extracts of different parts of Kelakai (Stenochlaena palustris) grown in Central Kalimantan – Indonesia. Asian Journal of Pharmaceutical and Clinical Research, Vol 9, Suppl. 2, 2016, 215-219.
29. Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal, 2013.
30. Sharma, A. (2011). Antibacterial activity of ethanolic extracts of some arid zone plants. International Journal of PharmTech Research, 283-286.
31. Light, M. E., Sparg, S. G. & van Staden, J. (2004). Biological activities and distribution of plant saponins. Journal of Ethnopharmacology, 219-243.
32. Li, A. N., Li, S., Zhang, Y. J., Xu, X. R., Chen, Y. M. & Li, H. B. (2014). Resources and biological activities of natural polyphenols. Nutrients, 6(12): 6020- 6047.
33. Sarker SD, Nahar L. (2007). Chemistry for Pharmacy Students General, Organic, and Natural Product Chemistry. England: John Wiley and Sons 2007; pp 283-359.
34. Kandhare, A. D., Bodhankar, S. L., Mohan, V., & Thakurdesai, P. A. (2015). Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice. Regulatory Toxicology and Pharmacology, 72(2):323-334.
35. Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R., Sharma, D., Subedi, K., & Malla, R. (2011).Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). Journal of Microbiology and Antimicrobials, 3(1):1-7.
36. Bolarinwa, I. F., Oke, M.O. , Olaniyan, S.A., and Ajala, A.S. (2016). A Review of Cyanogenic Glycosides in Edible Plants, Toxicology Marcelo L. Larramendy, IntechOpen, DOI: 10.5772/64886.
37. Buvaneswari K, Ramamoorthy D, Velanganni J. (2011) Preliminary phytochemical and antimicrobial activity studies on the leaves of the Indian plant Thevetia neriifolia Juss. World J Agric Sci., 7(6):659-66
38. Wang, M.R., Li, W., Luo, S., Zhao, X., Ma, C.H., and Liu, S.X. (2018). GC-MS Study of the Chemical Components of Different Aquilaria sinensis (Lour.) Gilgorgans and Agarwood from Different Asian Countries. Molecules, 23(9). pii: E2168.
39. Chen, X., Mukwaya, E., Wong, M. S., & Zhang, Y. (2014). A systematic review of biological activities of prenylated flavonoids. Pharmaceutical biology, 52(5): 655-660.
40. Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P., Roy, R., ... & Meyer, J. M. (2008). Antimicrobial activity of the crude extracts and five flavonoids from the twigs of Dorstenia barteri (Moraceae). Journal of Ethnopharmacology, 116(3): 483-489.
41. Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. Fitoterapia, 82(4): 513-523.
42. Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. HortScience, 35(4): 588-592.
43. Takechi M, Matsunami S, Nishizawa J, Uno C, and Tanaka Y. (1999) Hemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. Planta Medica, 65: 585– 586.
44. Saxena, M., Jyoti, S., Rajeev, N., Dharmendra, S., and Abhishek, G. (2013). Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry, 1: 168-182.
45. Okigbo N, Anuagasi C, Amadi J. (2009) Advances in selected medicinal and aromatic indigenous to Africa. Journal Medicinal Plants Research, 3(2): 86-95.
46. Ogbonna JDN, Ezema EB, Obimma CP, and Agibo MO. (2013). Phytochemical properties of methanol extract and antimicrobial study of less polar fractions of Lotanthus micranthus (Linn) parasitic on Alstonia boonei. The Pharma Innovation Journal, 2(8): 83-88.
47. Sheikh N, Kumar Y, Misra AK, and Pfoze L. (2013). Phytochemical Screening to validate the ethnobotanical importance of Root tubers of Dioscorea species of Meghalaya, North East India. Journal of Medicinal Plants Studies. 2013;.1(6): 62-69.
48. Lacaille- Dubois MA and Wagner H (2000). Bioactive Saponins From Plants: An Update.
49. Raghu V, George S, Krishna RV, Sindhu KK. (2013) Bioactive properties of phenolics present in Oroxylu mindicum - A review. J Pharmacol Phytochem, 2(3):23-7.
50. Scalbert A. (1991) Antimicrobial properties of tannins. Phytochemistry, 30(12):3875-83.

51. Ahmad I, Aqil F and Owais M. (2006) eds. Modern Phytomedicine: Turning Medicinal plants into drugs. Wiley-VCH Verlag GmbH & Co. KGaA. pp 41, 59, and 263.
52. Nathathe, B. and Ndip, R. (2011) Bioactivity of Hydana Africana on selected bacterial pathogens: Preliminary phytochemical screening. African Journal of Microbial Research, 5(18): 2820-2826.
53. Ngulde SI, Sandabe SI, Sandabe UK, Tijjani MB, Barkindo AA, and Hussaini IS. (2013) Phytochemical constituents, antimicrobial screening, and acute toxicity studies of the ethanol extract of Carissa edulis Vahl. root bark in rats and mice. American Journal of Research Communication, 1 (9): 99-110.
54. Kolodziej H and Kiderlen AF (2005). Antileishmanial Activity And Immune Modulatory Effects of Tannins and Related Compounds on Leishmania Parasitised RAW 264.7 Cells. Phytochemistry; 66:2056–2071.
55. Ashok, P. K., & Upadhyaya, K. (2012). Tannins are astringent. Journal of Pharmacognosy and Phytochemistry, 1(3): 45-50.
56. Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability, and biological effects. Molecular nutrition & food research, 53(S2): S310-S329.
57. Banso, A. & Adeyemo, S.O. (2007). Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. African Journal of Biotechnology. Vol. 6 (15), pp. 1785-1787.
58. Amin, M.M., Sawhney, S.S., Jassan, M.M. (2013) Quantitative and qualitative analysis of phytochemicals of Taraxacum officinale. Wudpecker J Pharm Pharmacol;2(1):1-5.
59. Simoben, C., Ibezim, A., Ntie-Kang, F., N Nwodo, J., & L Lifongo, L. (2015). Exploring cancer therapeutics with natural products from African medicinal plants, part I: xanthones, quinones, steroids, coumarins, phenolics, and other classes of compounds. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 15(9), 1092-1111.
60. Kaur, A., Nain, P., & Nain, J. (2012). Herbal plants used in the treatment of rheumatoid arthritis: a review. Int J Pharm Pharm Sci, 4(Suppl 4), 44-57.
61. Rosu, T., Pahontu, E., Pasculescu, S., Georgescu, R., Stanica, N., Curaj, A., Popescu, A., and Leabu, M. (2010). Synthesis, Characterization Antibacterial, and Antiproliferative Activity of Novel Cu(II) and Pd(II) Complexes with 2-hydroxy-8-R-tricyclo[7.3.1.0.(2,7)]tridecane-13-one thiosemicarbazone. European Journal of Medicinal Chemistry, 45(4):1627-34.
62. Wang, J., Liu, H., Gao, H., Zhao, J., Zhou, L., Han, J., Yu, Z., and Yang, F. (2011). Antimicrobial and Antioxidant Activities of the Flower Essential Oil of Halimodendron halodendron. Natural Product Communications, 6(11):1749-53.
63. Dvl, S and Rajarathinam, G. (2013). Evaluation of Antimicrobial and Antioxidant activity of Essential Oil of Trianthema decandra L. Journal of Pharmacy Research, 101106.
64. Rahimian, H., Rasekh, F., Sharifimehr, S. & Tajick, M. A. (2014). Some of the phytotoxic and antimicrobial compounds extracted from culture filtrates of Fusarium proliferatum FP85. Journal of Biodiversity and Environmental Sciences, 245-251.
65. Duraipandiyan, V., Gajendran, H., Girija, S., Kuppusamy, P. S., & Rajagopal, R. (2014). Chromatographic Characterization and GC-MS Evaluation of the Bioactive Constituents with Antimicrobial Potential from the Pigmented Ink of Loligo duvauceli. International Scholarly Research Notices.
66. Gergel, E., Konovalova, O. & V. H. (2013). GC-MS Analysis of Bioactive Components of Shepherdia argentea (Pursh.) Nutt. from Ukrainian Flora. The Pharma Innovation- Journal, 7-12.
67. Ahsan, T., Chen, J., Irfan, M., Wu, Y. & Zhao, X. (2017). Extraction and Identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express. 7(1):54.
68. Farzaei, M.H., Rahimi, R., Attar, F., Siavoshi, F., Saniee, P., Hajimahmoodi, M., Mirnezami, T., and Khanavi, M. Chemical Composition, Antioxidant and Antimicrobial Activity of Essential Oil and Extracts of Tragopogon graminifolius, A Medicinal Herb from Iran. Natural Product Communications, 9(1):121-4.
69. Plaza, M., Santoyo, S., Jaime, L., García-Blairsy Reina, G., Herrero, M., Señoráns, F.J., and Ibáñez, E. (2010). Screening for Bioactive Compounds from Algae. Journal of Pharmaceutical and Biomedical Analysis, 51(2):450-5.
70. Ceyhan-Güvensen, N., and Keskin, D. (2016). Chemical Content and Antimicrobial Properties of Three Different Extracts of Mentha pulegium Leaves from Mugla Region, Turkey. Journal of Environmental Biology, 37(6):1341-46.
71. Ponmathi S.A, Evanjaline, M. & Muthukumarasamy, S. Veerabahu, M. (2017). Determination of Bioactive Components Of Barleria Courtallica Nees (Acanthaceae) By Gas Chromatography-Mass Spectrometry Analysis. Asian Journal of Pharmaceutical and Clinical Research, 10(6), 273.
72. Jananie, R. K., Priya, V., & Vijayalakshmi, K. (2011). Determination of bioactive components of Cynodon dactylon by GC-MS analysis. NY Sci J, 4(4): 1-5.
73. Das, S., & Bisht, S. (2012). The Bioactive and Therapeutic Potential of Hemidesmus indicus R. Br. (Indian Sarsaparilla) Root. Phytotherapy Research, 27(6): 791–801. doi:10.1002/ptr.4788.

74. Bharathy V, Maria Sumathy B, and Uthayakumari F (2012). Determination of Phytocomponents by GC-MS In Leaves of Jatropha Gossypifolia L., Science Research Reporter, 2 (3), 286–290.
75. Sermakkani M, Thangapandian V, GC-ms analysis of Cassia italica leaf methanol extract, Asian J Pharm Clin Res, 2012; 5 (2): 90-94.
76. Musa, A.M., Ibrahim, M.A., Aliyu, A.B., Abdullahi, M.S., Tajuddeen, N., Ibrahim, H., Oyewale, A.O. (2015). Chemical Composition and Antimicrobial Activity of Hexane Leaf Extract of Anisopus mannii (Asclepiadaceae). Journal of Intercultural Ethnopharmacology, 4(2):129-33.
77. Jose, M., Cyriac, M. B., Pai, V., Varghese, I., & Shantaram, M. (2014). Antimicrobial properties of Cocos nucifera (coconut) husk: An extrapolation to oral health. Journal of Natural Science, Biology, and Medicine, 5(2), 359-364.
78. Abushelaibi, A. A., Al Shamsi, M.S., & Afifi, H.S. (2012). Use of antimicrobial agents in food processing systems. Recent Patents on Food, Nutrition & Agriculture, 4(1), 2-7.
79. Abdul Kaffoor, H., Arumugasamy, K., Jemimma, H. L. & Nantha Kumar, R. (2017). GC-MS Analysis of Root and Aerial Parts Ethanolic Extract of Phyllanthus vasukii Parthipan et al., Sp. Nov. (Phyllanthaceae). International Journal of Ayurvedic and Herbal Medicine, 2672-2684.
80. Adetutu, E.M., Ball, A.S., Weber, J., Aleer, S., Dandie, C.E., and Juhasz, A.L. (2012). Impact of Bacterial and Fungal Processes On 14C-Hexadecane Mineralisation In Weathered Hydrocarbon Contaminated Soil. Sci Total Environ. 1(414):585-91. DOI: 10.1016/j.scitotenv.2011.11.044.
81. Sun, J., Xu, L., Tang, Y., Chen, F., and Wu, X. (2012). Simultaneous Degradation of Phenol And N-Hexadecane by Acinetobacter Strains. Bioresour Technol, 123:664-8.
82. Yoon, M.A., Jeong, T.S., Park, D.S., Xu, M.Z., Oh, H.W., Song, K.B., Lee, W.S. and Park, H.Y., (2006). Antioxidant effects of quinoline alkaloids and 2, 4-di-tert-butylphenol isolated from Scolopendra subspinipes. Biological and Pharmaceutical Bulletin, 29(4), 735-739.
83. Kotan R, Cakir A, Dadasoglu F, Aydin T, Cakmakci R, Ozer H, Kordali S, Mete E, Dikbas N. (2010). Antibacterial activities of essential oils and extracts of Turkish Achillea, Satureja and Thymus species against plant pathogenic bacteria. Journal of the Science of Food and Agriculture, 90(1):145-60.
84. Kazemi, M. (2015). Phenolic Profile, Antioxidant Capacity, and Anti-Inflammatory Activity of Anethum graveolens L. Essential Oil. Natural Product Research, 29(6):551-3.
85. Ebrahimabadi, A.H., Movahedpour, M.M., Batooli, H., Ebrahimabadi, E.H., Mazoochi, A., and Qamsari, M.M. (2016). Volatile Compounds Analysis and Antioxidant, Antimicrobial and Cytotoxic Activities of Mindium laevigatum. Iranian Journal of Basic Medical Sciences, 19(12):1337-1344.
86. Richardson, J.S., Sethi, G., Lee, G.S., and Malek, S.N. (2016). Chalepin: Isolated from Ruta angustifolia L. Pers Induces Mitochondrial-Mediated Apoptosis in Lung Carcinoma Cells. BMC Complementary and Alternative Medicine, 16(1):389.
87. de Felício, R., de Albuquerque, S., Young, M.C., Yokoya, N.S., and Debonsi, H.M. (2010). Trypanocidal, Leishmanicidal and Antifungal Potential from Marine Red Alga Bostrychia tenella J. Agardh (Rhodomelaceae, Ceramiales). Journal of Pharmaceutical and Biomedical Analysis: 52(5):763-9.
88. Adeosun, T.E., Ogunwande, I.A., Avoseh, O.N., Raji, I.P., and Lawal, O.A. (2017). Composition and Anti-inflammatory Activity of Essential Oil of Jatropha curcas. Natural Product Communications, 12(3):439-440.
89. Cheng, M.C., Chang, W.H., Chen, C.W., Li, W.W., Tseng, C.Y., and Song, T.Y. (2015). Antioxidant Properties of Essential Oil Extracted from Pinus morrisonicola Hay Needles by Supercritical Fluid and Identification of Possible Active Compounds by GC/MS. Molecules, 20(10):19051-65. DOI: 10.3390/molecules201019051.\
90. Shibula, K., & Velavan, S. (2015). Determination of phytocomponents in methanolic extract of Annona muricata leaf using GC-MS technique. Int J Pharmacognosy Phytochem Res, 7(6):1251-1255.
91. Adesalu, T. A., Julies, M. L. & Temenu, T. O. (2016). Molecular characterization, lipid analysis, and GC-MS determination of bioactive compounds identified in a West African strain of the green alga Oedogonium (Chlorophyta). Journal of Pharmacognosy and Phytochemistry, 5(6):1-6.
92. Brindha, P. & Sivasubramanian, R. (2013). In vitro Cytotoxic, Antioxidant & GC-MS Studies on Centratherum punctatum Cass. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3):364- 367.
93. Akpuaka, Ekwenchi, M., Dashak, D., & Dildar, A. (2013). Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta indica A.Juss (Neem) Leaves. New York Science Journal, 119-124.
94. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules, 20(12), 21138-21156.
95. Chan, K. W., Iqbal, S., Khong, N. M., Ooi, D. J., & Ismail, M. (2014). Antioxidant activity of phenolics–saponins rich fraction prepared from defatted kenaf seed meal. LWT-Food Science and Technology, 56(1), 181-186.
96. Vadlapudi, V., & Kaladhar, D. S. V. G. K. (2012). Antimicrobial study of plant extracts of Datura metel L. against some important disease-causing pathogens. Asian Pacific Journal of Tropical Disease, 2, S94-S97.
97. Lee, M. T., Lin, W. C., Yu, B., & Lee, T. T. (2017). Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals- A review. Asian-Australasian Journal of animal sciences, 30(3), 299.