

IDENTIFICATION OF VOLATILE COMPOUNDS BY GC-MS AND
EVALUATION OF ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT
EUPHORBIA OSYRIDEA

Samar Ali^{*1}, Gulmeena Bazai², Nimra Fazal³, Aurangzeb Ashraf⁴, Sara Zameer⁵

^{*1,3,4,5}Department of Chemistry, University of Balochistan, Quetta- Pakistan

²Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta- Pakistan

^{*1}samar.chemistry@um.uob.edu.pk

DOI: <https://doi.org/10.5281/zenodo.22122718>

Keywords

Phytochemical screening; GC-MS; antibacterial activity; volatile compounds; *Euphorbia osyridea*

Article History

Received: 17 May 2026

Accepted: 13 June 2026

Published: 16 August 2026

Copyright @Author

Corresponding Author: *

Samar Ali

Abstract

The medicinal plant *Euphorbia osyridea* belongs to the family Euphorbiaceae and genus *Euphorbia*, which includes a number of plants used for therapeutic purposes. The species occurs in Afghanistan, India, Iran, and Pakistan. The present research evaluated the phytochemical profile, volatile compounds, and antibacterial effect of *E. osyridea*. Qualitative and quantitative phytochemical screening revealed the occurrence of coumarins, flavonoids, phenols, tannins, steroids, saponins, cardiac glycosides, and terpenoids. Among the investigated constituents, phenols showed the highest content (45%), followed by saponins (30%) in the ethyl acetate fraction and flavonoids (27%) in the methanolic extract. GC-MS analysis of the n-hexane fraction resulted in the identification of 38 compounds belonging to different chemical classes. The antibacterial capability of the raw extract and its fractions was assessed against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Identification of different phytochemicals and volatile compounds in *E. osyridea*, along with its antibacterial capability, shows that this plant may be a valuable source of a variety of potentially therapeutic compounds.

Introduction

Natural products obtained from plants, animals, microorganisms, and marine creatures, have been recognized as a vital source of biologically active substances. Plant derived compounds, in particular, plays a crucial role in traditional medicine systems and has generated a great deal of interest as possible sources of medicines. Their medicinal importance stems from the presence of various phytochemicals, such as saponins, flavonoids, alkaloids, glycosides, and tannins, which may produce different biological and health

benefits (Chariandy et al., 1999; Newman et al., 2000; Sarker & Nahar, 2007; Harborne, 1973).

The genus *Euphorbia* is one of the largest genera in the family Euphorbiaceae and contains over 2000 known species. Members of this genus produce many secondary metabolites, such as mono-, di-, and triterpenoids, as well as steroids. These types of compounds have kept scientists interested in *Euphorbia* species as sources of commercially useful natural products.

Euphorbia osyridea is a miniature herbaceous species that can be found in rocky places, gravelly soils, limestones cracks and sandy areas. It is

popularly called Rutazagh and Zhaarboti in Pashtu and has been reported from Pakistan, Iran, India and Afghanistan (Safa et al., 2012; Musakhel, 2007). The plant is known for its numerous applications in folk medicine. The roots of this plant are known for treating constipation. The leaves are believed to act as insect repellents and to treat allergies caused to animals (Safa et al., 2012; Musakhel, 2007). The latex of *E. osyridea* is also said to have a history of traditional usages (Lavie et al., 1968).

Even though there are traditional uses, not much data is available regarding the chemical compounds and biological effects of *E. osyridea*. Therefore, a breakdown of secondary metabolites present in the plant will provide significant information regarding the plant constituents responsible for various biological activities. The current study was carried out to analyze the phytochemical profile of *E. osyridea* using various methods such as phytochemical screening, GC-MS of the plant's volatile components, and analysis of the antibacterial activity of all crude extracts and their different fractions.

Materials and Methods

Plant materials

Whole plant material of *Euphorbia osyridea* was collected from Hazarganji National Park, Balochistan. The plant was authenticated using *Flora of Pakistan* with further confirmation by a

taxonomist from the Department of Botany, University of Balochistan, Quetta.

Extraction and fraction

Following identification, the plant material was shade-dried and subsequently ground into powder. The powdered material (7 kg) was extracted with methanol (30 L × 3) for seven days. The resulting extract was concentrated under reduced pressure using a rotary evaporator at 45 °C, yielding 80 g of crude extract. The crude methanolic extract was subsequently partitioned into fractions according to increasing solvent polarity. The obtained fractions comprised n-hexane (20 g), dichloromethane (DCM; 21.57 g), ethyl acetate (8.07 g), and n-butanol (10.07 g). The resulting fractions were maintained at room temperature until further analysis.

Qualitative Phytochemical Analysis

The crude methanolic extract and its solvent fractions were subjected to qualitative phytochemical screening using previously reported standard procedures. The analysis was performed to determine the occurrence of cardiac glycosides, saponins, tannins, alkaloids, terpenoids, steroids, coumarins, flavonoids, and total phenols. The respective phytochemical tests and their corresponding observations are presented in Table 1.

Table-1 Qualitative Analysis Tests and Methods

Test Name	Method	Inference
Alkaloids (Dragendorff's test)	Dilute HCl + fractions + stirred + filtered. 1ml of filtrate + 3 drops of dragendorff's reagent	Change of orange or reddish brown precipitates
Coumarins	3 ml of 10% NaOH + fraction + covered by filter paper for a period of time.	Yellow color
Cardiac glycoside (Keller-Killani test)	2mL of each fraction + 2mL of glacial acetic acid + 1 mL of conc H ₂ SO ₄ + 5 % FeCl ₃ .	Brownish red layer was formed between two liquids
Flavonoids (Sodium hydroxide test)	Few drops of 10% sodium hydroxide solution + each fraction.	Yellow coloration which turn into colorless by adding dilute hydrochloric acid
Phlobatannins	Each fraction + 2 % aq HCL + boiled.	Red colored precipitate

Quinones	Conc Hcl + 1-2 mL solution of each fractions	Red coloration
Saponins (foam or froth Test)	Plant fractions + 10mL of distilled water + shake +few drops olive oil.	Persistent froth or foam formation
Steroids	Plant Fractions + 2 mL chloroform + conc. H ₂ SO ₄ side of the wall of test tube.	Lower Chloroform layer appeared red whereas upper acid layer showed yellowish green
Terpenoids	Plant sample +2 mL +3 mL conc. H ₂ SO ₄ .	Change of reddish brown color at the colligation of the above two layers
Tannins (ferric chloride test)	Plant Fractions + 1% neutral ferric chloride (few drops)	Blue- black coloration

Quantitative Phytochemical Analysis

Determination of Total Flavonoid Content

For flavonoid determination, 1 g of sample was extracted with 8% methanol (10 mL) at room temperature. The extract was subsequently filtered, and the filtrate was dehydrated and weighed following the reported procedure of Bohm and Kocipai-Abyazan (1994).

Determination of Total Phenolic Content

One gram of plant extract was treated with n-hexane (20 mL) twice and filtered. The resulting filtrate was discarded, after which 10 mL of diethyl ether was added. The mixture was heated for 15 min, cooled, and filtered. Subsequently, 10 mL of 2% sodium hydroxide was added and the mixture was shaken thoroughly to facilitate separation of the organic and aqueous phases. The organic phase was washed with deionized water, whereas the aqueous phase was acidified by adding 2% HCl and extracted with 10 mL DCM. In the end, extract was dried and weighted according to reported method by Bohm and Kocipai (1994).

Determination of Total Saponin Content

The test sample was made to undergo processing by adding 5 mL of aqueous 1% ethanol to 1 g of the sample. The sample was heated with stirring in a water bath kept at 55 °C. After filtration, the residue was extracted again using another 5 mL of 1% ethanol. The resulting filtrate was evaporated to obtain about 20 mL of solution by heating at 90 °C. 1 mL of diethyl ether was added to the

concentrated solution and mixed well, which led to the formation of ether and aqueous layers. The ether was discarded and the aqueous phase was collected. n-Butanol was repeatedly used for purification with 3 mL aliquots in each purification. The n-butanol fraction was treated with 5% NaCl to get rid of any contaminants. Finally, the purified solution was dried and weighed.

Antibacterial Activity by Agar Well Diffusion

The antibacterial activity of the crude plant extracts and their various solvent solutions was examined through the agar well diffusion method. To obtain freshly grown cultures of the selected bacteria strains, their growth was adjusted to the ultimate turbidity level equivalent to ~ 0.5 McFarland standards. Sterile nutrient agar plates were made which were then, inoculated in a similar manner using a sterilized cotton swab. Inoculated agar plates were prepared with sterile wells having a diameter of around six millimeters each using sterile cork borer. Relevant concentration of the plant extracts or fractions were added to the wells. Ofloxacin was probably followed by corresponding solvents as negative control. After that inoculated plates were kept still to allow the diffusion of the extracts into the agar and then incubated at 37 °C for 1 day. Assessment of the antibacterial activity was done by measuring the diameter of the disinfectant zones spotted around the wells.

GC-MS Analysis

The gas chromatography-mass spectrometry (GC-MS) method was used to characterize the constituents of plant extract/fraction that are volatile and semi-volatile. The desired amount of sample was dissolved in the suitable solvent, filtered, and sent to the GC-MS machine. Helium gas was used to transport the sample through the system. The constituents were separated in accordance to their retention times in GC column, while the identification of constituents based on mass spectrometer results were achieved by matching the obtained spectra with NIST library mass spectra. The identification of constituents was done based on retention times

and area percentage of the constituents in the spectrum.

Results and Discussion**Qualitative Phytochemical Screening**

The qualitative screening result showed the presence of various metabolites in the crude methanolic extract of *Euphorbia osyridea* as well as its corresponding solvent fractions. Cardiac glycosides, coumarins, flavonoids, steroids, saponins, tannins, and terpenoids were found in the prepared solvent fractions, but alkaloids, phlobatannins, and quinones were absent in the test. Table 1 shows the distribution of the detected phytoconstituents.

Table 1: Qualitative phytochemical screening of *Euphorbia osyridea*

Phytochemicals	F1	F2	F3	F4	F5
Alkaloids	-	-	-	-	-
Cardiac glycosides	+	+	+	-	+
Coumarins	+	+	+	+	+
Flavonoids	+	-	+	+	+
Phlobatannins	-	-	-	-	-
Quinones	-	-	-	-	-
Steroids	+	+	+	+	+
Saponins	+	+	+	+	+
Phenols and Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+

(+) = Presence; (-) = Absence

Crude Methanolic extract = F1, n-hexane fraction = F2, Dichloromethane fraction = F3, Ethyl acetate fraction = F4, Butanol fraction = F5

Various types of secondary metabolites tell us about the chemical diversity of *E. osyridea*. These compounds are generally connected to various biological activities. They may play a cumulative role in the biological activities noted in plant extracts. Nevertheless, qualitative analysis offers only preliminary proofs about their presence without showing their activity.

Quantitative Phytochemical Analysis

Quantitative measurement revealed differences in the level of the key phytochemical groups in the tested fractions. The Total saponin content was maximum in ethyl acetate fraction (30%). The highest percentage of total phenolic content, which amounted to 34.5%, was found in methanol extract. The maximum percentage of flavonoids gained in crude methanol extract was recorded with a value of 27%. Indicated values for all analyzed fractions are shown in Table 2.

Table 2: Quantitative phytochemical screening of *Euphorbia osyridea* (%)

Phytoconstituent	F1	F2	F3	F4	F5
Total saponins	10	23.5	8	30	20

Total flavonoids	27	-	12.5	22	20.5
Total phenols	34.5	-	8	31.5	26

Crude Methanolic extract = F1, n-hexane fraction = F2, Dichloromethane fraction = F3, Ethyl acetate fraction = F4, Butanol fraction = F5

The variation observed between fractions may be related to differences in the polarity and solubility characteristics of individual phytochemical constituents. The presence of a higher quantity of phenolic and flavonoid compounds in the methanol extract as well as the higher concentration of saponins in the ethyl acetate fraction suggests that the type of solvent used played a role in the distribution of phytochemicals in *E. osyridea*.

Antibacterial activity

Antibacterial activity of both the crude methanolic extract and the fractions of the methanol extract was established using four types of bacteria. These include *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The size of the inhibition zone depended on the type of bacteria and the fraction of the extract used. The results are shown in Table 3.

Table 3: Antibacterial activity of *E. osyridea* (mm)

Microorganism	F1	F2	F3	F4	F5	Doxycycline (D-30)
<i>P. aeruginosa</i>	2	21	24	33	22	21
<i>E. coli</i>	26	23	25	33	24	19
<i>S. aureus</i>	33	28	33	33	30	23
<i>K. pneumonia</i>	25	20	26	33	24	20

Crude Methanolic extract = F1, n-hexane fraction = F2, Dichloromethane fraction = F3, Ethyl acetate fraction = F4, Butanol fraction = F5

Based on the results of the study, it was found that the ethyl acetate fraction exhibited the highest activity, leading to the formation of inhibition zones of 33 mm for the four test organisms. Equally impressive was the crude methanol extract that produced the highest level of activity against *S. aureus*, having the maximum zone of inhibition of 33 mm. The n-hexane and dichloromethane fractions also inhibited the tested organisms, although the magnitude of their activity varied between bacterial strains. The n-butanol fraction produced inhibition zones ranging from 22 to 30 mm in the reported assay. Doxycycline, used as the reference antibiotic, produced inhibition zones between 19 and 23 mm.

The observed antibacterial response suggests that constituents distributed among the different

solvent fractions may contribute to the activity of the plant. The comparatively pronounced response of the ethyl acetate fraction may be associated with the concentration of moderately polar phytochemicals in this fraction. Nevertheless, the present results represent activity of complex extracts and fractions; therefore, the contribution of individual compounds cannot be established solely from the inhibition-zone measurements.

GC-MS analysis

GC-MS analysis of the n-hexane fraction resulted in the detection of **38 constituents**, with their retention times and relative peak-area percentages reported in Table 4. The chromatographic profile showed numerous peaks across the analyzed

retention-time range, indicating the presence of chemically diverse volatile and semi-volatile

constituents. The chromatogram has showed the peaks of the detected compounds in **Figure 1**.

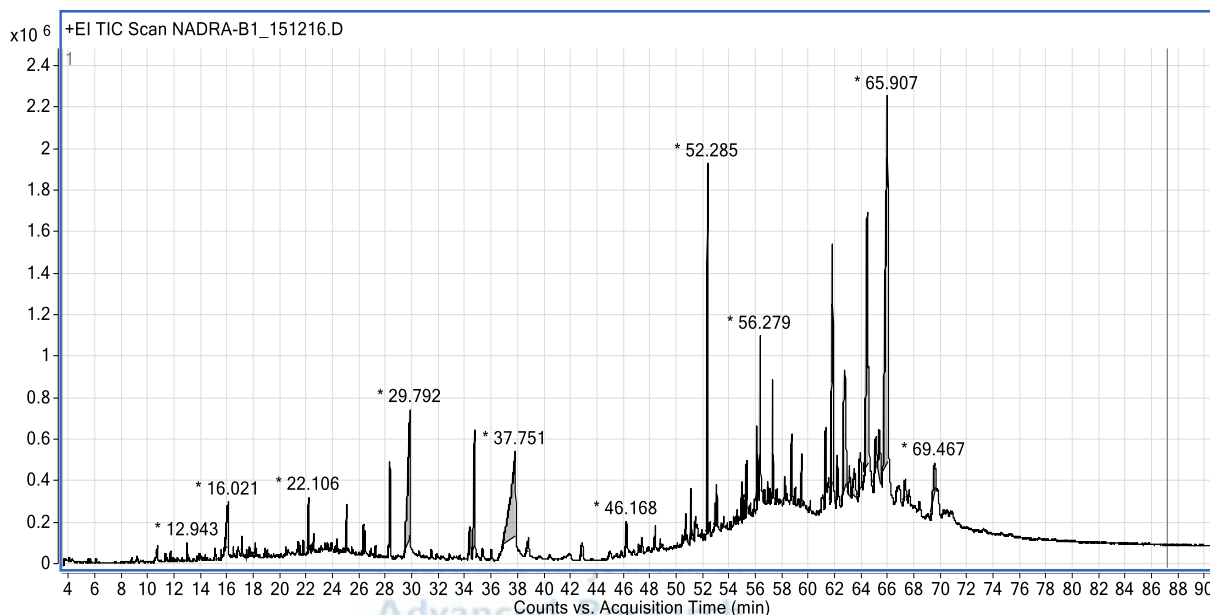


Figure 1: Chromatogram of n-hexane fraction

The GC-MS profile therefore indicates that the n-hexane fraction contains several major constituents together with a number of compounds occurring at comparatively lower abundance. The identification of these compounds provides a chemical basis for further investigation of the biological properties of the fraction.

The identified compounds included fatty acids and their derivatives, esters, aldehydes, alcohols, terpenoid/steroidal constituents, and other structurally diverse compounds. Among the

detected constituents, **Betulin** showed the highest reported peak area (49.73%), followed by **Lanosterol** (31.92%), **1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester** (22.08%), and **9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-** (16.89%). Other relatively abundant constituents included the compound at 56.283 min with an area of 11.32%, and **Lanosta-8,24-dien-3-one** with 6.83%. The identification of active constituents with their molecular formula, peak area (%), molecular weight and retention time are tabulated in **Table 4**.

Table 4: Constituents identified in the n-hexane fraction of *E.osyridea* by GC-MS

Retention time	Compound	% Area sum
5.650	Retinoic acid	1.73
10.675	Hexanoic acid, 3-ethyl-, methyl ester	0.44
11.681	2,4-Heptadienal, (E,E)-	0.36
12.947	5-Octen-2-yn-4-ol	0.63
16.003	Heptanediamide, N,N'-di-benzoyloxy	7.91
17.088	2-Decenal, (E)-	0.52
17.421	α -D-Glucopyranoside, O- α -D-glucopyranosyl-(1.fwdarw.3)- β -D-fructofuranosyl	0.40
18.112	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	0.61

21.353	Undecanoic acid, 10-methyl, methyl ester	0.32
21.792	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	0.50
22.098	Dodecanoic acid	3.20
22.42	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.47
24.262	Methyl tetradecanoate	0.36
24.967	Tetradecanoic acid	2.78
26.255	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.55
26.399	2-Pentadecanone, 6,10,14-trimethyl-	1.06
27.148	9,12-Octadecadienal, dimethyl acetal	0.59
28.266	Hexadecanoic acid, methyl ester	5.79
34.298	Methyl 10-trans, 12-cis-octadecadienoate	3.98
34.669	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	16.89
38.735	Octadecanoic acid	2.30
42.800	1H-Naphtho[2,1-b]pyran-8(4aH)-one, 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-	2.53
46.168	Podocarp-7-en-3 β -ol, 13 β -methyl-13-vinyl-	3.64
48.331	7-Methyl-Z-tetradecen-1-ol acetate	1.86
51.034	Androst-5-en-17-ol, 4,4-dimethyl-	3.04
51.363	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	1.80
52.267	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.08
52.868	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-, didehydro deriv.	1.39
52.986	9,19-Cyclocholestene-3,7-diol, 4,14-dimethyl-, 3-acetate	2.72
54.861	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	1.84
55.024	1HCyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a (1aH)-pentol, 3[(acetyloxy)methyl]1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate[1aR-(1a α ,1b β ,4a β ,5 β ,7a α ,7b α ,8 α ,9 β ,9a α)]-	2.36
55.180	Retinoyl- β -glucuronide 6',3'-lactone	1.37
56.033	Pregn-16-ene-11,14,18,20-tetrol, 3,9-epoxy-3-methoxy-, 11,20-diacetate, (3 α ,5 β ,11 α ,14 β ,20R)-	5.35
56.283	17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene	11.32
59.405	Vitamin E	3.80
61.236	Lanosta-8,24-dien-3-one	6.83
61.725	Lanosterol	31.92
64.999	Betulin	49.73

*RT: Retention Time; peak area (%) of compounds

Biological relevance of identified compounds

Several constituents detected in the GC-MS analysis have previously been associated with biological activities. The original literature cited in this study reports that **9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-** has been associated with anti-androgenic, antipyretic, antirheumatic,

cardioprotective, anticancer, and antioxidant activities (Mariswamy et al., 2011; Akpuaka et al., 2013).

Similarly, hexadecanoic acid methyl ester has been reported to possess antibacterial and antifungal properties (Chandrasekaran et al., 2011). The presence of this compound in the n-hexane

fraction may therefore be of interest in relation to the antibacterial activity observed in the present investigation. However, this association should be interpreted cautiously because the antibacterial assay was performed on the complex plant fractions rather than on isolated hexadecanoic acid methyl ester.

The GC-MS analysis also detected **1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester**, which has previously been reported in connection with cytotoxic and anticancer activity (Krishnan et al., 2014). Its occurrence in the investigated fraction adds to the chemical diversity of the extract and warrants further investigation.

Vitamin E was also identified in the n-hexane fraction. The literature cited in this paper refers to the antioxidant properties, cardiovascular properties, and anticancer properties of vitamin E (Lee et al., 2005). Similarly, octadecanoic acid and tetradecanoic acid also exhibit some antimicrobial properties (Rahuman et al., 2000).

Thus, it is concluded that the results from GC-MS analysis provide important information about the n-hexane fraction, it contains huge diversity of components that possess their biological activities according to the references provided in earlier. These statements correlate with the biological properties or capabilities of *E. osyridea* extracts. However, it is to be mentioned that just presence of the compound in GC-MS results is insufficient to confirm that the specific compound caused the observed antibacterial activity. Further, it is required to isolate the molecule from the mixture of chemical compounds and purify it in order to confirm its functionality.

Conclusion

The current investigation proved that *Euphorbia osyridea* comprises numerous phytochemical constituents and showcases antibacterial properties. The phytochemical evaluations established that *Euphorbia osyridea* contain many secondary-metabolite groups like phenols, flavonoids, saponins, tannins, terpenoids, steroids, coumarins, and cardiac glycosides. Out of all tested phytochemicals, the highest amount of total phenolic compounds was found in the crude

methanolic extract (34.5%), whereas the maximum amount of flavonoids was found in the same crude extract (27%), while ethyl acetate fraction contained the most saponins (30%).

Antibacterial assay displayed that the crude extract and its fractions produced an antibacterial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, but the ethyl acetate fraction was found to be the most effective in the present research.

In addition, in the results of analysis of n-hexane fraction done using GC-MS, 38 constituents were found representing a diverse array of chemical compounds, comprising esters, fatty acid derivatives, terpenoids and steroids.

In summary, the study suggests that *E. osyridea* offers rich sources of remarkable phytochemicals. Nonetheless, the biological properties of the crude extract of *E. osyridea* cannot be connected to the individual compounds identified using GC-MS technique without further investigation. Thus, further work should concentrate on extraction and identification

REFERENCES

- Ahmadi, E., Ghanadian, M., Saeidi, H., Rahiminejad, M., & Ayatollahi, A. (2012). Determination of triterpene contents of *Euphorbia osyridea*. *Research in Pharmaceutical Sciences*, 7(5), 748.
- Akpuaka, A., Ekwenchi, M. M., Dashak, D. A., & Dildar, A. (2013). Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. *Nature and Science*, 11(5), 141-147.
- Boham, A.B., & Kocipai, A.C. (1994). Flavonoid and condensed tannins from Leaves of Hawaiian *vaccinium vaticulum* and *vicalycinium*. *Pacific Sci.*, 48, 458-463.
- Chandrasekaran, M., Senthilkumar, A., & Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *European review for medical and pharmacological sciences*, 15(7), 775-780.

- Chariandy, C. M., Seaforth, C. E., Phelps, R. H., Pollard, G. V., & Khambay, B. P. S. (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *Journal of Ethnopharmacology*, 64(3), 265-270.
- Doughari, J. H., & Obidah, J. S. (2008). Antibacterial potentials of stem bark extracts of *Leptadenialancifolia* against some pathogenic bacteria. *Pharmacologyonline*, 3, 172-180.
- Doughari, J. H., Human, I. S., Benadé, A. J. S., & Ndakidemi, P. A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria.
- Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, 4(7), 685-688.
- Einbond, L. S., Reynertson, K. A., Luo, X. D., Basile, M. J., & Kennelly, E. J. (2004). Anthocyanin antioxidants from edible fruits. *Food chemistry*, 84(1), 23-28.
- Evans, W. C. (2002). Trease and Evans Pharmacognosy, "Aspects of Asian medicine and its practice in the west".
- Harborne JB (1973). *Phytochemical Methods: A Guide to Modern Techniques of plant Analysis*. Chapman and Hall Ltd, London. p. 279
- Jassbi, A. R. (2006). Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. *Phytochemistry*, 67(18), 1977-1984.
- Krishnan, K., Mani, A., & Jasmine, S. (2014). Cytotoxic activity of bioactive compound 1, 2-benzene dicarboxylic acid, mono 2-ethylhexyl ester extracted from a marine derived *Streptomyces* sp. VITSJK8. *International journal of molecular and cellular medicine*, 3(4), 246.
- Lavie, D., Jain, M. K., & Orebamjo, T. O. (1968). Terpenoids—VII. Constituents of *Euphorbia lateriflora* Schum and Thonn. *Phytochemistry*, 7(4), 657-660.
- Lee, I. M., Cook, N. R., Gaziano, J. M., Gordon, D., Ridker, P. M., Manson, J. E., & Buring, J. E. (2005). Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *Jama*, 294(1), 56-65.
- Mahesh, B., & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World journal of agricultural sciences*, 4(5), 839-843.
- Mariswamy, Y., Gnara, W. E., & Johnson, M. (2011). Chromatographic finger print analysis of steroids in *Aervalanata L* by HPTLC technique. *Asian Pacific journal of tropical biomedicine*, 1(6), 428-433.
- Musakhel, M.Y. (2007.) An Ethnobotanical knowledge among the tribals of torghar Area, District Qilla Saifulla Balochistan. Systematic survey Report [Online]. Habitat & Species Conservation Project, SUSG-CA Asia. 2007; 57p. Available from: URL <https://www.scribd.com/doc/33861226/Qila-Saifullah>
- Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery. *Natural product reports*, 17(3), 215-234.
- Nweze, E.L.; Okafor, J.L. & Njoku O. (2004). Antimicrobial Activities of Methanolic extracts of *Trumeguineesis* (Schum and Thorn) and *Morindalucinda* used in Nigerian Herbal Medicinal practice. *Journal of Biological Research and Biotechnology*. 2(1): 34-46.
- Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of pure and applied sciences*, 8(2), 203-208.

- Rahuman, A. A., Gopalakrishnan, G., Ghouse, B. S., Arumugam, S., & Himalayan, B. (2000). Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia*, 71(5), 553-555.
- Safa, O., Soltanipoor, M. A., Rastegar, S., Kazemi, M., Dehkordi, K. N., & Ghannadi, A. (2012). An ethnobotanical survey on hormozgan province, Iran. *Avicenna journal of phytomedicine*, 3(1), 64.
- Sarker, S., & Nahar, L. (2007). *Chemistry for pharmacy students: general, organic and natural product chemistry*. John Wiley & Sons.
- Sofowora, A. (1993). *Medicinal Plant and Medicine in Africa*, John Willey Spectrum, Ibadan Nigeria Pp. 281 - 285.
- Trease, G.E., & Evans, W.C. (1989). *Pharmacognosy*. London. Bailliere Tindall, London, Edn. 11, 45-50
- VandenBerghe, D.A.; Vlietinck, A.J. (1991). Screening methods for antibacterial and antiviral agents from higher plants. In: Dey, P.M., Harbone, J.D. (eds), *Methods in Plant Biochemistry*, Academic Press, London, p. 47-69

