Advances in Bioresearch
Adv. Biores., Vol 13 (5) September 2022: 29-38

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.5.2938

Advances in Bioresearch

ORIGINAL ARTICLE

GC-MS analysis, antimicrobial activity, and bioautography antioxidant assay in *Fagonia bruguieri* DC.

Piyush Panwar[1,5], Praveen Gehlot[2], Deepmala Verma[3], Manisha Mathur[4], K.K. Sharma[5], Raaz K. Maheshwari,[6]

[1]Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India .
[2]Department of Botany, Jai Narain Vyas University, Jodhpur, India.
[3]Department of Zoology, S SJain Subodh PG College, Jaipur, Rajasthan, India.
[4]Department of Botany, GD Girls College, Alwar, Rajasthan, India.
[5]Department of Chemistry, GD Girls College, Alwar, Rajasthan, India.
[6]Department of Chemistry, SBRM Govt PG College, Nagaur, Rajasthan, India.

ABSTRACT

In the present study, phytochemical analysis was done in a scarce plant Fagonia bruguieri DC. The plants were harvested from northwest states of Rajasthan, India. In vitro antimicrobial activity of different concentrations of acetone crude extract was done by agar well diffusion method. Two different nonpolar fractions (benzene and ethyl acetate) were also prepared for bioautography TLC assay. TLC bioautography divulged that Benzene fraction is not separating any antioxidant compound. With solvent system chloroform : ethanol, the ethyl acetate fraction of plant extract was showing three antioxidant compounds. In vitro antimicrobial activity reveals that the crude acetone extract of Fagonia bruguieri DC. is capable of inhibiting the growth of A. niger, E. floccosum, T. rubrum, P. aeruginosa on all selected concentrations and P. chrysogeum on 1mg/ml concentration. Furthermore GC-MS analysis of both non-polar fractions collectively identifies total 33 peaks. ethyl acetate fraction is showing the presence of citric acid, and hexadecanoic acid (organic antioxidant).

Keywords: antioxidant, antimicrobial, bioautography, medicinal plant, GC-MS.

Received 10.07.2022 Revised 30.07.2021 Accepted 21.08.2022

How to cite this article:
P Panwar, P Gehlot, D Verma, M Mathur, K.K. Sharma, R K. Maheshwari. GC-MS analysis, antimicrobial activity, and bioautography antioxidant assay in *Fagonia bruguieri* DC.. Adv. Biores. Vol 13 [5] September 2022. 29-38

.

INTRODUCTION

Species from the family of Zygophyllaceae are adapted to grow in arid and semi-arid environments. Some studies report approximately 285 species and 22 genera in the family of Zygophyllaceae[1]. These flowering plants are also reported from the Thar Desert, Rajasthan, India. Thar desert, which is located in the western part of India covers approximately 2,00,000 Km^2 of area. Study till now reports 10 to 11 species belonging to the family of Zygophyllaceae in Thar Desert [2]. *Fagonia bruguieri* DC. is reported as a rare plant species in this area [3]. The plant is 30 cm in height, flowers pale-pink in color. The plant is a biennial or perennial shrublet, pale-green in color. internodes 3 cm long, basal leaves are mostly trifoliolate and upper leaves unifoliolate. Stipular spines are mostly recurved, and longer than the leaves [4]. Local people know it by the name of "dhammas" and it is used as an herbal remedy for skin infections and itching. The plant is also mentioned as a herbal remedy in ayurvedic literature. It is a good source of antioxidants, anti-cancer agents. [5-7]. For the treatment of various infections and diseases, many developing countries are depended on herbal medicines. These are known as natural, less toxic, eco-friendly, affordable, and effective sources of treatment. Herbal medicines are also known as an alternative pathway to treat many medical conditions. These herbal medicines are also known for their antioxidant properties. Antioxidant compounds are those compounds that can act like armor to protect cells from reactive oxygen species [8]. Multiple drug resistance or antimicrobial resistance has now become a global problem, it is affecting global health, food security, and the economy [9-11]. There is an estimation of 4·95 million (3·62-6·57) deaths associated with antimicrobial resistance in 2019 [12]. It is imperative to

search for novel, efficacious and safe antibiotics. Many herbal medicines are also known for their antimicrobial activities [13]. Antimicrobial activity in *Fagonia bruguieri* DC. is yet obscure. Due to the scarcity of plants, the identification and characterization of nonpolar phytochemicals are not fully known. In the present study we identified antioxidant compounds by bioautography assay. Antioxidant compounds and other nonpolar compounds are further identified by GC-MS analysis. Apart from that, the antimicrobial activity of acetone crude extract was also done. The presence of moisture, ash, and primary metabolites was calculated.

MATERIAL AND METHODS

Collection of plant material

F. bruguieri was collected from Latitude: 27.364792 and Longitude: 72.510825 (Jaisalmer, Rajasthan, India). Identification- The plant was identified by the Botanical Survey of India, Jodhpur, Rajasthan, India (BSI), Identification Pl. Id. No. 402 (Figure 1). Washing and pulverizeding - To remove dust particles the plants were washed with tap water followed by distilled water. After cleaning the plants were shade dry at 25 degrees Celsius. The shade dried plants were powdered by pulverized machine.

Figure 1- Selected plant species: *Fagonia bruguieri* DC.

Moisture content, ash content, and estimation of primary metabolites

Moisture content- To calculate moisture content, in triplicate 5 grams of freshly harvested plant material were weighed and dried at room temperature. After drying the dried plant material was weighed. The following formula was used to calculate the moisture content percentage [14].

Moisture content = (fresh weight – dry weight)/ (fresh weight) X 100

Ash content- Ash content is helpful in the measurement of the inorganic non-burnable ions and material in plants. To calculate ash content, in triplicate 5 grams of dried plant material was kept in a crucible. The loaded crucibles were then kept in muffle furnace. The plant material was heated at 650°C (1202°F) for 7 hrs followed by cooling for 5 hrs. After burning the burnt plant material ash was weighed. The following formula was used to calculate the ash content percentage [15-16].

Ash content = (pre weight – ash weight)/ (pre weight) X 100

Carbohydrates - For estimation of free sugar, 0.5-gram plant material was mixed and crushed with 5 ml 80% ethanol. The mixture was then centrifuged at 12000 RPM for 15 minutes. Supernatant was collected and mixed with 5 ml concentrated sulphuric acid. Finally, one ml 5% phenol was added and kept at room temperature for 20 minutes. Glucose was used to set standard. Spectrophotometer was used to develop the standard. 80% ethanol was taken as a blank, Reading was taken at 490 NM wavelength. Lipid- Estimation of lipid was done by crushing 1 gm plant sample in 10 ml distilled water. Followed by that 20 ml trichloromethane (CHCl3) was added and crushed. After crashing 10 ml of

concentrated methanol was added, mixed and incubated at room temperature for 20 minutes. Finally, 2 ml of distilled water was added, and the mixture was filtered. The filtrate was collected and dried in a Petri plate. Dried filtrate was measured on weighing balance. Protein- For the estimation of protein Folin- Colcalteu reagent-based Lowry method was adopted. 100 mg of plant sample was mixed and crushed with 10 ml 0.2 molar phosphate buffer (72% of 0.2 M, mono sodium phosphate and 28% of 0.2 M, diabetes sodium phosphate). Bovine serum albumin was used as to set standard. The absorbance was measured at 660 NM [17-18].

Antimicrobial activity

Antimicrobial activity was done by the agar well diffusion method. Extract preparation- a modified maceration method was used to prepare the extract. Freshly powdered plant sample was mixed in acetone (1:10), incubated at room temperature (25 °C) for 24 hours, and filtrate was collected. Followed by that, Solid Residue was again mixed in acetone (1:10) and incubated at higher temperature (50°C) for 24 hours. Filtrate was collected and mixed. After incubation filtrate was dried in a petri plate. For bacteria nutrient Agar and for fungus potato dextrose Agar was used[19-20]. Three different concentrations of acetone crude extract (5 mg/ml, 2.5 mg/ml, and 1 mg/ml) and a control without extract were loaded in well. The well size was 4 mm inradius with a filling capacity of 120 µl. Microorganisms selected for experiment- Fungus like *Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum* were collected from Institute of Microbial Technology, Chandigarh, India. Bacteria and fungus like *Bacillus subtilis, Pseudomonas aeruginosa, Penicillium chrysogenum, Aspergillus niger, and Candida albicans* were collected from Sawai Man Singh Medical College, Jaipur, Rajasthan, India.

Isolation and characterization of phytochemical

Isolation of benzene fraction- 10 gram powdered plant sample was mixed in 100 ml of 30% hydrochloric acid and incubated at 37 °C for 4 hours. After incubation filtrate was discarded. Now to neutralize the pH from acidic to 7, solid residue was again mixed in distilled water. After neutralization of pH solid residue was collected and dried in a petri plate. After drying, solid residue was again mixed in 100 ml benzene and incubated at 37 °C for 24 hours. Finally, filtrate was collected and dried on a Petri plate [21-22].

Isolation of ethyl acetate fraction - For the ethyl acetate fraction, 10 gram powdered plant sample was mixed in 100 ml petroleum ether and incubated at 37°C for 24 hours. Subsequently, solid Residue was collected and dried on a petri plate. Now dried solid residue was mixed in 10 ml 85% ethanolic hydrochloric acid and incubated at 37°C for 4 hours. Now filtrate was collected and taken in a separating funnel. Subsequently, 50 ml ethyl acetate was added in separating funnel and mixed thoroughly. Now the upper layer was collected (three times). The upper layer was again transferred into a fresh separating funnel. To neutralize the pH of fraction (from Acidic to 7) the upper layer was washed with distilled water. Finally, ethyl acetate was again added in the mixture and the ethyl acetate layer was collected[23].

Antioxidant TLC bioautography assay- To separate bands on TLC plates two different solvent systems were used. For the benzene fraction, a freshly prepared mobile phase of n-hexane : diethyl Ether (1:1 v/v) was prepared and poured into the TLC chamber. Similarly for ethyl acetate fraction, freshly prepared mobile phase of Chloroform:ethanol (9:1 v/v) was prepared and poured in another TLC chamber. For better separation of bands both TLC chambers were saturated for 60 minutes. The TLC plates were activated by heating at 100 ºC for 60 min. After cooling of the TLC plate, both fractions were loaded on an activated TLC plate and run under a saturated TLC chamber. After separation of TLC bands the antioxidant compounds were identified by dipping the developed TLC plates into 0.05% methanolic DPPH[24].

Gas Chromatography-Mass Spectrometry analysis- GC column Rxi5 Si MS was used, thickness 0.25 um, length 30.0 m, diameter 0.25 mm, helium was used as a carrier gas, Pressure 66.8 kPa, total flow 23.9 mL/min, column flow 1.18 mL/min, linear velocity 39.4 cm/sec, purge flow 5.0 ml/min was taken. In MS ion source temp. was 250 °C. 2 µl of sample was injected, Total time for GC-MS was 30:75 minutes.

RESULT AND DISCUSSION

Moisture content and ash content – one gram fresh plant remains 0.535 gram after dehydration and ash production from 1 gram fresh plant sample is 0.060 gram. The moisture percentage in the plant is 46.5% and the dry weight percentage after dehydration of the plant is 53.5 %. Ash remains after blazing the dry plant is 12.1 %.

Primary metabolites- Primary metabolites like carbohydrates, fat, and protein are dominantly involved in the growth, development, and reproduction of any organism. In the present study, one gram of plant sample contains 1.97 Mg of free sugar (TSS), 198 Mg of nonpolar component (fat), and 40.92 Mg of protein. 18.3 % fat, 4.09 % of Protein, and 0.19% of free Sugar is present in Fagonia bruguieri DC.

Antimicrobial activity-The antimicrobial activity with acetone crude extract of *F. bruguieri* showed an inhibition zone with *T. rubrum, E. floccosum, A.niger* and *P. aeruginosa* at all the tested concentrations. *P. chrysogeum* is showing a zone of inhibition on 1 mg/ ml concentration, No zone of inhibition was observed with *M. canis , C. albicans* and *B. subtilis* (Table 1,2)[25]. It was observed that the highest zone of inhibition was documented (20 mm) with *T. rubrum* on 1 mg/ml and *E. floccosum* on 5 mg/ml Concentration. May be due to the sufficient presence of antimicrobial components in all concentrations no major changes in the size of the zone of inhibition was observed with *T. rubrum* and *E. floccosum.* The zone of inhibition was successively decreased when it treated acetone crude extract with *A.niger;* it was recorded 18 mm at higher concentration and successively decreased to 12 mm at lowest concentration. Similarly treatment of acetone crude extract with *P. aeruginosa* is showing zone of 16 mm on 5 mg/ml, 12 mm zone on 2.5 mg/ml concentration and no zone on lower concentration (table 2). *E. floccosum, M. canis* and *T. rubrum* are the fungus belonging to the phylum Ascomycota [25-26]. *T. rubrum* is one of the major causes of infections in humans like ringworm, jock itch, athlete's foot, infection in nail etc. [27]. Similarly in tropical and subtropical regions *E. floccosum* also cause an infection like tinea pedis, tinea corporis and onychomycosis on skin and nails[28]. Five pathogenic multiple drug resistance microorganisms including *P. aeruginosa* are steaking a major part in estimated 4·95 million deaths in 2019 [10].

TLC bioautography- When both fractions (benzene and ethyl acetate) were separated on silica gel based TLC plates, under visible light no bands were observed with benzene fraction and one band was observed with ethyl acetate fraction (Rf 0.62). Subsequently, treatment of TLC plate with DPPH reagent detected no band with benzene fraction and three pale-yellow bands on pinkish purple background were visible on TLC plate with ethyl acetate fraction (Rf 0.98, 0.76 and 0.59).

GC/MS analysis- Major phytochemical compounds present in both ethyl acetate and benzene fractions of *F. bruguieri* were identified by GC-MS analysis. Total of 28 compounds were identified from the complete plant of *F. bruguieri*. Their active principles with their retention time (RT), molecular formula, molecular weight, concentration of percentage, and chemical structure were presented in table 3,4.

Benzene fraction identified total 17 compounds, prevailing compounds in benzene fraction are Cyclohexane, methyl (0.94 %), Toluene (0.83), Heptane, 2,4-dimethyl (1.47), 2,4-Di-tert-butylpheno (0.71 %), Isopropyl myristate(0.55%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (1.10), 3-Eicosene, (E) (0.49%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.22 %), Dibutyl phthalate (5.13%), Tetrapentacontane (2.06%), Triphenylmethane (0.53%), Benzenemethanol, .alpha.,.alpha.-diphenyl (1.12%), Tetracosane (3.11%), 2-Methylhexacosane (1.83%), Hexatriacontane (15.49%), Bis(2-ethylhexyl) phthalate (1.46 %), and Tetracontane (4.52%). Ethyl acetate fraction also identified total 16 compounds, Heptane, 2,4-dimethyl (0.88%), 1-Propene-1,2,3-tricarboxylic acid, trimethyl ester, (E) (1.36%), Citric acid (1.32%), Methyl hexadec-9-enoate (0.63%), Hexadecanoic acid, methyl ester (8.38%), Dibutyl phthalate (1.80 %), 2(3H)-Furanone, 5-heptyldihydro (0.95%), 9,12-Octadecadienoic acid (Z,Z)-, methyl es (4.09%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (5.87%), Methyl stearate (1.78%), Triacontane, 1-iodo (3.61%), Hexadecanoic acid, methyl ester (2.05%), Triacontane, 1-iodo (2.78%), Hexatriacontane (5.82%), 9-Octadecenoic acid (Z)-, oxiranylmethyl ester (7.82%), 2-Methylhexacosane (8.57%), Docosanoic acid, and methyl ester (5.14%). Total of five compounds are common in both fractions they're Heptane, 2,4-dimethyl, Hexadecanoic acid, methyl ester, Dibutyl phthalate, 2-Methylhexacosane, and Hexatriacontane.

Bio activity- Hexadecanoic acid is an organic antioxidant compound, useful in hypercholesterolemia, nematicide and have pesticidal activity [29]. Dibutyl phthalate is useful for flexibility of material (fast-fusing plasticizers)[30] 9,12-Octadecadienoic acid (Z,Z)- is a fatty acid reported for its anti-inflammatory property, antihepatotoxicity property, useful in hypocholesterolemia, prevents from cancer, useful in treating symptoms of allergies, nematicidel, antieczemic, dihydrotestosterone blockers or 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, antiacne, antimicrobial, and insecticide in nature [31]. Hexatriacontane is possibly working as a antidepressant in mice [32]. 2-Methylhexacosane is antimicrobial in nature, and decreases blood cholesterol [33]. 2,4-Di-tert-butylphenol is reported as a phenolic compound showing antimicrobial activity against *A. niger, F. oxysporum,* and *P. chrysogenum*, and cytotoxicity against HeLa cells line of cancer [34-35]. Isopropyl myristate is reported as topical antifungal, anti-bacterial, anti-lice and anti-fleas [36]. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester or diisobutyl phthalate is reported as a antimicrobial, anti-inflammatory, antioxidant [37]. Docosanoic acid can be used in the production of biodiesel. 3-Eicosene, (E)- is reported as antimicrobial, antihyperglycemic, antioxidant, cytotoxic, and insecticidal [38]. Bis(2-ethylhexyl) phthalate is use in medical devices, toys and plastic wires etc [39]. Bio activity of tetracontane is reported as an antioxidant, and antibacterial compound [40].

Table 1- Antifungal activity acetone crude extract

	T. rubrum			*M. canis*			*E. floccosum*			*P. chrysogeum*			*C. albicans*			***A. niger***		
Conc (mg/ml)	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1
Zone of inhibition [mm]	16	18	20	+VE	NZ	NZ	20	18	14	NZ	NZ	12	NZ	NZ	NZ	18	16	12

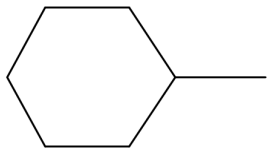
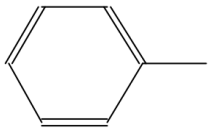
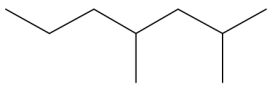
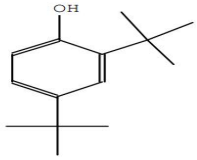
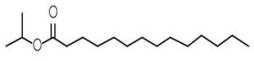
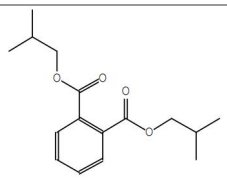
NZ- No zone of inhibition.

Table 2 -Antibacterial activity of acetone crude extract

	B. subtilis			*P. aeruginosa*		
Concentrations (mg/ml)	5	2.5	1	5	2.5	1
Zone of inhibition [mm]	NZ	NZ	NZ	16	12	+VE

NZ- No zone of inhibition.

Table 3- GC-MS assay of benzene fraction

S. No.	**RT**	**Compound name**	**Peak area (%)**	**Molecular formula**	**Molecular Weight g/mole**	**Structures**
1	2.894	Cyclohexane, methyl-	0.94	C_7H_{14}	98	
2	3.443	Toluene	0.83	C_7H_8	92	
3	3.911	Heptane, 2,4-dimethyl	1.47	C_9H_{20}	128	
4	17.790	2,4-Di-tert-butylpheno	0.71	$C_{14}H_{22}O$	206	
5	22.377	Isopropyl myristate	0.55	$C_{17}H_{34}O_2$	270	
6	22.923	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.10	$C_{16}H_{22}O_4$	278	

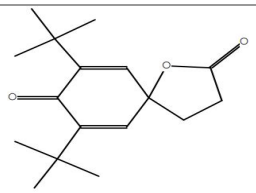
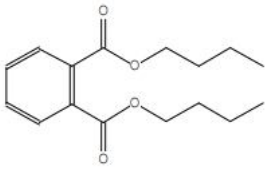
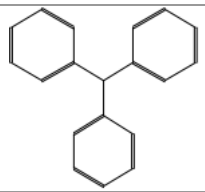
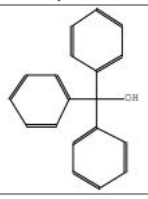
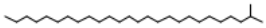
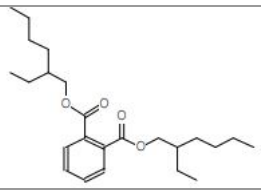
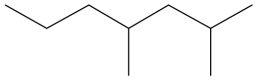
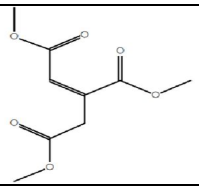
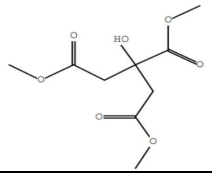
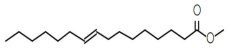
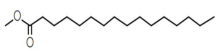
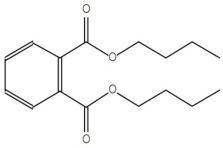
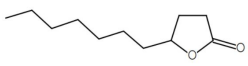
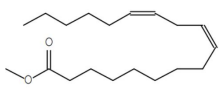
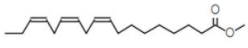
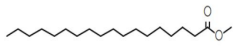
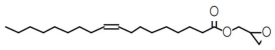
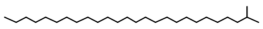
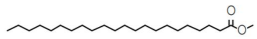
7	23.280	3-Eicosene, (E)-	0.49	$C_{20}H_{40}$	280	
8	23.553	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1.22	$C_{17}H_{24}O_3$	276	
9	24.091	Dibutyl phthalate	5.13	$C_{16}H_{22}O_4$	278	
10	24.145	Tetrapentacontane	2.06	$C_{54}H_{11}0$	758	
11	24.766	Triphenylmethane	0.53	$C_{19}H_{16}$	244	
12	26.454	Benzenemethanol, .alpha.,.alpha.-diphenyl	1.12	$C_{19}H_{16}O$	260	
13	27.372	Tetracosane	3.11	$C_{24}H_{5}0$	338	
14	28.530	2-Methylhexacosane	1.83	$C_{27}H_{56}$	380	
15	29.379	Hexatriacontane	15.49	$C_{36}H_{74}$	506	
16	29.800	Bis(2-ethylhexyl) phthalate	1.46	$C_{24}H_{38}O_4$	390	
17	30.417	Tetracontane	4.52	$C_{40}H_{82}$	562	

Table 4- GC-MS of assay ethyl acetate fraction

S. No.	RT	Compound name	Peak area (%)	Molecular formula	Molecular Weight g/mole	Structures
1	3.907	Heptane, 2,4-dimethyl	0.88	C_9H_{20}	128	
2	16.348	1-Propene-1,2,3-tricarboxylic acid, trimethyl ester, (E)-	1.36	$C_9H_{12}O_6$	216	
3	16.899	Citric acid, trimethyl este	1.32	$C_9H_{14}O_7$	234	
4	23.594	Methyl hexadec-9-enoate	0.63	$C_{17}H_{32}O_2$	268	
5	23.663	Hexadecanoic acid, methyl ester	8.38	$C_{17}H_{34}O_2$	270	
6	24.087	Dibutyl phthalate	1.80	$C_{16}H_{22}O_4$	278	
7	25.227	2(3H)-Furanone, 5-heptyldihydro	0.95	$C_{11}H_{20}O_2$	184	
8	25.579	9,12-Octadecadienoic acid (Z,Z)-, methyl es	4.09	$C_{19}H_{34}O_2$	294	
9	25.647	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	5.87	$C_{19}H_{32}O_2$	292	

10	25.906	Methyl stearate	1.78	$C_{19}H_{38}O_2$	289	
11	27.737	Triacontane, 1-iodo	3.61	$C_{30}H_{61}I$	548	
12	30.416	Triacontane, 1-iodo-	2.78	$C_{32}H_{65}I$	576	
13	28.530	Hexatriacontane	5.82	$C_{36}H_{74}$	506	
14	29.244	9-Octadecenoic acid (Z)-, oxiranyl methyl ester	7.82	$C_{21}H_{38}O_3$	338	
15	29.430	2-Methylhexacosane	8.57	$C_{27}H_{56}$	380	
16	29.708	Docosanoic acid, methyl ester	5.14	$C_{23}H_{46}O_2$	354	

CONCLUSION

The present study concludes that the acetone crude extract of *F. bruguieri* is capable of inhibiting the growth of *T. rubrum, E. floccosum, P. chrysogeum, A.niger,* and *P. aeruginosa.* Five pathogenic microorganisms (including *P. aeruginosa*) are steaking a major part in estimated 4·95 million deaths by multiple drug resistant microorganisms in 2019. Furthermore GC-MS analysis also identified phenolic compound like 2,4-Di-tert-butylphenol which is known for its antifungal activity against *A.niger.* The antioxidant TLC bioautography assay also reveals the towering presence of three antioxidant bands showing the retardation factor value of 0.98, 0.76 and 0.59 in ethyl acetate fraction. GC-MS analysis of ethyl acetate fraction confirms the presence of hexadecanoic acid (8.38 peak area %), and citric acid (1.32 peak area %) which are known for their antioxidant activity. GC-MS analysis also prevail bio active compounds like hexadecanoic acid, dibutyl phthalate, 9,12-Octadecadienoic acid (Z,Z)-, Hexatriacontane, 2-Methylhexacosane, 2,4-Di-tert-butylphenol, isopropyl myristate, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, docosanoic acid, 3-Eicosene, (E)-, Bis(2-ethylhexyl) phthalate, and tetracontane in benzene, and ethyl acetate fractions.

AUTHORS' CONTRIBUTION AND ACKNOWLEDGMENT

RK, DV, and PG conceptualized and designed the experiment. All the experiments were performed by PP. RK and PG supervised the experiment. PP analyzed the data and wrote the manuscript. The authors are thankful to Seminal Applied Sciences for providing microorganisms. The authors are also thankful to Dr. Ajay, Manipal University, Jaipur for GC-MS analysis.

REFERENCES

1. Christenhusz, M. J., and Byng, J. W. (2016). The number of known plants species in the world and its annual increase. Phytotaxa, 261(3), 201. doi:10.11646/phytotaxa.261.3.1.
2. Bhandari,M.M.(1990) Flora of the Indian Desert, p. 79.
3. Pandey,R. P.(1987) zygophyllaceae," Flora of india series 2(2), p. 167.
4. Pandey, R. P., Parmar, P. J. & Singh, V. (1987). 29. zygophyllaceae. *Flora of india series 2,* September, Volume 2, p. 161.
5. Shehab , N. G., Mahdy,A., Khan, S. A., and Noureddin, S. M. (2011). "Chemical constituents and biological activities of Fagonia indica Burm F." Research Journal of Medicinal Plant 5 (5): 531-546. doi:10.3923/rjmp.2011.531.546.
6. Bagban, I.M., Roy, S.P., Chaudhary, A., Das, S.K., Gohil, K.J. and Bhandari, K.K. (2012). "Hepatoprotective activity of the methanolic extract of Fagonia indica Burm in carbon tetra chloride induced hepatotoxicity in albino rats." Asian Pacific Journal of Tropical Biomedicine 2 (3): S1457-S1460. doi:https://doi.org/10.1016/S2221-1691(12)60437-7
7. Panwar, P. & Mathur, M., (2022). DPPH Radical Scavenging Activity, Antimicrobial Activity, and Screening of Plant Extract with Bioautography Assay in Fagonia. *Res. Jr. of Agril. Sci.,* 13(05), p. 1544–1547.
8. Buettner, G., (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics,* 300(2), pp. 535-543.
9. Poole, R. K., (1994). Bacterial multidrug resistance—emphasis on efflux mechanisms and Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy,* 1 October , 34(4), p. 453–456.
10. Murray , C. J. L. et al., (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lencet,* 12 Feb, 399(10325), pp. 629-655.
11. WHO, (2020). *Antibiotic resistance.* Online [Accessed 2022].
12. A. R. C., (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Antimicrobial Resistance Collaborators,* 399(10325), pp. 629-655.
13. Tambekar, D. & Dahikar, S., (2011). Antibacterial activity of some Indian ayurvedic preparations against enteric bacterial pathogens. *Journal of Advanced Pharmaceutical Technology & Research,* 21 april, 2(1), pp. 24-29.
14. Rathore, M., (2018). Variation in nutritional value of grewia tenax fruits from different regions of rajasthan, India. *Journal of Phytology,* pp. 12-14.
15. Aravantinos-Zafiris, G., Oreopoulou, V., Tzia, C. & Thomopoulos, C., (1994). Fibre fraction from orange peel residues after pectin extraction. *LWT - Food Science and Technology,* 27(5), pp. 468-471.
16. Harris, G. K. & Marshall, M. R., (2017). Ash analysis. In: *Food Analysis, 5th edn. Springer, New York.* s.l.:s.n., pp. 287-297.
17. Krishna, C. S., Sajeesh, T. & Parimelazhagan, T. (2014). Evaluation of nutraceutical properties of Laportea interrupta (L.) chew. *Food Science and Biotechnology,* 23(2), pp. 577-585.
18. Chandran, R., Nivedhini, V. & Parimelazhagan, T. (2013). Nutritional composition and antioxidant properties of Cucumis dipsaceus Ehrenb. ex Spach leaf. *The Scientific World Journal,* Volume 2013, pp. 1-9.
19. Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. International Journal of Infectious Diseases, 8(1), 39-45. doi:10.1016/j.ijid.2003.03.002
20. Okeke, M., Iroegbu, C., Eze, E., Okoli, A., & Esimone, C. (2001). Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. Journal of Ethnopharmacology, 78(2-3), 119-127. doi:10.1016/s0378-8741(01)00307-5
21. Kaul , B. & Staba, . E. J., (1968). Dioscorea tissue cultures. I. Biosynthesis and isolation of diosgenin from Dioscorea deltoidea callus and suspension cells.. *Loydia,* Volume 10, p. 171.
22. Sharma, M. & Khichar, . M. K., (2017). Isolation and identification of phytosterols from bignonia venusta (l.). *Asian J Pharm Clin Res,* 10(12), pp. 247-251.
23. Tomita, Y., Uomori, A. & Minato, H., (1970). Steroidal sapogenins and sterols in tissue cultures of dioscorea tokoro. *Phytochemistry,* 9(1), pp. 111-114.
24. Glavind, J., & Holmer, G. (1967).Thin-Layer Chromatographic Determination of Antioxidants α,α' Diphenyl-β-picrylhydrazy. Journal of the American Oil Chemists' Society, 44(9), 539-542. doi:10.1007/bf02679243
25. Rebell , G., Taplin , D. & Blank, H., (1964). *Dermatophytes: recognition and identification.* Miami, Florida: s.n.
26. Gräser, Y., Kühnisch, J. & Presber, W., (1999). Molecular Markers Reveal Exclusively Clonal Reproduction in Trichophyton rubrum. *Journal of Clinical Microbiology,* November, Volume 37(11), p. 3713–3717.
27. Zaugg, C. et al., (2009). Gene expression profiling in the human pathogenic dermatophyte Trichophyton rubrum during growth on proteins. *EUKARYOTIC CELL,* feb, Volume 8(2), p. 241–250.
28. Ahearn, D. G., (1988). Medical mycology: The pathogenic fungi and the pathogenic Actinomycetes. 260(12), p. 1794.
29. Siswadi, S. & Saragih, G. S., (2021). Phytochemical analysis of bioactive compounds in ethanolic extract of sterculia quadrifida R.Br.. *INTERNATIONAL CONFERENCE ON LIFE SCIENCES AND TECHNOLOGY (ICoLiST 2020).*
30. Lorz, P. M. et al., (2007). Phthalic acid and derivatives. *Ullmann's Encyclopedia of Industrial Chemistry.*
31. Diab, T. A., Donia, T. & Saad-Allah, K. M., (2021). Correction to: Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. *Beni-Suef University Journal of Basic and Applied Sciences,* 10(1).

32. Anon., (2017). Pharmacological repositioning of Achyranthes aspera as an antidepressant using pharmacoinformatic tools PASS and PharmaExpert: a case study with wet lab validation. *SAR and QSAR in Environmental Research,* pp. 69-81.
33. Khatua, S., Pandey, A. & Biswas, . S. J., (2016). Phytochemical evaluation and antimicrobial properties of Trichosanthes dioica root extract. *Journal of Pharmacognosy and Phytochemistry,* pp. 410-413.
34. Varsha, K. K. et al., (2015). 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus Sp. *International Journal of Food Microbiology,* Volume 211, pp. 44-50.
35. Torres, T. M. et al., (2021). Neuroprotective potential of extracts from leaves of Ora-pro-nobis (Pereskia aculeata) recovered by clean compressed fluids. *The Journal of Supercritical Fluids,* Volume 179, p. 105390.
36. Otunola, G. A. & Afolayan, A. J., (2018). Chemical Composition, Antibacterial and in vitro AntiInflammatory Potentials of Essential Oils from Different Plant Parts of Moringa oleifera Lam. *American Journal of Biochemistry and Biotechnology,* 14(3), pp. 210-220.
37. Beulah, G. G., Soris, P. T. & Mohan, V. R., (2018). GC-MS Determination of Bioactive Compounds of Dendrophthoe falcata (L.F) Ettingsh: An Epiphytic Plant. *International Journal of Health Sciences & Research,* 8(11), pp. 261-269.
38. Banakar , P. & Jayaraj, M., (2018). GC-MS analysis of bioactive compounds from ethanolic leaf extract of waltheria indica linn. and their pharmacological activities. *International Journal of Pharmaceutical Sciences and Research,* 9(5), pp. 2005-2010.
39. Shea, K. M., (2013). Pediatric exposure and potential toxicity of phthalate plasticizers. *Pediatrics,* 111(6), pp. 1467-1474.
40. pancholi, B., Gupta , S. & Verma, . B. K., (2022). bioactive constituents from bark of peltophorum pterocarpum baker ex k. heyne. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences,* Volume 24(2), pp. 427-433.