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Phytochemical Spectrum of Essential Oil of *Paganum harmala* by GC-MS and Antimicrobial Activity Using Sequential Solvents Fractions and Essential Oil

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Present study was designed to evaluate the antimicrobial potential of essential oil and extracts of different parts of *Paganum harmala*. Methanolic extract was prepared and extracted further using different organic solvents *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water. These extracts were then used for determining their antimicrobial potential. The sample found conspicuous antimicrobial activity of different extracts. In addition, extracted essential oil of this plant using super critical fluid extraction show antimicrobial activity. In essential oil, 1,2-*bis*[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine (13.29 %), methanol, [4-(1,1-dimethylethyl)phenoxy], acetate (11.29 %), Uridine, 5-tr hexyl-idecafluoro (8.56 %), Tetracosane (5 .40 %) and *bis*[phenylsulfonyl]-4-trichloromethylphenyl chloromethane (5.29 %) were the major constituents. Antimicrobial potential of *P. harmala* could be attributed to the compounds detected in essential oil.

Keywords: *Paganum harmala*, Antibacterial activity, GC-MS analysis, Medicinal plants.

INTRODUCTION

The use of medicinal plants to cure human diseases has been on rise. This increased interest of scientific and local community towards utilizing medicinal plants for treating ailments is due to the reason that plants are generally considered safer with no side effects[1]. Plants have different phytoconstituents which have as antioxidant and antimicrobial properties[2-4]. We are surrounded by a plethora of microorganisms which are known to cause severe illness in humans. These microorganisms have the tendency to develop drug resistance that renders humans prone to several diseases. However, medicinal plants are known to possess antimicrobial potential that could be utilized to combat these microbes and there are least chances of microbes to get resistance against plant extracts. Because antimicrobial activity of plant extract is known to be due to the presence of a number of compounds[5]. There are several reports in the literature that indicated the use of medicinal plants as antimicrobial agents[5]. *Paganum harmala* is widely utilized as medicinal plant. Antimicrobial potential of this medicinal plant has also been reported. The seeds are the chief portion recycled as a neurosensory drug and entertaining medicine and for the treatment of skin cancer, temperature, round worm, *etc*. Plant contains different chemical species, those may be sterols, anthraquinones, flavonoids, triterpenes, oxamides[7]. Furthermore, it is known that presence of these chemical compounds in essential oil could be responsible for its antimicrobial potential. As mentioned earlier, geographical distribution could affect the chemical constituents, therefore, we planned this study to evaluate the antimicrobial potential of organic extracts of different plant parts and probe the components present in essential oil[8].

Antimicrobial potential of this plant has been studied in India and other Asian countries. However the biochemical activity has not been widely reported in Pakistan. Some authors have studied chemical analysis and biological properties of various plants extracts in Pakistan[9-13]. Therefore, our aim was to report antimicrobial potential of *Paganum harmala* native in Pakistan. Furthermore, we were also interested to know chemical profile of this medicinal plant.

EXPERIMENTAL

Paganum harmala plants were grown in New Botanical Garden, University of Agriculture, Faisalabad, Pakistan. Plants were grown under natural conditions. The climate condition at the experimental site during the determination of different physiological attributes like day and night temperature was 39.28 ± 3 °C and 22.92 ± 4 °C, respectively. Photosynthetically available radiation (PAR) measured at non varied from 897 to 1364 μmol m^{-2} s^{-1}. And day night RH 33.1/55.1 %, respectively while geographical location of New Botanical Garden was latitude 30°30 N and longitude 73°10 E and altitude 213 from

sea level. Plants at reproductive stage were harvested and shade dry material of this plant was used for antimicrobial activity.

Nutrient agar, nutrient broth, potato dextrose agar, chloramphenicol and rifampacin from oxide, UK. *n*-Hexane, chloroform, ethyl acetate and *n*-butanol from Merck (Germany). All other chemicals and reagents were of at least analytical grade.

Organic extraction and fractionation: The shade-dried roots, stems and leaves were crushed and extracted with methanol at room temperature. The extract was evaporated in rotary evaporator to yield the residue. The whole residue was fractionated with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water[1].

Extraction of essential oil by using supercritical fluid extraction (SCFE): The extraction procedure was followed by the method of Cossuta *et al.*[14]. In the super critical fluid extraction (SCFE) process, the extraction was carried out by a solvent above its critical pressure and temperature. Carbon dioxide (CO_2) was used as extraction solvent and this process was carried out at at 100 bar pressure and temperature 40 °C at Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. The system used was (Deven Supercriticals Pvt. Ltd, Model No. PR-35). Antimicrobial activities of the essential oil against microorganisms was checked by using different standard methods.

GC-MS analysis of essential oils: GC-MS detection of prominent compounds was performed following the method of Delazar *et al.*[15].

Antimicrobial assay by disc diffusion method: Nutrient agar (Oxoid, UK) was prepared and then autoclaved. Before transferring this medium in sterilized Petri plates, 100 µL inoculum was added in medium while it was liquid and quite cool. Mixed and then pour into Petri plates. After this, 6 mm Wicks paper discs were laid flat on growth medium and 100 µL was put on each disc. The Petri plates were then incubated at 37 °C for 24 h, for the growth of bacteria. The extracts having antibacterial activity, inhibited the bacterial growth and clear zone of inhibition was formed. The zones of inhibition were measured in millimeters using zone reader and for fungal growth PDA (Oxide UK) was used. Antifungal activity of sample against selected fungal strains was determined by using disc diffusion method[16].

Fungal and bacterial strain and standard used: Fungal strains used were *Fusarium solani*, *Aspergillus niger*, *Trichoderma harizianum* and *Helmmentho sporiummydis* species and *Staphylococcus auraus* and bacterial strains were *Escherichia coli*, *Pasturella multocid*, *Bacillus subtilus* species, *etc*. were used for the assay. The chloramphenical and rifimpacine were used as standard.

Minimum inhibitory concentration (MIC mg/mL): Minimum inhibitory concentration of different samples was determined by resazurine method on the selected fungal and bacterial strains[17,18].

RESULTS AND DISCUSSION

Organic extracts of different solvents were tested against selected fungal strains (*Trichoderma harizianum*, *F. solnais*, *H. sporiummyedis*, *A. niger*). The organic extracts of different plant parts had shown significant antifungal activity. Of different solvents used to obtain organic extract and fractions, *n*-hexane fraction did not show any activity against *T. harizianum*. However, chloroform, ethyl acetate, *n*-butanol and aqueous fractions exhibited significant antifungal activity against *T. harizianum*. Maximal antifungal activity was recorded by leaf and stem extract of aqueous with MIC value 193.12 ± 0.19 and 187.5 ± 0.18 mg/mL, respectively. Different fractions extracted with organic solvents (*n*-hexane, chloroform, ethyle acetate, *n*-butanol and aqueous) had shown marked antifungal activity against *F. solani*. In contrast, root material extracted with ethyl acetate exhibited a significant activity against fungus *F. solani* with MIC 93.75 ± 0.09 mg/mL. The *n*-butanol extract were second on rank with respect to antifungal activity against *F. solani* with MIC value 95.62 ± 0.09 mg/mL. Likewise, results of present study of *n*-butanol extracts of leaf and root exhibited maximal antifungal activity against *H. sporiummyedis* (with MIC range). In contrast, ethyl acetate stem extract exhibited better activity against *H. sporiummyedis*. Maximum antifungal activity against *A. niger* was recorded in ethyl acetate extracts of leaf and *n*-butanol extracts of stem and roots (Table-1).with the MIC value 45 ± 0.04 and 47.34 ± 0.04 mg/mL, reactively.

Antifungal activity of different plant parts of *Paganum harmala* has been reported in the literature[16]. The water/aqueous soluble extracts caused a significant decrease in the mycelial growth of the majority of fungi tested with maximum activity detected for seed extracts. Additionally, water soluble seed extract inhibited spore germination of *Fusariumoxy sporum* f. sp. *melonis*. This study demonstrated the antifungal activity of *P. harmala* extracts on phytopathogenic fungi which can be used as an alternative for chemical compounds. Previous studies isolated four alkaloids from *P. harmala i.e.*, harmine, marmaline, harmalol and peganin in which harmine has shown some activity against algae, bacteria and fungi[19] and reported antifungal activity of *Paganum harmala* extract against fungal pathogen *Candida aldicans*.

Orgainc compounds extracted with different organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol and water) exhibited prominent antibacterial activity against *S. aureus*. Organic compounds of leaf stem and roots extracted with *n*-butanol and ethyl acetate exhibited maximal antibacterial activity against *S. aureus* (with MIC 24.37 ± 0.02 mg/mL.

Likewise, organic compounds of different plant parts extracted with ethyl acetate and *n*-butanol had shown maximal antibacterial activity against *B. subtilis* with MIC 11.95 ± 0.01 mg/mL.

Organic fractions of different plant parts obtained with *n*-butanol exhibited maximal antibacterial activity against *E. coli* MIC 95.62 ± 0.09 mg/mL. Organic fractions in other solvents exhibited moderate activity against this bacterial strain (*E. coli*). Of different organic extracts tested against *P. multocid*, chloroform stem extract had shown maximum antibacterial activity (Table-2). with MIC 90.52 ± 0.04 mg/mL.

Our results are in line with available literature that depicts the antimicrobial potential of different parts of *Paganum harmala* extracted in different organic solvents. In this context, Petrovic *et al.*[20] reported antimicrobial activity of ethyl acetate, water and ethanol extract against *Agrobacterium radiobacter sp. tumefaciens*, *Erwinia carotovora*, *Pseudomonas fluorescens*

TABLE-1
ANTIFUNGAL ACTIVITY OF *Paganum harmala* AGAINST SELECTED FUNGAL STRAINS

Samples	*Paganum harmala* against *Trichoderma harizianum*			*Paganum harmala* against *F. solnais*		
	Leaf	Stem	Root	Leaf	Stem	Root
n-Hexane	–	–	–	10 ± 0.32	10 ± 0.28	12 ± 0.12
Chloroform	–	–	10 ± 0.13	16 ± 0.35	12 ± 0.31	20 ± 0.32
Ethyl acetate	12 ± 0.14	20 ± 0.18	–	18 ± 0.37	14 ± 0.34	24 ± 0.31
n-Butanol	10 ± 0.12	12 ± 0.14	22 ± 0.22	20 ± 0.41	16 ± 0.35	18 ± 0.20
Aqueous	20 ± 0.18	20 ± 0.21	–	24 ± 0.45	20 ± 0.42	10 ± 0.24
Chloramphenicol	32 ± 0.24	32 ± 0.25	32 ± 0.18	32 ± 0.44	30 ± 0.53	32 ± 0.52
Samples	*Paganumharmala* against *H. sporiummyedis*			*Paganum harmala* against *A. niger*		
	Leaf	Stem	Root	Leaf	Stem	Root
n-Hexane	12 ± 0.06	–	14 ± 0.08	10 ± 0.12	10 ± 0.14	–
Chloroform	17 ± 0.08	–	20 ± 0.12	12 ± 0.11	18 ± 0.13	12 ± 0.01
Ethyl acetate	21 ± 0.21	16 ± 0.09	–	16 ± 0.05	14 ± 0.07	10 ± 0.03
n-Butanol	24 ± 0.12	12 ± 0.04	20 ± 0.07	14 ± 0.5	18 ± 0.05	14 ± 0.07
Aqueous	10 ± 0.11	–	–	13 ± 0.02	10 ± 0.03	–
Chloramphenicol	34 ± 0.05	34 ± 0.07	36 ± 0.08	30 ± 0.05	30 ± 0.02	36 ± 0.23

TABLE-2
ANTIBACTERIAL ACTIVITY IN TERMS OF ZONE OF INHIBITION
(MM) BY *Paganum harmala* Against Selected Bacterial Strains

Samples	*Paganum harmala* against *S. aureus*			*Paganum harmala* against *B. subtilis*		
	Leaf	Stem	Root	Leaf	Stem	Root
n-Hexane	10 ± 0.42	–	10 ± 0.5	–	10 ± 0.33	12 ± 0.22
Chloroform	12 ± 0.23	12 ± 0.17	16 ± 0.6	12 ± 0.13	10 ± 0.34	12 ± 0.28
Ethyl acetate	14 ± 0.02	16 ± 0.08	18 ± 0.4	18 ± 0.03	20 ± 0.22	14 ± 0.24
n-Butanol	18 ± 0.05	14 ± 0.61	14 ± 0.2	10 ± 0.25	22 ± 0.04	17 ± 0.22
Aqueous	12 ± 0.1	14 ± 0.03	12 ± 0.1	12 ± 0.30	18 ± 0.07	16 ± 0.29
Rifamycin	34 ± 0.09	34 ± 0.03	32 ± 0.05	34 ± 0.44	34 ± 0.38	34 ± 0.23
Samples	*Paganum harmala* against *E. coli*			*Paganum harmala* against *P. multocid*		
	Leaf	Stem	Root	Leaf	Stem	Root
n-Hexane	10 ± 0.03	–	12 ± 0.01	12 ± 1.11	10 ± 0.90	–
Chloroform	12 ± 0.05	–	18 ± 0.03	14 ± 0.12	22 ± 0.16	12 ± 0.25
Ethyl acetate	–	16 ± 0.03	10 ± 0.03	16 ± 1.1	10 ± 0.96	10 ± 0.41
n-Butanol	20 ± 0.08	18 ± 0.09	16 ± 0.11	14 ± 0.89	–	14 ± 0.16
Aqueous	–	10 ± 0.05	–	10 ± 0.85	12 ± 0.15s	–
Rifamycin	30 ± 0.05	30 ± 0.08	30 ± 0.06	30 ± 0.57	30 ± 0.75	30 ± 0.37

and *P. aeruginosa*. Of different extracts ethyl acetate extract was found to be very effective. Similarly, Nenaah[21] used bioassay-guided fractionation to extract β-carbolin alkaloids and tested their antibacterial and antifungal activity. He observed significant variations in compounds with respect to their antimicrobial potential. This variation was dependent on type of microorganisms tested and method of application. For example, inhibition zone ranging from 21.2 to 24.7 mm were observed in *Proteus vulgaris*, *Bacillus subtilis* and *Candida albicans* due to application of harmine. Furthermore, he reported that effectiveness of alkaloids was even more when applied as binary mixtures. For example he recorded 31.5 mm zone of inhibition on application total alkaloid extract. Likewise, *Paganum harmala* alkaloids were shown to possess antimicrobial activity against *S. aureus*, *E. coli* and *P. vulgaris*[22]. Seed extract of *Paganum harmala* was found to be very effective antimicrobial agents against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*. Of various extracts, ethyl acetate and ethanol were observed the most effective one[7].

Essential oil was extracted with the help of super critical fluid extraction method. GC-MS analysis of essential oil exhibited the presence of 39 components that make up to 98 % of total fraction. This volatile fraction consisted of a mixture of different classes of compounds. The major constituents were found to be 1,2-*bis*[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine (13.29 %), methanol, [4-(1,1-dimethylethyl)phenoxy]-, acetate (11.29 %), uridine, 5-*tr* hexyl-idecafluoro (8.56 %), tetracos (5.40 %) and *bis*[phenylsulfonyl]-4-trichloromethylphenyl chloromethane (5.29 %).

Antimicrobial activity of essential oil of *Paganum harmala* microbial strains: The antibacterial activity was evaluated by paper disc diffusion methods (Table-3). The qualitative antibacterial was carried out by the disc diffusion against four selected strain; *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Fusarium solnai* by using the method of Sarpeleh *et al.*[16].

Antimicrobial activity of essential oil of *Paganum harmala* exhibited significant antimicrobial potential against selected fungal and bacterial strain. Antimicrobial potential of essential of this medicinal plant could be attributed to the presence of different organic compounds in essential oil. Recently, Rizwan *et al.*[23] reported antimicrobial potential of essential oil was largely due to the presence of different organic compounds in

TABLE-3
CHEMICAL PROFILING BY GC-MS OF ESSENTIAL OIL FROM *Paganum harmala* EXTRACTED BY SUPERCRITICAL FLUID EXTRACTION

R.Time (Min)	Compound Name	Area %
3.398	Dimethoxybutane	5.24
4.501	3,3-Dimethoxy-2-butanone	0.35
4.850	3,3-Dimethoxy-2- butanone	0.41
4.970	1,1'-[ethylidene*bis*(oxy)]*bis*[2-methy1	0.3
8.690	Undecane	1.24
19.675	3,3-Dimethoxy-2-butanone	0.26
20.075	1,1'-[ethylidene*bis*(oxy)]*bis*[2-methy1	0.57
20.632	Undecane	1.16
20.783	Sulfur	0.54
20.858	1-(4,7 dihydro-2-methy1-7-oxopyrazolo [1,5-a]pyrimidin-5y1)-methyl Oster	0.21
20.892	3-Methyl heneicosane	0.62
21.042	2,6,10,15-Tetramethyl heptadecane	0.85
21.167	1,1,3,3,5,-hexadecamethyl	4.56
21.250	Chloroaniline-5 sulfonic acid	0.73
21.633	Pregna-5,16-dien-20-one, 3-[(trimethylisilyl)oxy]-, O-methyloxime,(3β)-	0.35
21.720	N-[(pentafluorophenyl)methylene]-3,4-*bis*[(trimethylsily)oxy]-	4.89
21.825	Tetratriacontane	3.9
21.908	Hexestrol di-TMS	2.78
21.958	[1-(3-butenylthio)-2-nitroethyl]	1.88
22.158	Tetracosane	5.4
22.308	1,2-*Bis*[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine	13.23
22.367	5-tridecafluorohexyl-	0.99
22.425	Uridine, 5-tridecafluorohexyl-	2.12
22.500	Tetracosane	2.6
22.566	Cholestano[3,5-c]isoquniolin-1 '(2'H)-one , 3',4'-dihydro-6',7'-dimethoxy	3.57
22.725	*Bis*[phenylsulfonyl]-4-trichloromethylphenyl chloromethane	5.29
22.775	Sarreroside	2.21
22.842	Verrucarin A, 7'-deoxo-7'-(1-hydroxyethyl)-	0.91
22.949	Uridine, 5-tr hexyl-idecafluoro	8.56
23.042	3,5,9- Trioxa-4-phosphapentacosan-1-aminium, 4-hydroxy-N,N,N-trimethyl-10-oxo-7-[(1-oxohexadacyl)oxy]-, hydroxi	1.77
23.204	Hexatriacontane	3.7
23.275	Butanedioicacid,2,3-*bis*[(*tert*-butyldimethylsily)oxy]-,*bis*(*tert*-butyldimethylsily) ester	1.42
23.333	Butanedioicacid,2,3-*bis*[(*tert*-butyldimethylsily)oxy]-,*bis*(*tert*-butyldimethylsily) ester	0.88
23.375	*Bis*[4-[4-hydroxy]pipperidino-3-nitrophenyl] sunlfone	1.34
23.450	Cholestano[3,2-c]isoquinolin-1'(2'H)-one, 3',4'-dihydro-6', 7'-dimethoxy-	1.2
23.517	Timethyl(4-*tert*.-butylphenoxy)silane	0.86
23.663	Methanol,[4-(1,1-dimethylethyl)phenoxy]-, acetate	11.29
23.817	Hexatriacontane	0.63
23.875	Ergostane-5,25-diol,3,6,12-*tris*[(trimethylsilyl)oxy]-,25-acetate,(3β, 5α, 6β, 12β)	0.71

TABLE-4
ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *Paganum harmala*

Essential oil	*Aspergillus niger*	*Fusarium solnai*	*Staphylococcus aureus*	*E. coli*
P. harmala	14 ± 0.1	18 ± 1.5	2o ± 1,2	22 ± 1.1
Standard	30 ± 0.9	30 ± 0.7	36 ± 0.3	36 ± 0.2

essential oil of *Agave attenuata*. The major compounds responsible for antimicrobial activity were found to be 1,2-*bis*[1-(2-hydroxyethyl)-3,6-diazahomoadamantanty-dene-9] hydrazine (13.29 %), methanol, [4-(1,1-dimethylethyl)-phenoxy]-, acetate (11.29 %), uridine, 5-*tr* hexyl-idecafluoro (8.56 %), tetracosane (5 .40 %) and *bis*[phenylsulfonyl]-4-trichloromethylphenyl chloromethane (5.29 %).

Conclusion

It could be inferred that *Paganum harmala* plant extracts possessed substantial antimicrobial activity. This antimicrobial activity might have been due to the presence of biologically important phyto constituents in different plant extracts.

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