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Evaluation of Fatty acid Composition and Antimicrobial Activity of Eight Medicinal Plants from Kashmir

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ABSTRACT

The seeds of *Abrus precatorius* Linn., *Amaranthus virdis* Linn., *Bunium persicum* Boiss., *Dioscorea deltoidea* Wall. *Ex Griseb*., *Malva neglecta* Wall., *Podophylum hexandrum* Royle., *Robina pseudoacacia* Linn. and *Teraxacum officinale* Weber were used for fatty acid composition and antimicrobial activity. By GC-MS analysis, the petroleum extracts of seed were rich in oleic acid with 60.2%, 58.9% and 57.5% for *A. precatorius*, *A. virdis* and *P. hexandrum* respectively. Linoleic acid was dominant in *M. neglecta* 57.4%, *T. officinale* 59.0% and *R. pseudoacacia* 45.6% and petroselinic acid in *B. persicum* 64.0%. The defatted seed extracts showed strong inhibition zones in (mm) against fungus *Aspergillus niger* (14.50-19.53 with nystatin 20.36), *Aspergillus fumigates* (18.03-21.06 with nystatin 25.56) and *Penicellium marneffei* (20.97-24.96 with nystatin 28.50) and strongest MIC values (µg/ml) of 150, 250 and 500 against bacteria *Esxherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study exhibited beneficial properties of these plants in food and pharmaceutical industries.

Keywords: Soxhlet extraction, Gas chromatography-mass spectrometry, Oleic acid, Linoleic acid, Antifungal, Antibacterial.

INTRODUCTION

Abrus precatorius Linn., *Amaranthus virdis* Linn., *Bunium persicum* Boiss., *Dioscorea deltoidea* Wall. *Ex Griseb*., *Malva neglecta* Wall., *Podophylum hexandrum* Royle., *Robina pseudoacacia* Linn. and *Teraxacum officinale* Weber are indigenous plants in Kashmir with low care cost. These plants are found in much abundance with numerous nutritional[1,2,3] and therapeutic[4-10] properties. Moreover the seeds of these plants could be an important source of fatty acids and antimicrobial agents. The traditional uses of these plants are given in Table 1. Lipids are the main constituents of food. They are most dominantly found in seeds of plants. The high degree of unsaturated fatty acids present in lipid helps to overcome various cardiovascular diseases[26]. Fatty acids mostly occur in natural fats and plant oils and

they contain important nutritional and metabolic substances in living organisms[27]. The unsaturated fatty acids linoleic acid and Linolenic acid are very important for human health[28], while as oleic and linoleic acids can act as vehicles for transfer of other active ingredients, dissolved or dispersed in oil-water type emulsions. The antioxidants could be blended with unsaturated fatty acids to increase their activity e.g. the blends of oleic acid with tocopherol have better protective effect than α-tocopherol itself. Moreover replacing saturated fats with unsaturated fats could decrease total and low density (LDL) cholesterol in foods. Excessive use of Antibiotics and synthetic drugs leads to serious health issues both in plants, humans and indirectly to whole food chain which leads to serious problems[29]. There is recent trend in search for the discovery of new drugs which are less expensive, eco-friendly, biodegradable, safer and natural[30,31]. Plants are a source of phytochemicals which can themselves used as pesticides which are biodegradable, less toxic to plants and animals[32]. Therefore keeping in consideration of their much abundance and tremendous biologically properties of *A. precatorius*, *A. virdis*, *B. persicum*, *D. deltoidea*, *M. neglecta*, *P. hexandrum*, *R. pseudoacacia* and *T. officinale* these plants were selected for the present work. Therefore we continue our laboratory work[33,34,35] on seed extracts of these plants. Moreover the defatted extracts of worked seeds were screened for antimicrobial activity and functional group analysis.

Table 1: Characteristics of the worked plants

S. No.	Common name	Botanical name	Family	Uses
1	Wanwagun	*P. hexandrum* Royle	Berberidaceae	It possesses anti-tumor and anti-oxidant activities[11,12,13]. It is Used in Ayurvedic system in the treatment of diseases like *Condylona acuminate*, *Taenia capitis*, monocytoid leukemia, Hodgkin's diseases, cancer[14,15,16].
2	Jangli Zeer	*B. persicum* (Bioss.)B.	Apiaceae	This plant is used as spice and flavouring agent in foods due to its earthy aroma[17,18]. Seeds are used for treatment of toothache, dyspepsia, diarrhea and jaundice[19].
3	Shangir	*A. precatorius* L.	Fabaceae	Its seeds are used for tuberculosis and painful swellings[20]. It is an excellent source of vitamins, minerals, amino acids and carbohydrates[21]. It is used as aphrodisiac, laxative, bronchodilator, anti-malarial, anti-convulsant, antioxidant, anti-inflammatory, analgesic[22,23,24,25].
4	Hand	*T. officinale* Weber.	Asteraceae	This plant is used as a food[26]. Its leaf extract is used against obesity and cardiovascular diseases[27]. It is used as an anti-inflammatory medicine[28] and to treat infections, bile, liver problems and diuretic[29].
5	Charleri	*A. virdis* L.	Amaranthaceae	Entire plant is used to treat dysentery and inflammation[30]. It possesses anti-oxidant activities[31].
6	Singli Mingli	*D. deltoidea* Wall.	Dioscoreaceae	This plant is used to treat various digestive disorders such as diarrhea, irritability, abdominal pain[32]. It is used as traditional medicine and steroidal drugs in western india[33].
7	Suchal	*M. neglecta* Wallr.	Malvaceae	Aerial parts of this are used to treat skin afflications and gastrointestinal disorders[34]. It is used to treat asthma, colds, digestive, urinary and abdominal problems[35].
8	Kikur	*R. pseudoacacia* L.	Fabaceae	The plant is used as an antioxidant, anti-spasmodic, febrifuge, diuretic, antitumor, antimicrobial and laxative[36]. Its leaves are used for treatment of wounds.

MATERIALS AND METHODS

Materials and chemicals

The seeds of all worked plants were collected from northern parts of Kashmir (India) in September-October 2018. The plants were identified by Dr. Sajad Gangoo and Dr. Tahir Mushtaq, faculty at Forest department, SKAUST-K. Seeds were allowed to dry out at room temperature until they get fully dried. All the chemicals and reagents were purchased from Sigma-Aldrich, USA.

The fungal strains used in this study viz., *Aspergillus niger* (MTCC-281), *Aspergillus fumigates* (MTCC-343) and *penicillium marneffei* (recultured) were obtained from the section of plant pathology, Botany department, Aligarh Muslim University and different strains of bacteria, viz. *Escherichia coli*

(ATCC-25922) *Pseudomonas aeruginosa* (PA01) and *Staphylococcus aureus* (ATCC-29213) were collected from Agricultural Microbiology Department, Aligarh Muslim University, Aligarh (India).

Soxhlet extraction of seeds

The seeds of *A. precatorius*, *A. virdis*, *B. persicum*, *D. deltoidea*, *M. neglecta*, *P. hexandrum*, and *R. pseudoacacia* and *T. officinale* were dried, grinded into powder in mixer grinder. The oil was extracted by using 180 mL of petroleum ether (40-60°C) in Soxhlet apparatus (J-SIL Scientific Industries, Agra India). The solvent was evaporated by Rota vapor (Butchi R-300, Mumbai India). The samples were stored at 4°C until further use. Defatted seed cakes of all plants were extracted separately with chloroform and methanol (1/1, v/v). The extracts were then dried over anhydrous Na_2SO_4, filter and solvent evaporated. These extracts were used for antifungal and antibacterial activities. The extraction time was about 6-8 h for each extraction. The percentage oil yield (%) of seed oil was calculated. The analytical values of the seed extracts were determined in accordance with AOCS[36].

Synthesis of esters of fatty acid (FAMEs)

one gram of oil was saponified separately by refluxing with 0.5N potassium hydroxide (KOH) solution prepared in ethanol and the reaction was observed in thin layer chromatography (TLC). After completation of reaction, unsaponifiable matter was separated out with diethyl ether and remained part was acidified with 6N hydrochloric acid (HCl). Finally diethyl ether was used to separate out the mixed fatty acids (MFAs) in separating funnel.

In second step, of the reaction, MFAs are added with an excess amount of absolute methanol with sulphuric acid as a catalyst. The subsequent mixture was diluted to cloud point by ice chilled water in ice bath. The overall mixture was extracted repeatedly with ether. All the ethereal extracts were washed with 5% aqueous sodium bicarbonate and they are passed through anhydrous sodium sulphate to yield (FAMEs). Normal column chromatography was used to purify FAMEs and the eluent used was n-hexane and diethyl ether (98/2, v/v). The FAMEs were identified by Fourier transform infrared spectroscopy (FTIR) as given in (Figure S1).

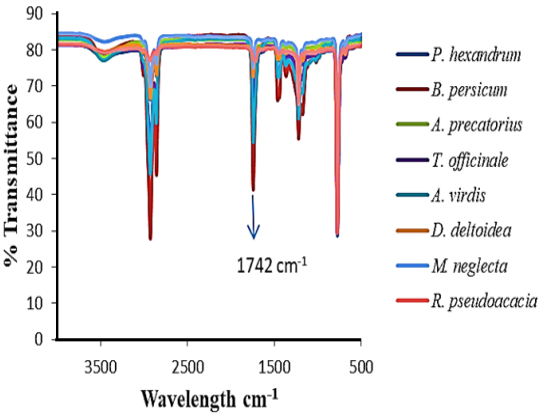

Fig. S1. FTIR spectrum of FAMEs of *P. hexandrum*, *B. persicum*, *A. precatorius*, *T. officinale*, *A. virdis*, *D. deltoidea*, *M. neglecta* and *R. pseudoacacia*

Chromatography

TLC was carried on thin (0.5mm) plates of glass (10 cm × 5 cm) dimensions, which are polished with uniform, layer of silica gel (60-120, Merck, Mumbai, India) formed in ethanol. Petroleum ether/ diethyl ether/acetic acid (80/20/1, v/v) were used as developer in TLC analysis in iodine vapor chamber. The separation of FAMEs was done by column chromatography with silica gel (Merck, Mumbai, India, 60-120 mesh).

Instrumentation

Fourier Transform Infrared Spectrometer (Perkin–Elmer,UK) fitted with zinc selenide crystal was used for functional group analysis. FAMEs were analyzed by using Gas chromatography (GC, Clarius-680, Perkin Elmer) with flame ionization (FI) and mass spectrometer (MS) as its detectors. Helium was used as a carrier gas, at a flow rate of 0.5 mL/min through an Elite-5MS (0.25mm × 30mm) capillary column. Oven temperature was initially 180°C for 2 min, raised to 200°C for 2°C/min, to a final temperature of 215°C at 2°C/minute. injector and detector temperatures are set at 180°C and 280°C respectively.

Determination of antimicrobial activity

Antifungal assay by disc diffusion technique

The defatted seed extracts were studied for antifungal activity against *A. fumigatus*, *A. niger* and *P. marneffi* by disc method as per clinical laboratory standards (NCCLS) on filamentous fungi diffusion[37]. The fungal cultures were developed on czapexdox broth (diffco). Twenty mL of agar media was taken

into petri dishes and stand to solidify. The lawning of individual fungal strain was prepared on the surface of agar media around the disc. The sterilized discs (6mm in Whatman paper, 42) were transferred in added standard (S, S/2 and S/4) concentrations. A disc without seed extract was used as negative control while as standard nystatin drug was used as positive control. The mycelium mats of *A. niger*, *A. fumigatus* and *P. marneffi* of old culture (7- day) were washed carefully and kept in normal saline solution. Finally they are filtered aseptically through wool glass. The inoculum was adjusted to 1-5 × 100 mL, and colony forming units/mL of suspension of test fungi were determined out. The conidial appearance was used for *in vitro* antifungal assay tests. All the tests were repeated (× 3). The dishes were incubated at for 48 h at a temperature of 28°C and inhibition zones of fungus (mm) were determined.

Antibacterial activity by broth dilution method

The antibacterial activity of defatted seed extracts against two gram positive *E. coli*, *S. aureus* and *Gram negative P. aeruginosa* bacteria was done to determine the lowest concentration which inhibit growth entirely also called Minimal Inhibitory Concentration (MIC) by broth dilution method[38] with a concentration of 10^4 CFU. Initially the samples were diluted in Muller Hinton medium with a concentration 500 µg/mL, which were serially and subsequently diluted to attain 250, 125, 62.5 and 31.25 µg/mL concentrations. Ten microliters of bacterial cultures were added to each. After 20 h of incubation at 37°C.

Statistical analysis

Antimicrobial observations found were statistically evaluated by using analysis of variance (ANOVA) by using the IBM SPSS Statistics 20 software. Duncan's post hoc tests were performed to observe the differences between groups at 5% significance level.

RESULTS AND DISCUSSION

Physicochemical properties

Saponification value (SV) is a good tool for the determination of average molecular weight of fatty acid in a triglyceride[39]. Lower the SV of the oil the large is the chain length of triglyceride and hence molecular weight. From Table 2 the highest saponification values was shown by *B. persicum* and *R. pseudoacacia* followed by *T. officinale*, *A. precatorius, D. deltoidea, M. neglecta, P. hexandrum* and *A. virdis*. Iodine value gives the idea of degree of unsaturation. As listed in Table 2 *R. pseudoacacia, T. officinale* and *M. neglecta* shows maximum iodine values followed by *B. persicum, D. deltoidea, P. hexandrum* and *A. virdis* respectively.

Table 2: Oil % (w/w), S.V and I.V of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia* seed oils

Plants	Oil percentage (w/w)	Saponification value	Iodine value
P. hexandrum	6.9	120.60	59.88
B. persicum	6.83	177.70	93.90
A. precatorius	16.78	122.19	56.93
T. officinale	3.8	127.49	109.83
A. virdis	2.38	117.52	53.98
D. deltoida	1.6	121.13	89.20
M. neglecta	17.19	121.13	109.47
R. pseudoacacia	8.44	151.70	161.90

Fatty acid composition

The composition of fatty acid was determined by gas chromatography (GC) and individual fatty acids were identified by mass spectroscopy (MS) as given in (Fig. S2 to Fig. S16). Fatty acid composition of all seed oils of plants are shown in Table 3. The seed oils were mostly dominant in unsaturated acids with (62.7%, 84.6%, 64.0%, 65.8%, 60.9%, 62.2%, 62.6% and 77.5%) composition of total unsaturated fatty acids (TUFA) for *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia respectively. Oleic acid* was the most dominant monounsaturated fatty acid in *A. precatorius, A. virdis, P. hexandrum* and *D. deltoidea* with (60.2%, 58.9%, 57.5% and 21.7%) respectively, while as it is found in smaller amounts in *T. officinale* 6.0%, *M. neglecta* 3.7% and *R. pseudoacacia* 1.3%. The dominant amount 64% petroselinic acid was found in seed oil of *B. persicum*. Among polyunsaturated fatty acids, linoleic acid was most dominant in *T. officinale*, *M. neglecta, R. pseudoacacia, D. deltoidea* and *B. persicum* with (59%, 57.4%, 45.6%, 39.3% and 15.9%) respectively, while it is found in minor amount in *P. hexandrum* 3.7% and *A. virdis* 0.9%. The linolenic acid was dominant in *R. pseudoacacia* 30.6% while low percentage in *P. hexandrum* 1.1%, *A. precatorius* 0.6% and *M. neglecta* 0.7%. Among SFAs palmitic and stearic acid were the main fatty acids found. Palmitic acid includes (25.6%, 22.7%, 20.2%, 14.9%, 11.1%, 11.0%, 8.1% and 3.9%) for M. *neglecta, P. hexandrum, D. deltoidea, A. virdis, A. precatorius,*

T. officinale, B. persicum and *R. pseudoacacia* respectively and stearic acid includes (10.7%, 8.1%, 4.8%, 4.5%, 3.5%, 3.5% and 1.2%) for *P. hexandrum, M. neglecta, T. officinale, A. precatorius, A. virdis, D. deltoidea* and *R. pseudoacacia* respectively. The fatty acid composition of *P. hexandrum* is quiet similar to that of *Sterculia tomentosa* from Sudan[40]. In *B. persicum* and *R. pseudoacacia* the major fatty acids found are comparatively similar in composition as reported in *Olea europea* from Palestine and Temperate growing *Cannabis sativa* respectively[41,42]. The fatty acid composition of *D. deltoidea* is similar to cotton seed which is used in pharmaceutical and cosmetic preparation[43].

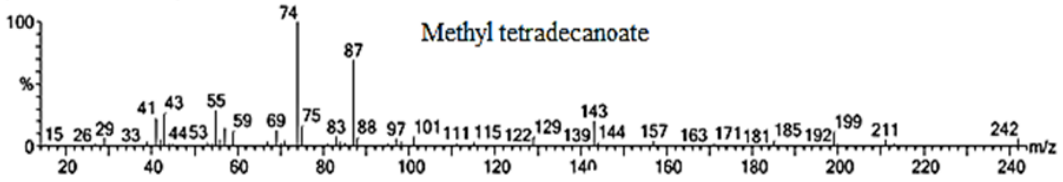

Fig. S2. Mass spectrum of Methyl tetradecanoate

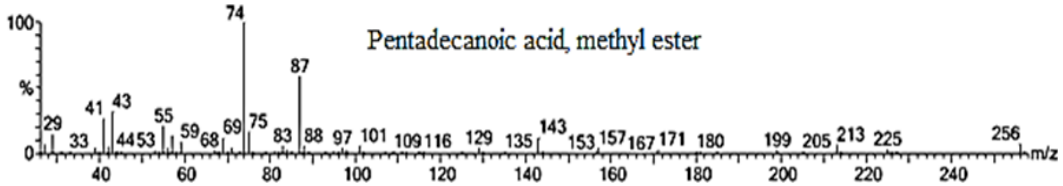

Fig. S3. Mass spectrum of Pentadecanoic acid, methyl ester

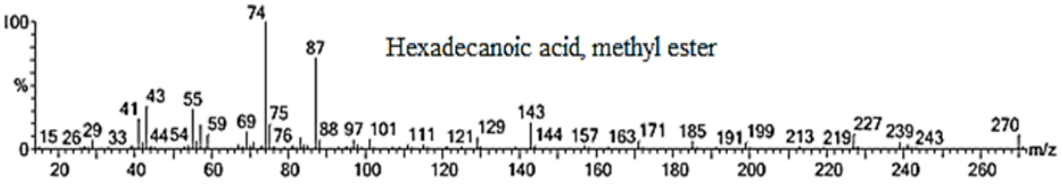

Fig. S4. Mass spectrum of Hexadecanoic acid, methyl ester

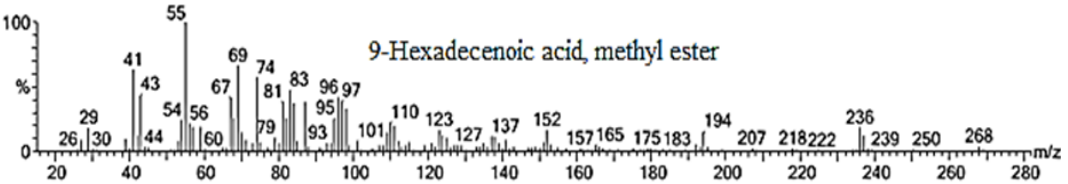

Fig. S5. Mass spectrum of 9-Hexadecenoic acid, methyl ester

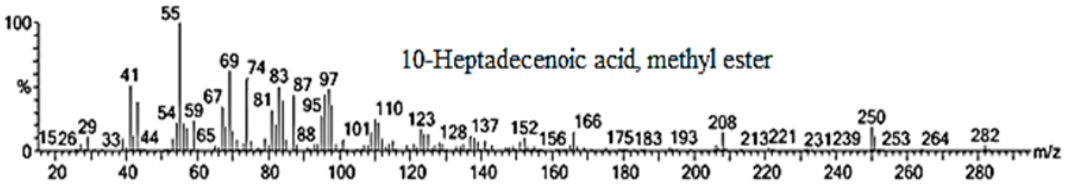

Fig. S6. Mass spectrum of 10-Heptadecenoic acid, methyl ester

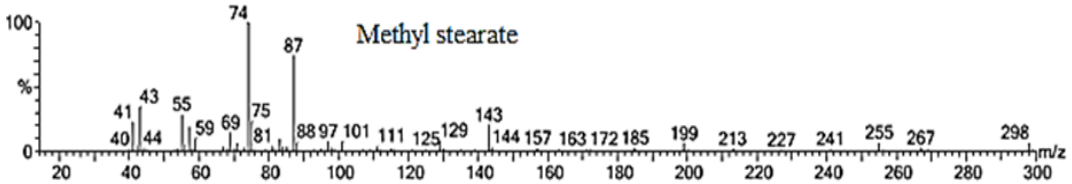

Fig. S7. Mass spectrum of Methyl stearate

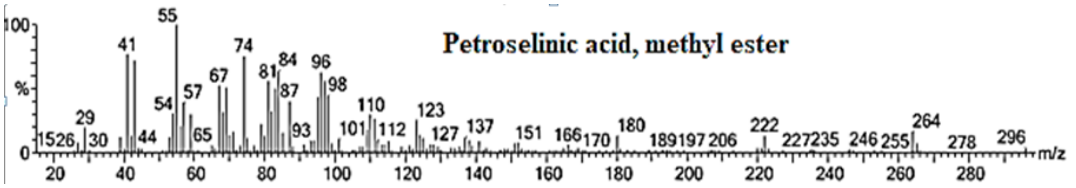

Fig. S8. Mass spectrum of Petroselinic acid, methyl ester

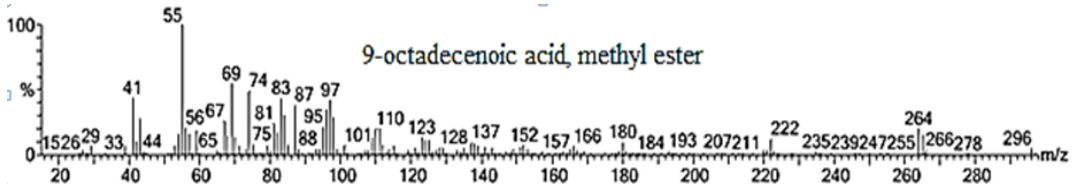

Fig. S9. Mass spectrum of 9-octadecenoic acid, methyl ester

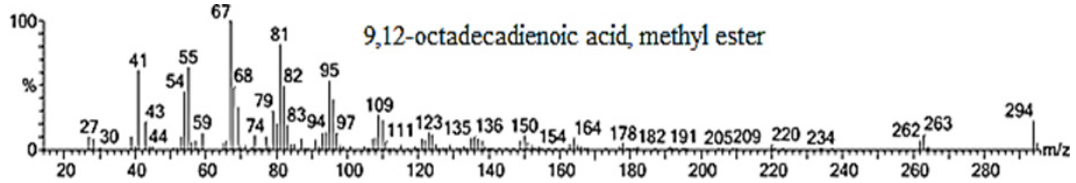

Fig. S10. Mass spectrum of 9,12-octadecadienoic acid, methyl ester

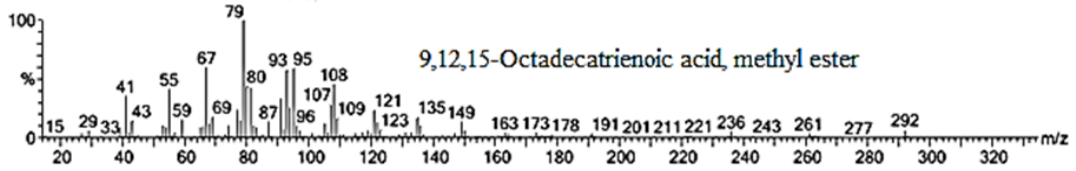

Fig. S11. Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester

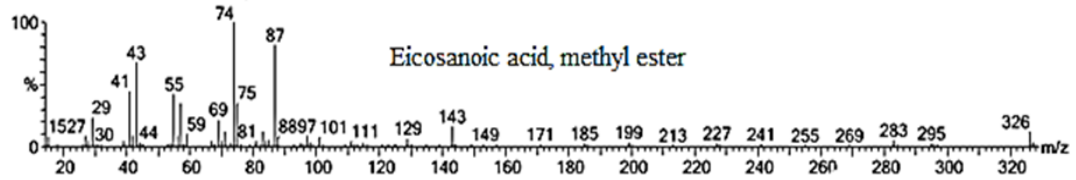

Fig. S12. Mass spectrum of Eicosanoic acid, methyl ester

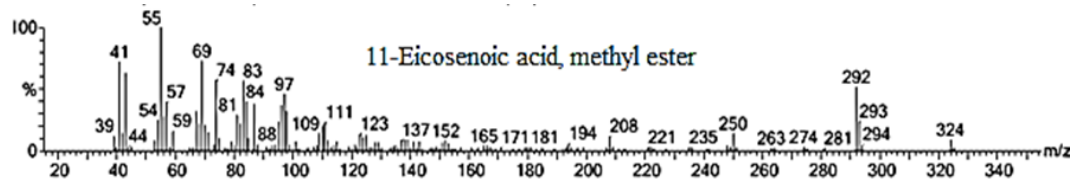

Fig. S13. Mass spectrum of 11-Eicosenoic acid, methyl ester

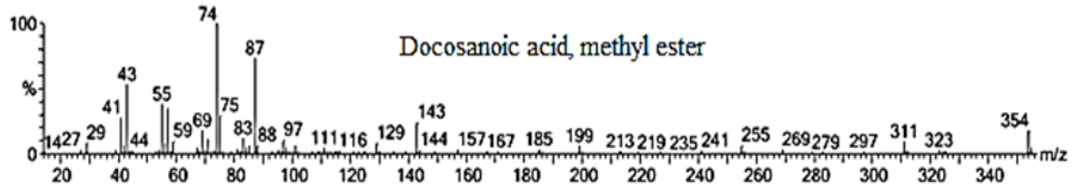

Fig. S14. Mass spectrum of Docosanoic acid, methyl ester

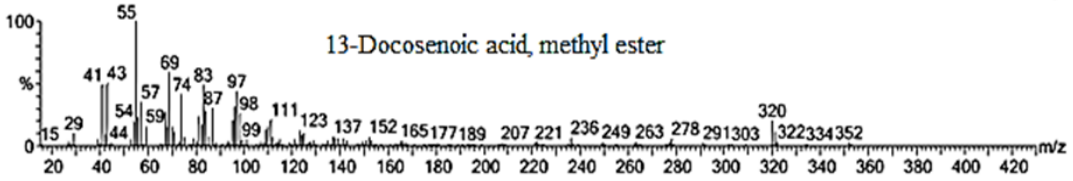

Fig. S15. Mass spectrum of 13-Docosenoic acid, methyl ester

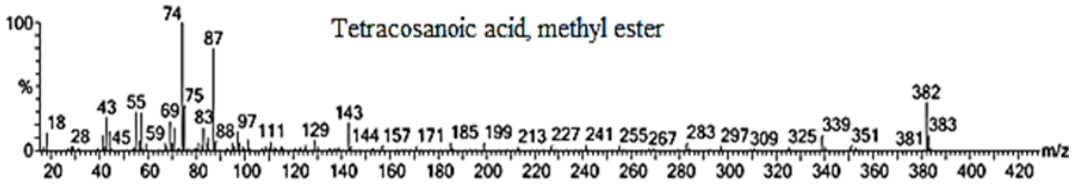

Fig. S16. Mass spectrum of Tetracosanoic acid, methyl ester

Table 3. Fatty acid composition of seed oils of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia*

Common and systematic names	Area (%)							
	P. hexandrum	*B. persicum*	*A. precatorius*	*T. officinale*	*A. virdis*	*D. deltoida*	*M. neglecta*	*R. pseudoacacia*
Myristic acid14:0	0.4	-	-	-	0.6	1.2	0.5	-
Pentadecanoic acid 15:0	-	-	-	-	1.1	-	-	-
Palmitic acid 16:0	22.7	8.1	11.1	11.0	14.9	20.2	25.6	3.9
Palmitoleic acid16:1	-		-	0.8	0.5	0.7	0.3	-
Heptadecenoic acid 17:1	-	-	-	-	-	-	0.5	-
Stearic acid 18:0	10.7	-	4.5	4.8	3.5	3.5	8.1	1.2
Petroselinic acid 18:1 Δ6	-	64.0	-	-	-	-	-	-
Oleic acid 18:1 Δ9	57.5	-	60.2	6.0	58.9	21.7	3.7	1.3
Linoleic acid 18:2	3.7	15.9	-	59.0	0.9	39.3	57.4	45.6
Linolenic acid 18:3	1.1	-	0.6	-	-	-	0.7	30.6
Arachidic acid 20:0	0.4	0.7	1.4	3.1	1.4	0.9	-	-
Gondoic acid 20:1	-	1.5	2.6	-	0.6	0.5	-	-
Behenic acid 22:0	-	0.6	7.1	2.3	-	2.3	-	-
Erucic acid22:1	-	3.2	0.6	-	-	-	-	-
Lignoceric acid24:0	-	0.3	3.5	-	-	1.2	-	-
Total unsaturated acids	62.7	84.6	64.0	65.8	60.9	62.2	62.6	77.5
Total saturated acids	33.8	9.7	27.6	21.2	21.5	29.3	34.2	5.1

Functional group analysis by FTIR

Transmission spectra of defatted seed extracts of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia* are shown in (Fig. S17) and peak values are given in (Table 4). The IR peaks for *B. persicum* seed extracts are pointed out while as IR spectrum of rest of seed extracts are given in (Fig. S17). The presence of various functional groups as depicted by IR confirm various active substances present e. g defatted seed extracts of these plants which could be responsible for their biological activities.

Antifungal activity of defatted seed extracts

All the defatted seed extracts showed good antifungal activity at all concentrations, but most dominant activity was shown at Standard solution S (Table 5). The defatted seed extracts of *T. officinale, B. persicum* and *M. neglecta* displayed most dominant effect with zone of inhibition 19.53 mm, 18.82 mm and 18.61 mm respectively against *A. niger* with respect to nystatin 20.36 mm. Against *A. fumigates* the inhibition zone was found to be 21.42 mm, 21.06 mm and 21.05 mm for *T. officinale, B. persicum* and *M. neglecta* respectively with nystatin displayed 25.56 mm inhibition zone. Against *P. marneffei* the most dominant effect was shown by *T. officinale* 24.96 mm, *M. neglecta* 24.22 mm followed by *R. pseudoacacia* 23.93 mm, *B. persicum* 23.81 mm, *A. precatorius* 22.46 mm with respect to nystatin e.g 28.35 mm (Table 5).

Table 4: Regions of FTIR spectrum of defatted seed extracts of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia*

P. hexandrum		*B. persicum*		*A. precatorius*		*T. officinale*		*A. virdis*		*D. deltoida*		*M. neglecta*		*R. pseudoacacia*	
Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups	Peak value cm^{-1}	Functional groups
3375.9	Alchols	3478	Alchols	3379.02	Alchols	3413.88	Alchols	3436.22	Alchols	3394.84	Alchols	3422.72	Alchols	3377.47	Alchols
3010	cis RHC=CHR	3010	cis RHC=CHR	3008.51	cis RHC=CHR							2934.2	$-CH_2-$	3012.05	cis RHC=CHR
2932	$-CH_2-$	2932	$-CH_2-$	2926.24	$-CH_2-$	2925.42	$-CH_2-$	2924.77	$-CH_2-$	2925.7	$-CH_2-$	2864.94	$-CH_2-$	2927.19	$-CH_2-$
		2856	$-CH_2-$												
2854.86	$-CH_2-$	1740	–C=O (ester)	2855.43	$-CH_2-$	2854.79	$-CH_2-$	2853.88	$-CH_2-$	2855.86	$-CH_2-$	2075.72	Non-assigned	2856.18	$-CH_2-$
1707.74	–C=O (acid)	1610	Non-assigned	1739.98	–C=O (ester)			1710.07	–C=O (acid)	1711.87	–C=O (acid)	1710.03	–C=O (acid)	1742.15	–C=O (ester)
1627.86	C=C cis-olefins	1516	$-C-H\ (-CH_2)$	1714.29	–C=O (acid)	1712.77	–C=O (acid)					1642.85	C=C cis-olefins	1643.71	C=C cis-olefins
1495.98	$-C-H\ (-CH_2)$	1460	$-C-H\ (-CH_2)$	1645.59	C=C (cis)			1460.39	$-C-H\ (-CH_2)$	1461.22	$-C-H\ (-CH_2)$	1456.64	$-C-H\ (-CH_2)$	1459.19	$-C-H\ (-CH_2)$
1461.01	$-C-H\ (-CH_2)$	1372	$-C-H\ (-CH_3)$	1460.25	$-C-H\ (-CH_2)$	1459.64	$-C-H\ (-CH_2)$					1399.32	=C–H cis	1378.55	$-C-H\ (-CH_3)$
1398.09	$-C-H\ (-CH_3)$	1220	$-C-H\ (-CH_2)$	1362.39	O–H	1378.29	$-C-H\ (-CH_3)$					1219.74	$-C-H\ (-CH_2)$	1219.38	$-C-H\ (-CH_2)$
1210.37	$-C-H\ (-CH_2)$	1170	–C–O	1219.42	$-C-H\ (-CH_2)$	1219.55	$-C-H\ (-CH_2)$	1251.91	$-C-H\ (-CH_2)$	1219.42	$-C-H\ (-CH_2)$	1055.04	–C–O	1071.4	–C–O
1076.48	–C–O	1052	–C–O	1057.16	–C–O	1043.27	–C–O	1091.14	–C–O	1063.46	–C–O	1032.38	–C–O		
932.74	–HC=CH– (trans)	848	0			793.33	–C–H	778	–C–H			1014.05	–C–O	674.04	$-C(CH_2)n$
896.55	–HC=CH– (cis)	542	Non-assigned	673.24	Non-assigned	675.16	Non-assigned					630.88	Non-assigned		

Table was constituted according to References[45,46,47].

Table 5: Antifungal activity of extracts of defatted seeds of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia against A. niger, A. fumigates and P. marneffei*

Seed extracts	*A. niger*			*A. fumigates*			*P. marneffei*		
	S/4	S/2	S	S/4	S/2	S	S/4	S/2	S
P. hexandrum	9.57 ± 1.15 d	13.59 ± 0.56 c	14.70 ± 1.1 c	10.60 ± 1.28 d	16.14 ± 1.06 c	19.74 ± 0.83 c	12.38 ± 0.35 d	18.53 ± 0.42 d	21.95 ± 0.24 d
B. persicum	11.39 ± 1.2 c	14.38 ± 0.4 c	16.95 ± 0.49 b	14.69 ± 1.06 b	17.25 ± 0.37 b	21.06 ± 1.22 b	14.21 ± 1.13 c	19.53 ± 0.12 c	23.81 ± 0.16 c
A. precatorius	8.26 ± 1.02 d	13.63 ± 0.28 c	14.67 ± 0.50 c	8.60 ± 0.72 e	13.71 ± 0.50 d	18.03 ± 0.36 d	12.4 ± 0.52 d	18.51 ± 0.59 d	22.46 ± 0.52 d
T. officinale	15.52 ± 1.55 b	16.39 ± 1.12 b	19.53 ± 0.61 a	14.54 ± 1.11 b	18.35 ± 0.80 b	21.42 ± 1.22 b	16.42 ± 1.27 b	22.52 ± 0.34 b	24.96 ± 0.20 b
A. viridis	8.21 ± 1.01 d	12.21 ± 0.25 d	14.50 ± 1.06 c	9.31 ± 0.32 d	12.50 ± 1.22 e	18.19 ± 1.00 d	11.30 ± 0.69 d	16.67 ± 0.32 e	20.42 ± 0.80 e
D. deltoidea	8.82 ± 0.49 d	13.65 ± 1.00 c	16.39 ± 0.53 b	9.36 ± 0.98 d	13.80 ± 0.15 d	18.65 ± 0.37 c	12.53 ± 0.72 d	16.70 ± 0.24 e	20.97 ± 0.25 e
M. neglecta	12.81 ± 0.54 c	15.20 ± 1.00 b	17.21 ± 0.37 b	12.64 ± 0.41 c	15.62 ± 0.36 c	21.05 ± 0.24 b	15.33 ± 0.74 b	22.57 ± 0.12 b	24.22 ± 0.99 b
R. pseudoacacia	11.50 ± 1.00 c	14.25 ± 0.61 c	16.45 ± 0.37 b	12.18 ± 0.03 c	16.71 ± 0.22 c	19.48 ± 0.42 c	13.49 ± 0.38 c	19.49 ± 0.17 c	23.93 ± 0.17 c
Nystatin	20.36 ± 0.13 a			25.56 ± 0.27 a			28.35 ± 0.21 a		

S= Standard Solution; S/2= Half of Standard; S/4= one fourth of standard

Each value represents the mean of three determinations (n=3) ± standard deviation.

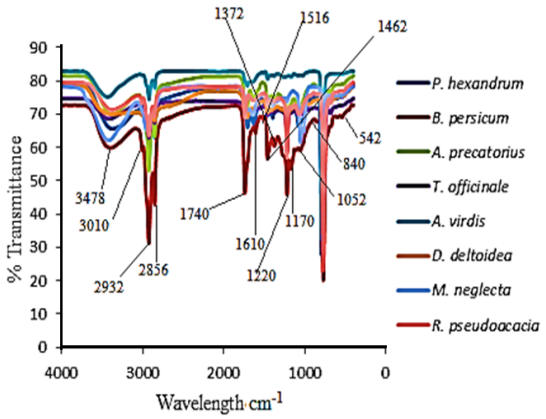

Fig. S17. FTIR analysis of the defatted seed extracts of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia*

Table 6: Antibacterial activity of extracts of defatted seeds of *P. hexandrum, B. persicum, A. precatorius, T. officinale, A. virdis, D. deltoidea, M. neglecta* and *R. pseudoacacia against E. coli, S. aureus* and *P. aeruginosa*

S. No.	Seed extracts	Minimal Inhibitory Concentration (µg/ml)		
		E. coli	*S. aureus*	*P. aeruginosa*
1	*P. hexandrum*	250	250	250
2	*B. persicum*	125	250	125
3	*A. precatorius*	125	125	250
4	*T. officinale*	250	250	125
5	*A. viridis*	250	125	250
6	*D. deltoidea*	250	125	125
7	*M. neglecta*	125	250	125
8	*R. pseudoacacia*	500	500	250
9	*Streptomycin*	5	5	10

Antibacterial activity of defatted seed extracts

The results of this study are shown in (Table 6). The defatted seed extracts of *B. persicum, A. precatorius* and *M. neglecta* displayed lowest MIC 125 µg/mL, while other defatted extracts showed MIC 250 µg/mL except *R. pseudoacacia* which showed 500 µg/mL. Inhibition against *S. aureus* defatted seed extracts of *A. precatorius*, *A. virdis* and *D. deltoidea* showed lowest MIC 125 µg/mL, while defatted extracts of other plants (Table 6) showed MIC 250 µg/mL except *R. pseudoacacia* showed 500 µg/mL. Against *P. aeruginosa* (*Gram-negative* bacteria) defatted seed extracts of *B. persicum, T. officinale, D. deltoidea* and *M. neglecta* showed lowest MIC 125 µg/mL while other seed extracts of plants showed MIC 250 µg/mL.

CONCLUSION

From the above results, this research concluded that these plants contains high percentage of oleic, linoleic and significant amount of palmitic acids. Moreover the defatted seed extracts showed powerful antifungal and antibacterial activities. Based on above results we conclude that seed extracts of these plants could be used in food industry, cosmetic industry and source of new natural fungicides and bactericides in pharmaceutical industry. However further work need to be carried on isolation, purification and characterization of active compounds in defatted seed extracts.

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Conflict of interest

The authors proclaim no conflict interest.

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