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Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L.

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Abstract. – *Objectives:* **The fatty acid methyl esters (FAME extract) from *Sesuvium (S.) portulacastrum* was studied for its fatty acid composition and antimicrobial activity against human pathogenic microorganisms.**

Materials and Methods and Results: **The gas chromatographic analysis of FAME extract revealed the presence of palmitic acid with the highest relative percentage (31.18%), followed by oleic acid (21.15%), linolenic acid (14.18%) linoleic acid (10.63%), myristic acid (6.91%) and behenic acid (2.42%). The saturated fatty acids were higher than the unsaturated fatty acids. FAME extract showed the highest antibacterial and anticandidal activities and moderate antifungal activity against the tested microorganisms. The highest mean zone of inhibition (16.3 mm) and the lowest MIC (0.25 mg/ml) and MBC (0.5 mg/ml) values were recorded against *Bacillus subtilis*. The lowest mean zone of inhibition (8.8 mm) and the highest MIC (8 mg/ml) and MFC (16 mg/ml) values were recorded against *Aspergillus fumigatus* and *Aspergillus niger*.**

Conclusions: **The results of the present study justify the use of *S. portulacastrum* in traditional medicine and the FAME extract can be used as a potential antimicrobial agent against the tested human pathogenic microorganisms.**

Key Words:

Sesuvium portulacastrum, Halophyte, Fatty acid methyl esters, Antimicrobial activity.

Introduction

Antibiotic resistance is the biggest challenge in the treatment of infectious diseases. The wide range of occurrence of antibiotic resistance suggests that, in principal, any organism could develop resistance to any antibiotics[1]. The antibiotic resistance to antibiotic drugs increases the cost of treatment and often results in treatment failure. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem; for example, to continue the studies to develop new antimicrobial agents especially from traditionally used medicinal plants.

Mangroves and/or halophytes are of great importance to many people who live along tropical shorelines. Extracts and chemicals from mangroves are used mainly in folkloric medicine for range of diseases and these practices continue to this day. However, the extraction of novel chemical compounds from mangroves, in addition to those already known to the pharmacopoeia of the people is in its infancy[2]. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents but because such information may be of values in disclosing new sources of already known biologically active compounds.

Sesuvium portulacastrum (also known as "sea purslane"), a halophyte belongs to the family Aizoaceae and grows naturally in the sub tropical, mediterranean coastal and warmer areas around the world. The plant has a long history of use in folk medicine. In traditional medicine, it has been used for the treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy and purgative and also used to cure toothache[2]. Traditional healers in Zimbabwe and South Africa use this plant to treat various infectious diseases and kidney problems[3].

Fatty acids and lipids are constituents of all plant cells, where they function as membrane compounds, storage products , metabolites and as a source of energy[4]. Fatty acids are widely occurring in natural fats and dietary oils and they are important nutritious substances and metabolites of living organisms[5] which may mediate the chemical defense against microorganisms[6-8]. Fat-

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ty acid compositions of some mangrove species from Pichavaram mangrove forest has been reported already[9]. However, the fatty acid composition and its antimicrobial activity of *Sesuvium portulacastrum* have not been reported previously. So, the present study was aimed to find out the fatty acid composition and antimicrobial efficacy of the FAME extract from the leaves of *Sesuvium portulacastrum* against human pathogenic microorganisms.

Materials and Methods

Plant Material

The leaves of *Sesuvium portulacastrum* L. were collected from the mangrove forest of Pichavaram in the Vellar-Coleroon estuarine complex and the voucher specimen is deposited at the Herbarium of the Department of Botany, Annamalai University, India. The collected plant samples were brought to laboratory using separate polythene bags. First they were washed with tap water and surface sterilized in 10% sodium hypochlorite to prevent the contamination of any microbes. Then they were washed with sterile distilled water and air dried at room temperature. The dried samples were ground into a fine powder and used for the present study.

Preparation and Analysis of Fatty Acid Methyl Esters (FAME Extract)

Twenty grams of plant powder were refluxed with a mixture of methanol: benzene: concentrated sulfuric acid (200:100:10 v/v) for 2 h. The filtrate was transferred to a separating funnel and 60-70 ml of distilled water was added. Then a small amount of hexane was added and pooled. The hexane fraction was separated into two layers and the lower layer was removed. The upper layer was washed with 50 ml of 10% sodium bicarbonate solution and shaken two times vigorously and the lower layer was removed. The upper layer was washed two times with saturated 0.9% sodium chloride solution. The upper layer was saved, passed through sodium sulfate and saved for further analysis. The residue was dissolved in hexane and analysed by gas chromatography (Varian GC# 1, Varian Inc. Scientific Instruments, Palo Alto, CA, USA). CP-Wax 5 g (Chrompack, Varian India Pvt. Ltd., Mumbai, India) (50×0.20 mm) capillary column was used to analyse the fatty acids. The temperature of the injector and the detector were kept at 210 and 220°C respectively. The temperature of the oven was programmed from 180°C and N_2 was used as carrier gas. 2 µl of methyl esters was introduced onto the column. The constituents of the FAME extract were identified by comparison of their relative retention times with those of authentic standards from Sigma-Aldrich Chemical Co (St Louis, MO, USA).

Antimicrobial Assay

Microorganisms Used

The antimicrobial activity of FAME extract of *S. portulacastrum* was investigated against four strains of Gram positive bacteria *viz. Bacillus subtilis* (NCIM 2063), *Bacillus pumilus* (NCIM 2327), *Micrococcus luteus* (NCIM 2376) and *Staphylococcus aureus* (NCIM 2901) and against three strains of Gram negative bacteria *viz. Pseudomonas aeruginosa* (NCIM 5031), *Klebsiella pneumoniae* (NCIM 2957) and *Escherichia coli* (NCIM 2256). These standard strains were obtained from National Collection of Industrial Microorganisms (NCIM), Biochemical Sciences Division, National Chemical Laboratory, Pune, India. Ten isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and reference standard of MRSA (NCTC 6571) were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, India. The stock cultures were maintained on nutrient agar medium at 4°C. Four human pathogenic yeast type fungi such as *Candida albicans, Candida krusei, Candida tropicalis* and *Candida parapsilosis* and three mould fungi *viz. Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus* were also obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, India. The stock cultures were maintained on Sabouraud dextrose agar medium at 4°C.

Disc Diffusion Method

The agar diffusion method[10] was followed for antibacterial and antifungal susceptibility tests. Petri plates were prepared by pouring 20 ml of Mueller Hinton agar, Mueller Hinton agar supplemented with 4% sodium chloride and Sabouraud dextrose agar and allowed to solidify for susceptibility tests against bacteria, MRSA and fungi respectively. Ciprofloxacin (5 µg/disc) for bacteria, methicillin (5 µg/disc), oxacillin (1 µg/disc) and vancomycin (30 µg/disc) for MRSA and amphotericin-B (100 units/disc) for fungi

were used as positive controls and 5% dimethyl sulfoxide (DMSO) was used as blind control in these assays. Finally, the inoculated plates were incubated at 37°C for 24 h (for bacteria), 35°C for 24-48 h (MRSA), 28°C for 24-48 h (yeasts) and 28°C for 72-96 h (mycelial fungi). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated four times.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the FAME extract was tested in Mueller Hinton broth for bacteria, Mueller Hinton broth supplemented with 4% sodium chloride for MRSA, yeast nitrogen base for yeasts and Sabouraud dextrose broth for mycelial fungi to get the concentrations of 16-0.06 mg/ml by the broth macrodilution method[11]. The culture tubes were incubated at 37°C for 24 h (bacteria), 35°C for 24-48 h (MRSA), 28°C for 48 h (yeasts) and 72-96 h (mycelial fungi).

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC of the FAME extract was determined[12] by plating 100 µl samples from each MIC assay with growth inhibition into freshly prepared Mueller Hinton agar (for bacteria), Mueller Hinton agar supplemented with 4% sodium chloride (for MRSA) and Sabouraud dextrose agar (for yeasts and mycelial fungi). The plates were incubated at 37°C for 24 h (bacteria), 35°C for 24-48 h (MRSA), 28°C for 48 h (yeasts) and 72-96 h (mycelial fungi).

Statistical Analysis

All the data of antibacterial and antifungal activities were examined as mean ±SD. One-sample T test was carried out to determine the significant differences ($P< 0.05$) between the means. The analysis was carried out using Statistical Package of Social Sciences (SPSS package software, Version 11.5, Chicago, IL, USA).

Results

The fatty acid composition of *Sesuvium portulacastrum* was determined by gas chromatography and the relative percentages are presented in Table I and the chromatogram is given in Figure 1. The saturated fatty acid content was higher than the unsaturated fatty acids recorded. Among

Table I. Fatty acid composition of the leaves of *Sesuvium portulacastrum*.

Peak No.	Name of the fatty acid	No. of carbon atoms	Relative percentage
1.	Lauric acid	C12:0	1.67
2.	Tridecanoic acid	C13:0	0.53
3.	Myristic acid	C14:0	6.91
4.	Pentadecanoic acid	C15:0	0.14
5.	Palmitic acid	C16:0	38.18
6.	Heptadecanoic acid	C17:0	0.08
7.	Stearic acid	C18:0	ND
8.	Oleic acid	C18:1	21.15
9.	Linoleic acid	C18:2	10.63
10.	Linolenic acid	C18:3	14.18
11.	Nonadecanoic acid	C19:0	ND
12.	Arachidic acid	C20:0	0.27
13.	Heneicosanoic acid	C21:0	0.01
14.	Behenic acid	C22:0	2.42
	Saturated fatty acids		50.21
	Unsaturated fatty acids		45.96
	Unidentified fatty acids		3.83
	Total		**100.00**

ND = Not deductable.

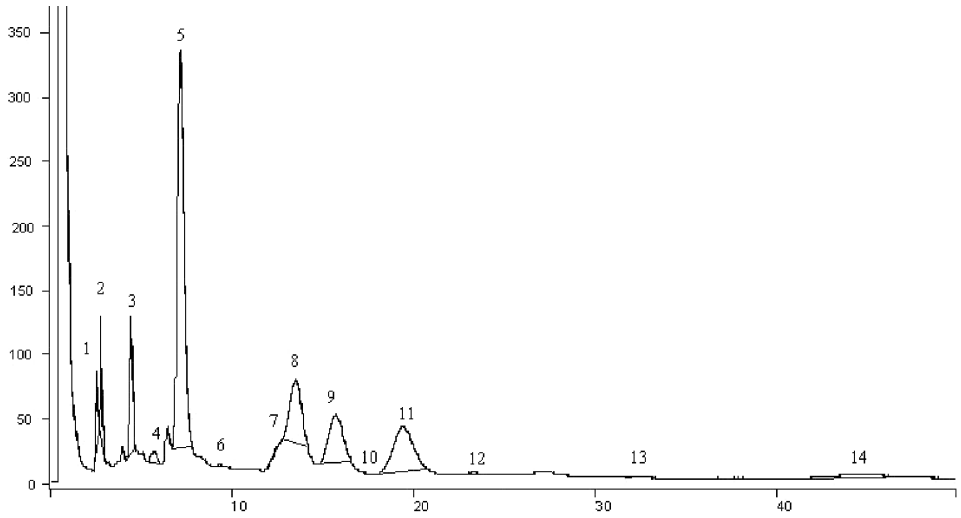

Figure 1. Gas Chromatogram of fatty acid methyl esters from the leaves of *S. portulacastrum*.

the saturated fatty acids, palmitic acid (38.18%) was recorded with the highest relative percentage followed by myristic acid (6.91%), behenic acid (2.42%), lauric acid (1.67%), tridecanoic acid (0.53%), pentadecanoic acid (0.14%), arachidic acid (0.27%), heptadecanoic acid (0.08%) and heneicosanoic acid (0.01%). Saturated fatty acids such as stearic acid and nonadecanoic acid could not be deducted in the FAME extract of *Sesuvium portulacastrum*. Among the unsaturated fatty acids, oleic acid (21.15%) was with the highest relative percentage followed by linolenic acid (14.18%) and linoleic acid (10.63%).

FAME extract from the leaves of *Sesuvium portulacastrum* possessed significant antimicrobial activity against all the microorganisms tested when compared to the respective positive controls (Ciprofloxacin for bacteria; methicillin, oxacillin and vancomycin for MRSA and amphotericin-B for fungi) (Table II) (Significant at $P<0.05$ level). In the present study, a potent antibacterial and anticandidal activity and a moderate antifungal activity were recorded. FAME extract of *Sesuvium portulacastrum* recorded the highest mean zone of inhibition (16.3 mm) and the lowest MIC (0.25 mg/ml) and MBC (0.5 mg/ml) values against *Bacillus subtilis*. The FAME extract also showed antimicrobial activity against *Micrococcus luteus* (15.3 mm; MIC = 0.5 and MBC = 1 mg/ml), MRSA (15.3 mm; MIC = 0.5 and MBC = 1 mg/ml), *Staphylococcus aureus* (14.8 mm; MIC = 0.5 and MBC = 1 mg/ml), *Bacillus pumilus* (13.8 mm; MIC = 0.5 and MBC = 1 mg/ml), *Klebsiella pneumoniae,* (12.8 mm; MIC = 0.5 and MBC = 1 mg/ml), *Pseudomonas aeruginosa* (12.0 mm; MIC = 0.5 and MBC = 2 mg/ml) and *Candida albicans* (11.0 mm; MIC = 0.5 and MBC = 1 mg/ml). The FAME exract also showed moderate antifungal activity against *Aspergillus flavus* (9.5 mm; MIC = 4 and MBC = 8 mg/ml), *Aspergillus fumigatus* (8.8 mm; MIC = 8 and MBC = 16 mg/ml) and *Aspergillus niger* (8.8 mm; MIC = 8 and MBC = 16 mg/ml).

Discussion

Analysis of fatty acid composition of *Sesuvium portulacastrum* by gas chromatography revealed the presence of higher amount of saturated fatty acids than the unsaturated fatty acids. Palmitic acid seems to be a major fatty acid. Similar results were reported in *Arthrocnemum indicum, Suaeda maritima* and *Suaeda monoica*[13]. The mesocarp and seed of *Hippophae rhamnoides* and *Myrtus communis* have been reported to be rich in palmitic acid and palmitoileic acid as well as oleic and linoleic acids[14]. In our previous report higher amount of palmitic acid, was recorded in some mangroves from the Pichavaram mangrove forest[9]. The presence of

Table II. Fatty acid composition of the leaves of *Sesuvium portulacastrum*.

S. No.	Microorganisms	Mean Zone of inhibition* (mm)**					FAME extract	
		FAME extract		Ciprofloxacin		Amphotericin-B		
		Concentration of the disc						
		200 µg/disc	100 µg/disc	50 µg/disc	5 µg/disc	100 units/disc	MIC mg/ml	MBC/MFC mg/ml
1.	*Bacillus subtilis*	16.3 ± 1.3	14.3 ± 1.0	11.5 ± 0.6	31.5 ± 2.4	NT	0.25	0.5
2.	*Bacillus pumilus*	13.8 ± 1.0	12.0 ± 0.8	9.5 ± 0.6	34.3 ± 2.1	NT	0.5	1
3.	*Micrococcus luteus*	15.3 ± 1.0	14.0 ± 0.8	11.5 ± 0.6	30.5 ± 2.4	NT	0.5	1
4.	*Staphylococcus aureus*	14.8 ± 1.0	13.3 ± 1.0	11.5 ± 0.6	29.0 ± 1.8	NT	0.5	1
5.	MRSA	15.3 ± 1.0	13.3 ± 1.0	11.5 ± 0.6	25.0 ± 1.7	NT	0.5	1
6.	*Pseudomonas aeruginosa*	12.0 ± 0.8	10.5 ± 0.6	9.3 ± 0.5	30.0 ± 1.8	NT	0.5	2
7.	*Klebsiella pneumoniae*	12.8 ± 1.0	11.0 ± 0.8	9.5 ± 0.6	32.5 ± 1.7	NT	0.5	1
8.	*Escherichia coli*	9.5 ± 0.6	7.8 ± 0.5	6.8 ± 0.5	30.3 ± 1.7	NT	1	2
9.	*Candida albicans*	11.0 ± 0.8	9.5 ± 0.6	7.5 ± 0.6	NT	17.5 ± 1.3	0.5	1
10.	*Candida krusei*	9.5 ± 0.6	7.8 ± 0.5	6.8 ± 0.5	NT	18.8 ± 1.3	2	4
11.	*Candida tropicalis*	9.8 ± 0.5	8.5 ± 0.6	7.5 ± 0.6	NT	17.3 ± 1.0	2	4
12.	*Candida parapsilosis*	11.0 ± 0.8	8.8 ± 0.5	6.8 ± 0.5	NT	18.3 ± 1.0	2	4
13.	*Aspergillus niger*	8.8 ± 0.5	6.8 ± 0.5	NA	NT	15.3 ± 1.0	8	16
14.	*Aspergillus flavus*	9.5 ± 0.6	8.3 ± 0.5	7.3 ± 0.5	NT	16.5 ± 1.3	4	8
15.	*Aspergillus fumigatus*	8.8 ± 0.5	7.3 ± 0.5	NA	NT	15.5 ± 1.0	8	16

*Diameter of mean zone of inhibition (mm) including disc diameter of 6 mm; **Mean of four assays (Significant at $P < 0.05$ level); ± = Standard deviation; NT = Not tested; NA = No activity.

unsaturated fatty acid, oleic acid, was reported in the fresh leaves of some mangroves[15]. Linoleic and linolenic acids were also reported in some members of Chenopodiaceae[9].

Reid et al[16] stated that most of the active fractions contained a mixture of fatty acids and related compounds. In the present study, FAME extract from the leaves of *Sesuvium portulacastrum* exhibited antimicrobial activity against all the microorganisms tested. Similar observations were made with the FAME extract of leaves of *Ipomoea pes-caprae*[13]. Fatty acid fraction of seed lipids of *Zizyphus spina-christi* showed activity against *Bacillus subtilis, Streptococcus pyogenes* and *Escherichia coli*[17]. Long chain unsaturated fatty acids, including linoleic acid are well known to inhibit Gram negative bacteria such as *Escherichia coli*[18,19,20]. This large difference in the fatty acid sensitivities between Gram positive and Gram negative bacteria may be resulted from the impermeability of the outer membrane of the Gram negative bacteria since the outer membrane of the Gram negative bacteria is an effective barrier against hydrophobic substances[21-23].

In the present investigation, the highest mean zones of inhibition were recorded at the concentration of 200 mg/disc. As the disc dosage level increased, the inhibitory effect also increased. Similar results were reported in some members of Chenopodiaceae[24]. Linolenic, linoleic acids isolated from *Schotia brachypetala, pelargonium* sp., and *Pentanisia prunelloides,* respectively, were found to have antibacterial activity[25-27]. Oleic and arachidonic acids were known to possess antimicrobial activity against human pathogens[28]. Also, lauric acid is a potential antimicrobial agent, suitable for external application. In our study, FAME extract from the leaves of *Sesuvium portulacastrum* contained one or more of the above said fatty acids in higher amount. There were only a few studies on the antimicrobial activity of FAME extract of marine plants, though there were many studies on the antimicrobial activity of individual fatty acids[13,24]. Since the FAME extract of *Sesuvium portulacastrum* showed potential activity against all the microorganisms tested, the FAME extract of *Sesuvium portulacastrum* could be used as a potential natural antimicrobial agent against the tested human pathogenic microorganisms.

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