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RESEARCH ARTICLE

ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF *Sesuvium portulacastrum* EXTRACTS AGAINST LEATHER CONTAMINATING ORGANISMS

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ABSTRACT

In this study the antimicrobial activity of leaf extracts obtained from genus *Sesuvium* L. was examined *in vitro* against eight strains of bacteria and three strains of fungi. Four extracts of *S.portulacastrum* obtained by extraction in methanol, chloroform, petroleum ether and ethyl acetate solvents respectively, were compared for their antimicrobial and antifungal activity. These activities were assessed by measuring the diameter of inhibition zones, minimum inhibitory concentration (MIC) values.

INTRODUCTION

Sesuvium portulacastrum (*S.portulacastrum*) is commonly known as sea purslane, a halophyte belongs to the family Aizoaceae and grows naturally in the subtropical, Mediterranean, coastal and warmer areas around the world[1]. It is native to Africa, Asia, Australia, North America, and South America[2]. The plant has a long history of use in folk medicine, in the treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy and purgative and also used to cure toothache[3]. One study demonstrates that extraction of essential oil from the fresh leaves of *S.portulacastrum* and the essential oil exhibited antibacterial and antifungal activity[4]. Nabikan *et al.* showed that the effect of extracts from tissue culture derived callus and leaf of the saltmarsh plant S.portulacastrum on synthesis of antimicrobial silver nanoparticles using $AgNO_3$ as a substrate[5]. Chandrasekaran *et al* expressed the fattyacid methyl esters (FAME) extract from *S.portulacastrum* can be used in traditional medicine as a potential antimicrobial agent[6]. The aim of this study is to investigate the antibacterial and antifungal activity for different plant extracts using methanol, chloroform, petroleum ether and ethyl acetate solvents, against microorganisms frequently involved in spoilage and processesing of leather.

MATERIALS AND METHODS

The plant material was collected from Chidambaram District and used for the study.

Processing of sample

Two hundred fifty grams of withered leaves of *S.portulacastrum* were washed with tap water to eliminate dust. They were left in the shade dry for 15 days. The dried material was ground to fine powder using a blender mixer. The powder was passed through a sieve of pore size 0.5mm, which was extracted at room temperature with methanol (70%), ethyl acetate (80%), petroleum ether (95%), and chloroform (90%) by dissolving 10gms of powder in 50ml of solvents respectively, on orbital shaker for 24 hours. All the extracts were filtered and were concentrated using rotary evaporator.

Antimicrobial Assay

Microorganisms Used

The antimicrobial activity of leaf extract of *S.portulacastrum* was investigated against two gram positive bacteria viz, *Bacillus subtilis, Staphylococcus aureus* and four strains of gram negative bacteria viz, *Escherichia coli, Klebsiella pneumonia, Vibrio cholera, Pseudomonas aeroginosa.* These isolates, isolated from raw hides obtained from tannery division and confirmed by various biochemical tests. Three mould fungi viz, *Aspergillus niger, Aspergillus flavus, Trichoderma* species isolated from leather, by growing on potato dextrose agar and Sabouraud dextrose Agar.

Disc diffusion Method

The agar diffusion method[7] was followed for antibacterial and antifungal susceptibility tests. Petriplates were prepared by dissolving 2.8gms of agar in 100ml distilled water and sabouraud dextrose agar and allowed to solidify for susceptibility tests against bacteria and fungi. Chloromphenical (10µg/disc), penicillin ((10µg/disc), ciprofloxacin (10µg/disc) for bacteria were used as positive control. Finally, the inoculated plates were incubated at 37^0c for 24 hours. The zone of inhibition was observed and measured in millimetres. Each assay in this experiment was repeated four times.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of leaf extract of *S.portulacastrum* was tested in nutrient broth, Muller Hinton Agar for bacteria, Potato dextrose Agar for fungi by adding 2.5ml of different plant extract to 100 ml of sterilised PDA medium, allowed to solidify. The plates were incubated at 3-14 days at room temperature.

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RESULTS

The plant extract exhibited a notable antibacterial activity against all the bacterial species tested in Disc diffusion Method. However *Klebsiella pneumonia* and *Pseudomonas aeroginosa* showed least zone of inhibition in petroleum ether and chloroform extract and the maximum zone of inhibition of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Vibrio cholera* (Table 1).

Table 1. Antibacterial activity of *Sesuvium portulacastrum*

Pathogens	Inhibition zones (mm) 100µg coccentration per disc			
	Organic solvents			
	Methanol*	Chloroform	Petroleum ether	Ethyl acetate*
B. subtilis	-	6	6	-
E.coli	-	8	8	-
K. pneumonia	-	3	2	-
P. mirabilis	-	6	7	-
P. vulgaris	-	7	6	-
P. aeroginosa	-	3	2	-
S.aureus	-	6	1	-
V.chlerae	-	5	4	-

*- No Inhibition Activity

Minimum Inhibitory Concentration (MIC)

In Minimum Inhibitory Concentration, 10µl, 100µl, 200µl of chloroform extract was used and control plates were prepared. 100 millilitre of bacterial culture was used. The plates were incubated for 24 hours at 37^0 C, low number of colonies was counted from 200µl of chloroform added plates. The counted colonies were compared with control plates and using the formula the percentage of inhibition was calculated (Table 2).

Table 2. Antibacterial activity of *Sesuvium portulacastrum* Minimum Inhibitory Concentration

Pathogens	Control CFU/ml	Test	Percentage of inhibition (%)
B. subtilis	798	21	97.3
E.coli	873	13	98.5
K. pneumonia	1019	38	96.2
P. *mirabilis*	907	18	98
P. vulgaris	1017	31	96.3
P. aeroginosa	872	184	78
S.aureus	1630	36	97
V.chlerae	992	172	82.6

Test – Chloroform extract at 200 mg of plant extract

Antifungal activity of *S.portulacastrum*

S.portulacastrum has notable antifungal activity against all the four fungal species tested. It was observed that sesuvium extract inhibited the reproductive activity in *Aspergillus niger, Aspergillus flavus* and inhibited the growth in *Trichoderma* species.

DISCUSSION

Chemical control of phytopathogenic bacteria practiced for ages, but the constant use of chemicals lead to undesirable pollution, since they release toxic residues that might lead to the chemicals by the pathogens. Natural products are considered an important source of new antimicrobial agents. Drugs derived from unmodified natural products or drugs semi- synthetically obtained for natural sources correspond to 78% of the new drugs approved by the FDA between 1983 and 1994. Halophytes comprising approximately a half of the total global biodiversity, large scale screening will continue to play an important role in development of new drugs. Some of the phenolic and alcoholic constituents of Aizoceae are pharmacologically important either individually.

Conclusion

The chloroform extract was found to be most effective against growth of leather borne pathogen. In the chloroform extract 200 ml was Minimum Inhibitory Concentration against leather borne pathogens. The chloroform, methanol and ethyl acetate extract from the plant of *S.portulacastrum* showed a fungistatic activity against a leather borne fungi like *Aspergillus niger, Aspergillus flavus,* the fungicidal activity against a *Trichoderma* species.

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