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SHORT COMMUNICATION

ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS USED IN SAUDI ARABIA

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ABSTRACT

The antimicrobial activity of four plants' extracts (Thyme leaves (*Thymus vulgaris*), Sage leaves (*Salvia officinalis*), Myrrh exudates (*Boswelia carterii*) and Oliban (*Boswelia carterii)*), used in traditional medicine in Saudi Arabia and other Middle East countries, were evaluated against the following seven bacterial species, *Streptococcus* sp.*, Staphylococcus aureus, Vibrio tubiashii, Micrococcus luteus* ATCC 9341, *Cellulosimicrobium cellulans, Bacillus cereus* and *Legionella pneumophila* and two fungi species, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *Lycopersicii.* Our results showed that the highest antimicrobial activity was observed for the extracts of Thyme, Myrrah and Sage, and the Oliban extracts did not present any antimicrobial activity at any concentration. The minimum inhibitory concentration ranged from 2.0-4.0 % (v/v) for Thyme and Myrrh. The fungal species tested differed significantly in their susceptibility to plant extracts, with complete inhibition by Thyme to all tested microorganisms.

Keywords: Antimicrobial activity, medicinal plants, plant extract.

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria has the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). This is a cause for concern as the numbers of patients in hospitals have suppressed immunity, due to new bacterial strains, which are multi resistant. Consequently, new infections can occur in hospitals resulting in high mortality. Herbal medicines are widely used for the treatment and prevention of various illnesses (Taha Al-Sayed, 2000), and it is adapted to local environment and conditions, compared with any introduced species (Mahasneh and El-Oqlah, 1996, 1999; Jaouhari *et al*., 2000). Several kinds of extracts and metabolites from various Gulf region plants have been isolated and their chemical structure has been elucidated (Al-Easa *et al.,* 1990; Mahasneh, 2000; Saadabi, 2006; Abou-zeid *et al.*, 2008; Fardos, 2009; Nehal and Rokayah, 2009). However, the biological activity of such extracts and compounds against certain bacteria and fungi are poorly investigated. The literature search has indicated a total absence of information about the antimicrobial activity of Saudi medicinal plants. In this study an attempt was made to investigate the four commonly used plants in traditional medicine for their possible antimicrobial and antifungal activities.

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MATERIALS AND METHODS

Medicinal plant materials

Samples of four medicinal plants i.e. Thyme leaves (*Thymus vulgaris*), Sage leaves (*Salvia officinalis*), Myrrh exudates (*Boswelia carterii*) and Oliban (*Boswelia carterii)* were collected during summer 2010 from different stores in Dammam, Saudi Arabia and identified by Pharmacology Department, in King Saud University, Riyadh. The plants were brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and dried in shade then stored until use.

Plant Extract Preparation:

The plants were finely grinded to powder form and homogenized in 200 ml of ethanol (96%) and distilled water (20:80 V: V) for 10 min., then left in dark glass bottles for 72hours for tissue maceration. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 3 min. for ethanol evaporation. The collected extracts were then stored in refrigerator at 5°C until needed (Table 1).

Microbial Culture and Growth Conditions:

Tested microorganisms included seven bacterial species: *Vibrio tubiashii, Staphylococcus aureus, Streptococcus* sp., *Micrococcus Luteus* ATCC9341, *Cellulosimicrobium cellulans, Bacillus cereus, Legionella pheumophila* and two fungal species. *Aspergillus flavus* and *Fusarium oxysporum* f sp. *Lycoperscii.* Cultures of bacteria were

grown for 12hours in 50ml nutrient broth (NA) (Difco, USA) at 37°C and were maintained on nutrient agar (Difco, USA) at 4°C. Cultures of fungi were grown in malt broth (Oxoid, UK) at 28°C and were maintained on Potato dextrose agar (PDA) (Difco, USA) plates. All the test organisms were obtained from Biological Sciences Department of the University of Dammam.

Antimicrobial Activity Assay:

The plant extracts were added to conical flasks containing sterilized PDA (Potato dextrose agar) for fungi culture and NA (Nutrient agar) for bacterial culture before its solidifying to obtain the proposed concentrations of 2,4,6,8,10 % (v/v). Twenty ml of amended media were poured into 9cm diameter, Petri dishes, and another set of untreated PDA, and NA plates were used as control (Nehal and Rokayah, 2009). All plates were inoculated individually with 0.5cm diameter of the tested organisms' culture. Plates with fungal species were incubated in the dark at 25 ± 2°C till the organism reached full growth whereas the bacterial cultured plates were incubated at 30-32°C for 24hours. Antimicrobial activity was recorded as the width (in millimeters) of the growth in the agar. The results were reported as positive (+) if there was inhibition of growth and negative (-) if there was no inhibition of growth. Triplicate sets of plates were prepared and the mean of three readings was calculated and used in the analysis for Minimum Inhibitory Concentration (MICs) which were determined after 24hours for the bacteria and after 48hours for fungi. The MICs were determined as the lowest concentration of plant extract inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was regarded as no growth (Mitscher *et al*., 1987; Kawther, 2007).

RESULTS AND DISCUSSION

The results (Table 2) showed that the inhibitory activity increases with increased concentrations of plants' extracts. The extracts from Thyme, Sage, Myrrah and Oliban presented antimicrobial activity to the most of the

Table 1. Botanical classification and active principles of the plants.

Common Name	Scientific Name	Plant Parts Used	Descriptions
Thyme	*Thymus vulgaris L.*	Stripped and dried and flowers	Chemical oils (thymol and carvacrol), flavonoids, tannins and triterpenes
Sage	*Salvia officinalis L.*	Leaves	rosmarinic, caffeic, chlorogenic acids, carnosol, flavonoids, essential oil (thuyone and cineole)
Oliban	*Boswelia carterii*	Stem exudates	Resin, gum, essential oil (filandrin, painen) and oliben compound
Myrrh	*Commiphora molmol*	Stem exudates	Resin, gum and essential oil (Determine B-eudesmol and α –copaene)

Table 2. Antimicrobial activity caused by plant extracts through agar Diffusion method.

Micro-organisms	Thyme					Extract Sage					Myrrh					Oliban				
	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Fusarium oxysporum	+	++	+++	+++	+++	-	-	-	-	+	-	-	+	++	++	-	-	-	-	+
Aspergillus flavus	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-
taphylococcus aureus	++	++	+++	+++	+++	-	+	++	+++	+++	-	+	++	++	++	-	+	+	++	++
Vibrio tubiashii	++	++	+++	+++	+++	-	+	++	+++	+++	++	++ +	+++	+++	+++	-	+	++	+++	+++
Streptococcus sp.	++	++	+++	+++	+++	-	+	++	+++	+++	++	+ +	++	+++	+++	-	+	+	++	++
Cellulosimicrobium cellulans	++	+++	+++	+++	+++	-	+	++	+++	+++	+	+	++	++	++	-	+	+	++	++
Micrococcos luteus	++	+ ++	+++	+++	+++	+	++	+++	+++	+++	-	+	+	+	++	+	++	++	+++	+++
Legionella pneumophila	++	+ ++	+++	+++	+++	-	+	++	+++	+++	++	++	+++	+++	+++	-	++	+++	+++	+++
Bacillus cereus	+	++	++	++	++	-	+	++	+++	+++	+	++	++	+++	+++	-	+	++	++	++

Data are presented as follows, - = No inhibition of fungal growth, + = Slight inhibition, ++ =Moderate inhibition, +++= Strong inhibition.

tested microorganisms. The extracts from Thyme and Myrrah presented the highest activities followed by Sage and then Oliban. However the tested bacteria showed high significant susceptibility to these extracts more than the tested fungi. The Oliban had no activity against *Aspergillus flavus* and slightly inhibitory action at concentration of 10% (v/v) against *Fusarium oxysporum* f. sp. *Lycopersici.* The minimum inhibitory concentration ranged from 4 – 6 %(v/v) for Thyme and Myrrh, 6 -8 %(v/v) of Sage, and from 8 -10 %(v/v) of Oliban.

This present study, showed high inhibitory action of plants extract to tested microorganisms (Table 2). There was high positive correlation between the increased concentration and an antimicrobial activity until a certain limit (Cimanga *et al.,* 2002; Marcelo *et al*., 2006; Nehal and Rokayah, 2009). Thyme extract showed the highest activity against the two fungal species and seven bacterial species (Dorman and Deans, 1999; Gislene *et al.,* 2000; Marcelo *et al.,* 2006). Moreover Essawi and Srour (2000) and Al-Turki (2002) added that the activity was attributed to the presence of several compounds working together such as, thymol, carvacrol, flavonoids and tannins. Our results showed that Thyme and Myrrh demonstrated higher anti microbial activity compared with Sage, this data is in agreement with Hammer *et al.* (2001), Al-Turki (2002) and Mahasneh (2002). The fungal strains tested differed significantly in their susceptibility to plants extract. On the contrary, Oliban extract showed no inhibitory action on two fungal strains and lowest inhibitory action on bacterial strains compared with another plants extract that can be traced back to the Resin, Gum, essential oil, filandrin and painen.

Antimicrobial phytochemicals are rosmarinic, caffeic, chlorogenic acids carnosol, flavonoids, rein, gum and essential oils plus other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisms vary and depends on these compounds (Mossa *et al.*, 2001; Rios and Recio, 2005; Aly and Bafiel, 2008). Their mechanism of action may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical often leading to inactivation of the protein and loss of function. They have the ability to intercolate with DNA, formation of ion channels in the microbial membrane (Ali, 1999), competitive inhabitation of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999). Therefore, it is important that the plant species which have demonstrated growth inhibiting activity in this assay be further investigated to evaluate the significance of these extracts, clinical role and the medical system of indigenous people. Additional research is also necessary to isolate and characterize their active compounds for pharmacological testing.

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