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ANTIMICROBIAL ACTIVITIES OF SOME SAUDI ARABIAN HERBAL PLANTS

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Abstract

Background: Several edible plants are used in Kingdom of Saudi Arabia since early time to control microbial infections. In the present study, twenty-four Saudi Arabian medicinal plants d according to traditionally used were select and investigated for the antimicrobial activities
Materials and Methods: This study was designed at evaluating the antimicrobial activities of the methanol extracts of twenty-four species of sixteen plant families used in the traditional medicine by Saudi Arabian people for the treatment of numerous ailments of the microbial and non-microbial origin against four Gram-positive, four Gram-negative bacteria and four fungi and yeast using the agar well diffusion method.
Results: Of most of the plants tested were found to be active against two to eight organisms. Five plants were active against eight organisms. The data appeared that extracts of *Echium arabicum* (SY-176), *Rhantarium epapposum* (SY-180), *Rumex vesicarus* (SY-181), *Ziziphus nummularia* (SY-188), *Caylusea hexagyna* (SY-197) and *Artemisia monosperma* (SY-198) have anti-microbial activity against the most of tested bacteria, fungi and yeast. Whereas (SY-181), the extracts of *Teucrium oliverianum* (SY-175), *Zilla spinosa* (SY-187), and *Rhazya stricta* (SY-195) have poor action against the tested bacteria, fungi and yeast.
Conclusion: The antimicrobial activity of plant extracts against bacteria was more effective than against fungi

Key words: Gram-positive bacteria, Gram-negative bacteria, fungi, yeast, medicinal plants, Saudi Arabia

Introduction

Medicinal plants comprise an immense potential for producing new drugs of great benefit to mankind and represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Different parts of medicinal plants were used for extract as raw drugs and they possess various medicinal properties (Mahesh and Satish, 2008). The rising failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of numerous medicinal plants for their prospective anti-microbial activity.

Additionally, the developing countries, synthetic drugs are not only expensive and insufficient for the treatment of diseases but also have deception and side effects (Elizabeth, 2005). The kind of this situation stresses need to search a novel drug for treating such disease (Sieradzki et al., 1999). Therefore, researchers are gradually turning their consideration to natural products in search of new leads to develop superior drugs against the infection of microbes (Saravanan, *et al*., 2011). A lot of remedial plants have been used as nutritional supplements and as well in the treatment of several diseases lacking proper data of their function.

Several spices, herbs and herb extracts have been shown to possess antimicrobial properties. By now, garlic, onion, ginger, mustard and pepper have been documented as antimicrobial activity against several types of bacteria (Al Mofleh, 2010).

In previous study, different extracts of twenty-seven medicinal plants collected from different localities belong to eighteen different plant families and tested for their ability to induce NAD(P)H: quinoneoxidoreductase in murine hepatoma cells grown in microtiter plate wells (Shahat, et al., 2013, Shahat et al., 2016).

Antioxidant activity of six medicinal plants Asteraceae family collected from different areas of Kingdom of Saudi Arabia was evaluated (Shahat, *et al*., 2014, Shahat et al., 2015). The current study was carried out to describe preliminary screening to demonstrate the existence of bactericidal (Gram-positive) and bacteriostatic (Gram-negative) activities in the 80% extracts of twenty-four Saudi Arabian medicinal plants.

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Materials and Methods

Plant Materials

Twenty-four species of 16 plant families used in the traditional medicine by Saudi Arabian people were collected from different localities in April 2013. Identification of the plants was done by the Plants Taxonomy and Herbarium Unit. Voucher specimens have been deposited at the Herbarium of the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia (Table 1).

Table 1: Medicinal plants used in this study

Index	(Voucher noumber)	Plants (Family)	Yield (%)
SY-175	15930	*Teucrium oliverianum* (Lamiaceae)	20
SY-176	15931	*Echium arabicum* (Boraginaceae)	14.5
SY-177	15932	*Haplophyllum tuberculatum* (Rutaceae)	19.6
SY-178	15933	*Senna italic* (Caealpiniaceae)	14.9
SY-179	15934	*Pulicaria crispa* (Asteraceae)	6.7
SY-180	15935	*Rhantarium epapposum* (Asteraceae)	3.1
SY-181	15936	*Rumex vasicanus* (Polygonaceae)	13
SY-182	15937	*Ducrosia anethifolia* (Aplaceae)	26
SY-183	15938	*Heliotropium ramosissimum* (Boraginaceae)	2.58
SY-184	15939	*Picris cyanocarpa* (Asteraceae)	19.5
SY-185	15940	*Anthemis deserti* (Asteraceae)	10.8
SY-186	15945	*Cleome ambliocarpa* (Cleomaceae)	14.9
SY-187	15946	*Zilla spinosa* (Brassicaceae)	13.5
SY-188	15947	*Ziziphus nummularia* (Rhamnaceae)	11.6
SY-189	15949	*Neurada procumbens* (Neuradaceae)	8.6
SY-190	15951	*Trigonella hamosa* (Papilionaceae)	22
SY-191	15952	*Achillia fragrantissima* (Asteraceae)	6.2
SY-192	15953	*Convolvulus prostates* (Convolvulaceae)	15.3
SY-193	15954	*Cltrullus colocynthis* (Cucurbitaceae)	14.2
SY-194	15955	*Emex spinosa* (Polygonaceae)	16.8
SY-195	15957	*Rhazya strict* (Apocynaceae)	22
SY-196	15958	*Scrophularia hypericifolia* (Scrophulariaceae)	12.12
SY-197	15959	*Caylusea hexagyna* (Resedaceae)	13.04
SY-198	15960	*Artemisia monosperma* (Asteraceae)	16.4

Extracts Preparation

100 gm of the dried aerial part of the plants were macerated twice in 300 mL aqueous methanol (80%) for 72 h at room temperature. The extracts were filtered and concentrated under reduced pressure at 40°C using a rotary evaporator. The obtained dry extract was weighed and the percentage yield was expressed in terms of air dried weight of plant materials.

Material for Antimicrobials

The bacterial, fungal and yeast strains were personally obtained from the microbiology Lab, Botany Dept., Fac. of Sci. (Al- Azhar Univ. Assiut, Assiut Univ. and Minia Univ.).

Microorganisms:

No	Microorganisms	Source
Gram-negative bacteria		
1	*Klebsiella pneumonia (Kp)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
2	*Proteus vulgaris (Pv)*	
3	*Pseudomonas aeruginosa (Pa)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
4	*Serratiamarcescens (Sm)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
Gram-positive bacteria		
1	*Bacillus cereus (Bc)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
2	*Micrococcus luteus (Ml)*	Department of Botany, Faculty of Sciences, Minia University**,** Minia, Egypt.
3	*Micrococcus roseus (Mr)*	

4	*Staphylococcus aureus (Sa)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
Fungi and yeasts		
1	*Aspergillus flavus (Af)*	Botany Dept., Fac. of Sci. Minia Univ. Department of Botany, Faculty of Sciences, Minia University, Minia , Egypt.
2	*Aspergillus ochraceus(Ao)*	Department of Botany, Faculty of Sciences, Al-Azhar University, Assiut, Egypt.
3	*Fusarium moniliforme (Fm)*	
4	*Candida albicans (Ca)*	

Methods of antibacterial activity
Determination of antibacterial activity:

The agar well diffusion method was used to test the antibacterial activity of the prepared extracts (Oke *et al*., 2009) [7]. Of all tested bacteria, stock cultures were grown in nutrient broth for 18 h. Final cell concentrations were standardized until 10^7 –10^8cfu/ml. One milliliter of this inoculum was added to each plate containing nutrient agar. When the agar was solidified, 4 wells (6 mm diameter) were formed in every plate. Crude extracts were prepared at a concentration of 10 mg/ml with dimethyl sulphoxide (DMSO) as solvent; 50µl of each extract was applied into each well. The control sample was prepared using DMSO and Cephradine (CE); 30µg/disk and cefotaxime (CTX) 30µg/disk were used as standard antibacterial agent. After 12–15 min of diffusion time at room temperature, the plates were incubated at 37 °C for 48 h. At the end of the incubationperiod the antibacterial activity was evaluated by measuring the inhibition zones. The diameter of the inhibition zone was measured in 3 directions and the average values were tabulated. The experiment was made in three replications.

Antifungal activity

According to Ndukwe *et al*. (2004), the antifungal activity was assayed using method of agar well diffusion. Aliquot of 100 µl spores suspension ($1x10^8$ spores/ml) of each testing fungi were streaked in radial patterns on the surface of complete media plates (potato dextrose agar (PDA) media). A six mm in diameter wells were performed in the media, and then each well is filled with definite concentration (10 mg/ml) with dimethyl sulphoxide (DMSO) as solvent of each tested extract. We used the DMSO as control for the extracts and the DMSO as negative control for the extracts and Nystatin (30 µg /disk) was used as positive control. The cultured plates were incubated at 20ºC for 3-5 days. Radius of the zone of inhibition was measured in two directions at right angles to each other. Three replicates per treatment were carried out and each treatment was repeated at least twice.

Results and Discussion

Data in Table (2) showed preliminary screening of the Antimicrobial activities of some selected traditional Saudi Arabian medicinal plants. The data appeared that extracts of (SY-176, SY-180, SY-181 SY-188, SY-197 and SY-198) have antimicrobial activity against most of tested bacteria, fungi and yeast. Whereas, the extracts of (SY-175, SY-187, and SY-195) have poor activity against tested bacteria, fungi and yeast.

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Table 2: Diameters of inhibition zones (mm) of extracts against some selected

Microbial / Exracts	**Bc**	**Kp**	**Ml**	**Mr**	**Pv**	**Pe**	**Sm**	**Sa**	**Af**	**Ao**	Fm	Ca
SY-175	9±1.5	9±2.0	-	-	-	-	-	-	-	-	-	-
SY-176	-	8±2.0	10±1.0	8±1.0	9±1.0	10±3.5	14±2.6	7±2.6	-	-	23±2.6	-
SY-177	9±0.6	8±2.0	-	-	-	11±2.6	-	-	-	-	-	12±2.6
SY-178	9±2.0	12±2.6	11±1.7	-	-	10±3.0	12±4.4	-	-	-	-	-
SY-179	10±2.0	11±2.0	9±1.7	9±1.0	-	-	-	-	-	-	13±1.7	-
SY-180	8±3.5	11±2.0	-	-	12±2.6	14±5.6	15±5.0	9±2.0	9±1.0	10±2.0	-	-
SY-181	8±3.0	8±1.0	-	8±1.0	9±1.7	10±2.6	12±1.0	8±1.0	-	-	12±1.0	-
SY-182	-	8±2.6	-	8±3.0	-	-	-	9±2.6	-	-	15±4.6	-
SY-183	9±1.7	9±2.0	8±1.7	-	9±2.6	-	-	8±1.0	-	-	12±4.0	-
SY-184	8±2.6	8±2.0	-	8±2.6	9±2.6	-	12±2.6	9±2.0	-	-	14±2.6	-
SY-185	-	10±2.0	-	-	-	-	-	9±3.2	-	-	-	-
SY-186	8±0.577	9±2.645	-	-	-	-	-	9±1.527	-	-	-	-
SY-187	-	-	-	-	9±1.5	-	-	-	-	-	11±1.0	-
SY-188	9±1.0	11±2.6	13±1.0	7±1.0	10±3.0	11±1.7	15±2.6	10±1.0	-	-	-	-
SY-189	-	-	-	-	-	-	10±4.0	8±1.7	10±2.0	-	13±3.6	-
SY-190	-	11±1.0	-	-	-	10±3.0	-	-	10±1.7	-	-	-
SY-191	8±2.0	9±1.0	9±2.6	-	10±3.0	-	16±4.0	-	-	-	12±2.0	-
SY-192	-	9±3.0	-	-	9±2.6	11±1.0	15±3.0	10±2.6	-	-	12±1.0	-
SY-193	-	-	-	-	-	-	-	10±3.0	-	-	13±1.7	9±1.0
SY-194	8±2.0	9±4.6	-	-	10±1.7	-	-	-	-	-	11±1.000	-
SY-195	-	10±2.6	±-0.0	-	-	-	-	9±3.6	-	-	-	-
SY-196	-	-	-	-	9±2.0	-	-	-	11±1.7	-	13±2.6	-
SY-197	10±2.6	10±2.0	-	9±3.5	-	-	17±2.6	8±2.0	-	15±3.0	13±1.7	-
SY-198	9±3.5	10±3.6	10±2.0	9±2.6	9±2.0	-	15±4.6	9±1.0	-	-	14±3.6	-
CE 30µg	6±0.0	6±0.0	11±0.67	6±0.3	6±0.0	8±0.0	6±0.0	6±0.0	-	-	-	-
CTX 30µg	14±0.6	13±0.6	14±0.9	19±0.8	26±0.6	17±0.7	47±1.5	15±0.3	-	-	-	-
Nys. 30µg	-	-	-	-	-	-	-	-	21±0.9	19±1.3	27±0.2	24±2.1

Values are mean inhibition zone (mm) ± S.D of three replicates.

The most susceptible Gram-positive bacteria were *Bacillus cereus* and *Staphylococcus aureus*, while the most resistance Gram-negative bacteria were *Proteus vulgaris* and *Pseudomonas aeruginosa*, these variations might be associated with the differences in cell surface structures between Gram-negative and Gram-positive bacteria (Cowan, 1999).

The methanolic extract of SY-188 exhibited the highest antibacterial activity against all tested bacteria this may due to alkaloids, flavonoids, glycosides and saponins in extract of *Zizyphus nummularia* (SY-188) (Duby, et al., 2010), but did not inhibit tested fungi and yeast. Also, data indicated that SY-176 extract displayed antimicrobial activity against all tested microorganisms except Bacillus *cereus, Aspergillus flavus, Aspergillus ochraceus*, and *Candida albicans.* SY-175 was effective only against *Bacillus cereus* and *Klebsiella pneumonia*. SY-175, SY-178, SY-185, SY-186, SY-188 and SY-195 extracts showed no inhibition zone against all tested fungi and yeast, indicating that they did not posses any antifungal activity. *Candida albicans* was more resistant to all extracts with the exception of SY-177 and SY-193. Additionally, *Klebsiella pneumonia* exhibited sensitive to most extracts, *Serratia marcescens* displayed inhibition zone ranged between 10-17 mm. The antimicrobial activity of plant extracts against bacteria was more effective than against fungi, which is similar to the results reported by Avato and Ertürk (Avato *et al*., 1997; Ertürk *et al*., 2003).

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