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***In vitro* Antimicrobial Activity of Some Saudi Arabian Plants Used in Folkloric Medicine**

Abdulmoniem M.A. Saadabi, A.G. AL-Sehemi and K.A. AL-Zailaie
Department of Science, Abha Teachers College, P.O. Box 249,
Abha, Kingdom of Saudi Arabia

Abstract: Methanolic, chloroform and aqueous extracts of 26 medicinal plants used in folklore medicine in Saudi Arabia were screened for *in vitro* activity against some Gram-positive and Gram-negative pathogenic bacteria using the cup-plate agar diffusion method. The extracts at concentration of 0.1 mL cup showed varying degrees of inhibitory activity against the test organisms. Extracts from *Withania somnifera* showed the highest activity, followed by *Datura stramonium*, while *Zygophyllum portulacoides* demonstrated the least activity when compared to 40 µg mL⁻¹ Ampicillin control antibiotic. The bacteria tested differed significantly in their susceptibility to plant extracts, with *Staphylococcus aureus* and *Bacillus subtilis* have been completely inhibited. The plants which exhibited a marked antibacterial activity were shown to be rich in flavonoids, tannins and alkaloids. These results support the traditional use of the plants in the treatment of some bacterial infections.

Key words: Medicinal plants, folkloric medicine, antibacterial activity, Saudi Arabia

INTRODUCTION

Higher plants have been used for centuries as remedies for human diseases (Azaizeh *et al.*, 2003). This has encouraged research into screening of plants for antibacterial and antifungal activities (Omer and Elnima, 2003). In the literature a number of compounds have been isolated from plants and their chemical structures were fully elucidated and many of them were tested for possible biological activities (Crombie *et al.*, 1990; Mitscher *et al.*, 1972). Many of the active compounds has found place in modern therapy and compounds vary from alkaloids, terpenes, flavanoids, glycosides, to many minor classes of plant constituents (Vardamides *et al.*, 2001; Almagboul *et al.*, 1985; Edeoga and Ikem, 2002).

As yet, plants in Saudi Arabia have not been thoroughly investigated with regard to their biological activities (Mossa, 1986; Al-Taweel *et al.*, 2004; Kadriya *et al.*, 2004). Therefore, an attempt was done to investigate plants collected from different regions for possible antibacterial activities and these plants are commonly used in traditional medicine.

MATERIALS AND METHODS

Plant materials: Plants were collected from the South area of the Kingdom of Saudi Arabia in January 2002 and were authenticated by the Department of Botany, University of

Kartoum, Sudan; A voucher specimens were deposited at Science Department Herbarium.

Preparation of plant extracts: Ten gram of the coarsely powdered plant material were successively Soxhlet extracted with CHCl₃ and MeOH for 24 h. The extracts were evaporated under vacuum and the residues were separately dissolved or suspended in the same extracting solvent (10 mL) and kept in refrigerator till use. In addition, water extracts were prepared by adding distilled water to 10 g of coarsely powdered plant material in a conical flask and left to soak overnight. The residue was then filtered and the final volume was adjusted to 10 mL with distilled water and the solution used immediately

Screening for antibacterial activity: The plant extracts were tested against four types of bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The bacteria was cultured on nutrient broth (Oxoid) at 37°C for 24 h. Each extract was tested against each organism using the cup-plate agar diffusion method. (Groove and Randall, 1955) and the inhibition zones were measured. The means of the diameters of the inhibition zones are reported in Table 1.

Phytochemical screening: Phytochemical screening was carried out using the methods adopted by Crombie *et al.*, (1990) in similar surveys.

RESULTS AND DISCUSSION

Seventy eight plant extracts representing 26 plants belonging to 20 families were tested (Table 1 and 2). The

results of the antibacterial screening of plants used in Saudi Arabia folk medicine are shown in Table 1. The plant extracts which possessed 15 mm inhibition zones or more against one or more micro-organisms were

Table 1: Antibacterial activity of certain Saudi Arabian plants: *in vitro* tests

Plant (Botanical family)	Part (s) used	Zone of inhibition (mm)											
		Ch				Me				H ₂ O			
		B.s	S.a	E.c	P.a	B.s	S.a	E.c	P.a	B.s	S.a	E.c	P.a
<i>Abutilon figarianum</i> Webb (Malvaceae)	L	13	13	13	15	17	17	16	15	18	16	17	15
<i>Argemone mexicana</i> L. (Papaveraceae)	L	14	13	15	13	15	16	16	17	-	-	-	-
<i>Cadaba farinosa</i> Forsk (Capparaceae)	St	14	15	15	13	16	17	12	12	-	14	-	-
<i>Cassia acutifolia</i> Del. (Leguminaceae)	Fr	15	16	16	15	14	15	15	13	15	15	13	14
<i>Cyperus rotundus</i> L. (Cyperaceae)	W.P	14	13	15	14	16	17	17	18	15	15	19	17
<i>Datura stramonium</i> L. (Solanaceae)	L	14	13	15	15	16	17	17	18	15	15	19	17
<i>Fagonia cretica</i> L. (Zygophyllaceae)	L	-	-	-	-	14	15	17	16	14	15	18	14
<i>Gnidia kraussiana</i> Meisn. (Thymelaceae)	W.P	15	12	13	17	14	17	16	18	14	15	12	16
<i>Grewia flavescens</i> Juss (Tiliaceae)	L	15	16	15	14	17	15	17	16	13	14	15	15
<i>Grewia tenax</i> (Forsk) Fiori (Tiliaceae)	L	-	-	-	-	18	16	19	18	14	16	17	15
<i>Grewia villosa</i> Willd. (Tiliaceae)	L	15	13	15	15	20	16	18	16	22	16	17	21
<i>Hyoscyamus muticus</i> L. (Solanaceae)	L	16	15	14	13	13	14	15	14	12	15	14	15
<i>Ipomoea blepharosepala</i> Hochst ex. A. Rich (Convolvulaceae)	W.P	14	-	15	14	15	-	16	17	13	-	14	16
<i>Momordica balsamina</i> L. (Cucurbitaceae)	L	13	14	14	15	15	17	17	16	14	16	14	14
<i>Murraya exotica</i> Koem. ex L. (Rutaceae)	W.P	14	13	11	12	17	16	15	16	13	14	13	13
<i>Nymphaea lotus</i> L. (Nymphaeaceae)	W.P	16	15	15	15	21	21	20	20	14	17	16	15
<i>Peganum harmala</i> (Caryophyllaceae)	L	13	12	13	12	13	13	15	14	14	12	14	13
<i>Plicosepalus acaciae</i> Zucc. (Loranthaceae)	W.P	16	16	16	18	20	20	20	15	16	15	13	16
<i>Polycarpha corymbosa</i> (L.) Lam (Caryophyllaceae)	W.P	15	15	15	17	13	14	18	17	12	13	17	16
<i>Premna resinosa</i> (Hochst) Schan. (Verbenaceae)	L	-	-	-	-	13	14	14	12	16	15	14	16
<i>Salvadora persica</i> L. (Salvadoraceae)	St	15	13	14	12	17	15	17	13	13	14	15	14
<i>Sterculia steigerera</i> Del. (Sterculiaceae)	Fr	16	17	16	15	16	14	15	15	13	13	12	12
<i>Vahila dichotoma</i> (Murr.) Kurtze (Saxifragraceae)	W.P	12	14	12	11	17	16	16	15	18	21	20	15
<i>Vigna fragrans</i> Bak. F. (Papilionaceae)	R	12	14	13	14	18	16	18	18	16	18	16	15
<i>Withania somnifera</i> (L.) Dun. in DC. (Solanaceae)	L	15	13	15	15	20	16	18	16	22	16	17	21
<i>Zygophyllum portulacoides</i> Forsk (Zygophyllaceae)	St	-	-	-	-	13	14	15	14	12	15	14	15
Ampicillin 40 ug mL ⁻¹										16	24	15	20

*B.s, *Bacillus subtilis*, a, *Staphylococcus aureus*, c, *Escherichia coli*, P.a, *Pseudomonas aeruginosa*, concentration of extracts 0.1 mL⁻¹ cup (100 mg mL⁻¹), inhibition zones are the mean of three replicates, not tested, L = Leaves, St = Stem, W.P = Whole Plant, Fr = Fruit, Ch = Chloroform, Me = Methanol, H₂O = Water

Table 2: Comparative antimicrobial activity of the tested plant families

Botanical family	Antimicrobial activity*	No. of plants tested
Malvaceae	+	1
Papaveraceae	+	1
Capparaceae	+	1
Leguminaceae	+	1
Cyperaceae	+	1
Solanaceae	++	3
Zygophyllaceae	++	2
Thymelaceae	+	1
Tiliaceae	++	3
Convolvulaceae	+	1
Cucurbitaceae	+	1
Rubiaceae	+	1
Nymphaeaceae	++	1
Caryophyllaceae	++	2
Loranthaceae	+++	1
Verbenaceae	+	1
Salvadoraceae	++	1
Sterculiaceae	+	1
Saxifragraceae	+	1
Papilionaceae *	+	1

+++ = Active + = Moderate activity - = Low activity (if any)

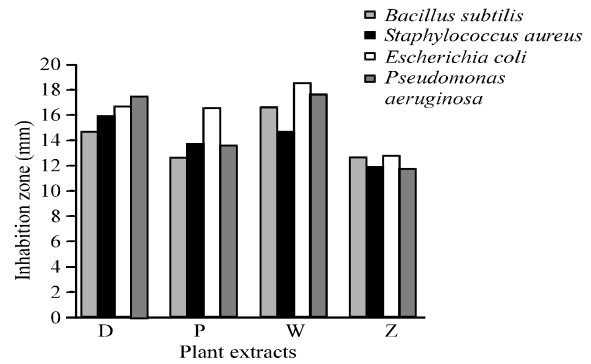


Fig. 1: *In vitro* inhibitory effects of different plant extracts i.e., *Datura stramonium* (D), *Peganum harmala* (P), *Withania somnifera* (W) and *Zygophyllum portulacoides* (S) on different four types of bacteria

considered to be active. Out of the 78 extracts examined 76% showed significant antibacterial activity. Sixty four percent of extracts showed significant activity against *Bacillus subtilis*, 76% against *Staphylococcus aureus*, 84% against *Pseudomonas aeruginosa* and 76% against *Escherichia coli*.

It is evident that the two Gram-negative organisms, i.e., *E. coli* and *P. aeruginosa*, showed a slightly higher sensitivity to the plant extracts compared to the two Gram-positive organisms used *B. subtilis* and *S. aureus*. Out of the 26 plants tested, 14 plants showed a high activity against the four types of bacteria tested. Extracts of 7 plants were active against the two Gram-negative bacteria (28%). Out of these 7 plants, 3 were active against *E. coli* and *P. aeruginosa* and 3 against *P. aeruginosa* alone and only one plant was active against *E. coli*. Extracts of 5 plants were active against both *B. subtilis* and *S. aureus*. Out of these 5 plants, one was active against *B. subtilis* and *S. aureus*, 3 against *S. aureus* and only one against *B. subtilis* (Fig. 1).

Out of the most active 46 extracts, methanol extracts exhibited a well marked antibacterial activity (43%),

followed by chloroform (39%) and aqueous extracts (17%). This data is in agreement with previous reports elsewhere using the same plants (Almagboul *et al.*, 1985, 1988). Out of the 26 plants tested against *S. aureus* and *P. aeruginosa*, 10 plants exhibited a marked antibacterial activity. These are *Momordica balsamina*, *Cyperus rotundus*, *Plicosepalus acaciae*, *Nymphaea lotus*, *Vigna fragrans*, *Vahila dichotoma*, *Gnidia kraussia*, *Grewia tenax*, *Withania somnifera* and *Datura stramonium*.

Some of the above-mentioned plants were phytochemically screened and the results are shown in Table 3. All the plant extracts tested showed the presence of sterols and/or triterpenes. Five of the most active plants contain alkaloids, flavanoids and tannins, 6 plants contain flavanoids and alkaloids. Tannins and flavanoids were detected in the same plants. None of the plants tested was found to contain anthraquinones. *Plicosepalus acaciae* was found to be the most active plant, it is rich in flavanoids, tannins, sterol and/or triterpenes. It also contains traces of cyanogenic glycosides. None of the plants tested is rich in saponins.

Table 3: Preliminary phytochemical screening of the most active antibacterial plant samples

Plant species	Part used	Sterols and or triterpenes	Cyanog-enic glycosides	Anthra-quinones	Saponins	Tannins	Flavonoids	Alkaloids
<i>Momordica balsamina</i>	L	+	-	-	-	+	+	+
<i>Cyperus rotundus</i>	W.P.L	±	-	-	+	+	+	+
		+	-	-	-	-	+	+
<i>Plicosepalus acaciae</i>	L	+++	±	-	+	+++	+++	-
	S	++	±	-	+	+++	+++	-
	W.P	++	-	-	+	+++	+++	-
<i>Nymphaea lotus</i>	W.P	+	-	-	-	+	++	++
<i>Vigna fragrans</i>	R	++	±	-	+	-	-	-
<i>Vahila dichotoma</i>	W.P	+	+	-	-	+	+	+
<i>Gnidia kraussiana</i>	R	+	+	-	-	+	+	+
	S	+	+	-	+	+	-	+
<i>Grewia tenax</i>	L	++	-	-	±	-	+++	+++

Constituents: + = Low concentration: ++ = Medium concentration, +++ = High concentration, - = Not detectable, Traces = ±

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