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ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS

By

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SUMMARY

In this study, the alcoholic and lyophilized aqueous extracts from twenty wild medicinal plants belonging to two families from the Qassim area were studied. The sensitivity of eighteen microbes (five Gram-positive and six gram-negative bacteria, five fungi and two yeasts) to the prepared extracts at concentrations of 10, 25, 50, 100 and 200 mg/ml was investigated. The minimum inhibitory concentrations (MIC) for different active extract were also investigated against the tested bacteria.

Alcoholic and aqueous extracts of the stuided plants exhibited a strong antibacterial activity against *Staph. aureus, Staph. aureus* (methicillin resistant), *Strept.* types B and D, *Salm.* type C, *E. coli, H influenza Pr. mirabilis* and *Ps. aeruginosa.* The values of MIC for *C. bruguierana, R. stricta, P. harmala, C. coccineum* (No. 6) and *W. somnifera* extracts against *H. influenza* were 8.36, 8.42, 8.47, 23.77 and 23.87 mg/ml, respectively. It has been also observed that fungi and yeasts were less sensitive, with the exception of *T. mentagrophytes* which was slightly sensitive to some plant extracts.

INTRODUCTION

Plants have been used by man as medicines for treatment of many diseases for a long time. Medicinal plants are being used either directly by folk medicine practitioners, or alternatively, their active principles are included in suitable standerdized pharmaceutical forms. In recent years, there has been a wide range of worldwide systematic investigation of plants for biological activities, among these investigations is the antimicrobial activity. As a matter of fact, antimicrobial screening methods are quick and of low costs (Al-Meshal et al. 1982, Al-Yahya et al. 1983, Zaki et al. 1984). It is worth to mention that several local medicinal plants are being used to control pathogenic microbes, in addition to their other uses. Among these plants are *Astragalus spinosus, Francoeuria crispa, Capparis cartilaginea* and *Calligonum comosum* (Banoub and El-Sheikh 1982).

This report is an attempt to elucidate the antibacterial and antifungal activities of some selected local medicinal plants. Those showing significant activities will be subjected to detailed phytochemical investigations to find out their constituents responsible for such activities.

MATERIALS AND METHODS

Plant Materials:

Plants used in this study were collected from different areas of the Qassim province (Table 1). The identity of plants was verified by staff of the Botany Department, college of science, King Saud University, Riyadh. (Migahid, 1978) Voucher specimens are deposited in the Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim, Saudi Arabia.

Preparation of the Alcoholic Extracts:

The air-dried parts (200 g) of each plant were

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powdered and extracted at room temperature with 95% ethanol till exhaution. The alcoholic extract of each plant was evaporated under reduced pressure using a Buchi rotary evaporator. For antimicrobial screening, aqueous solutions of the alcoholic extracts were prepared using Tween 80.

Preparation of the Lyophilized Aqueous extracts:

The air-dried powdered parts (200 g) of each plant were extracted with hot distilled water for 5 times. The combined aqueous extract of each plant was filtered then lyophilized using a Labconco freeze dryer-18 (model 75018). These lyophilized aqueous exracts were dissolved in distilled water.

Preparation of the Extract of Centaurea bruguierana:

The air-dried powdered aerial parts were extracted with 95% ethanol till exhaustion. The solvent evaporated under vaccum. The residue was solved in 20% aqueous ethanol. The hydroalholic solution was extracted successively light petrolium ether and chloroform. The reming aqueous layer was lyophilized using the Lconco freeze dryer-18. The residue was dissolin methanol, to give methanol insoluble (rejected) and the methanol soluble part was used in this study.

Micro-organisms:

1) Bacteria: *Staphylococcus. aureus, Staphylococcus. aureus* (methicillin resistant), *Streptococcus.* types B and D, *Bacillus subtilis, Salmonella.* type C, *Escherichia. coli, Proteus mirabi pseudomonas. aeruginosa, Haemophilus influenza* and *Klebsiella pneumoniae.*

Table 1: Medicinal plants, name, Parts and extracts used for Microbiological Sceening.

No.	Plant name	Family	Plant part	Extract (s)
1.	*Rhanterium epapposum*	Compositae	Aerial parts	Alc &Aq.
2.	*Centaurea bruguierana*	Compositae	Aerial parts	Aq.**
3.	*Euryops arabicus*	Compositae	Leaves	Aq.
4.	*Artemisis judaica*	Compositae	Aerial parts	Aq.
5.	*Cynomorium coccineum*	Cynomoriaceae	Peels of flower*	Alc.
6.	*Cynomorium coccineum*	Cynomoriaceae	Peels of stems & roots*	Alc.
7.	*Cynomorium coccineum*	Cynomoriaceae	Inner pulp*	Alc.
8.	*Withania somnifera*	Solanaceae	Leaves	Alc & Aq.
9.	*Fagonia cretica*	Zygophyllaceae	Aerial parts	Aq.
10.	*Tribulus terrestris*	Zygophyllaceae	Aerial parts	Aq.
11.	*Peganum harmala*	Zygophyllaceae	Aerial parts	Aq.
12.	*Blepharis ciliaris*	Acanthaceae	Aerial parts	Aq.
13.	*Cassia italica*	Leguminosae	Leaves	Aq.
14.	*Astragalus spinosus*	Leguminosae	Aerial parts	Aq.
15.	*Capparis spinosa*	Capparaceae	Leaves	Alc & Aq.
16.	*Cleome amblyocarpa*	Capparaceae	Aerial parts	Aq.
17.	*Catharanthus roseus*	Apocynaceae	Leaves	Aq.
18.	*Rhazya stricta*	Apocynaceae	Leaves	Aq.
19.	*Heliotropium bacciferum*	Boraginaceae	Aerial parts	Aq.
20.	*Arnebia decumbens*	Boraginaceae	Aerial parts	Alc.
21.	*Calligonum comosum*	Polygonanceae	Leaves	Aq.
22.	*Chrozophora verbascifolia*	Euphorbiaceae	Aerial parts	Aq.

* Three alcoholic extracts were prepared. One from the fresh peels of flower heads, another from fresh peel of roots and stems. The third from the inner pulp of roots and stems. The produced alcoholic extract in each case was freeze-dried after evaporating alcohol.
** See preparation of the extracts.

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Table (2): Antibacterial activity of medicinal plant extracts against certain Gram-positive bacteria (n=5) (mean ± S.E).

Plant	Extract	Conc.(mg/ml)	Diameter of inhibitory zone (mm) Staph. aureus	Staph. group B	Strept. group D	Staph. aureus Meth Resist.	Bacillus subtilis
Rhanterium epapposum	Aq	100	-	-	-	-	11.4±0.25
	Alc.	200	-	-	-	-	12.8±0.37
		100	-	-	-	-	11.0±0.00
		200	-	-	-	-	13.8±0.37
Cynomorium coccineum	Alc	25	10.8±0.20	9.8±0.20	-	-	-
	(No. 5)	50	12.4±0.25	11.4±0.25	10.4±0.25	13.4±0.25	-
		100	13.2±0.20	13.4±0.25	13.8±0.37	17.8±0.37	12.0±0.45
		200	15.4±0.25	15.8±0.37	17.2±0.37	20.2±0.37	15.8±0.37
	Alc	25	-	-	-	12.6±0.25	-
	(No. 5)	50	-	-	12.4±0.25	17.0±0.00	-
		100	10.6±0.25	12.6±0.25	14.4±0.40	22.0±0.00	11.4±0.25
		200	13.8±0.37	15.8±0.37	16.4±0.40	27.4±0.25	15.2±0.20
	Alc	25	-	-	-	13.4±0.25	-
	(No. 5)	50	-	-	13.2±0.20	17.8±0.37	-
		100	12.4±0.25	13.6±0.40	15.8±0.37	23.0±0.00	12.0±0.45
		200	14.6±0.40	15.6±0.25	17.8±0.37	28.4±0.25	15.6±0.40
Withania somnifera	Aq	25	-	-	-	10.8±0.20	-
		50	-	-	-	15.4±0.25	-
		100	-	-	-	18.4±0.40	-
		200	-	12.8±0.37	-	22.0±0.00	-
Centaura bruguierana	Aq	25	-	-	-	10.6±0.25	-
		50	-	-	-	13.4±0.25	18.8±0.37
		100	-	-	-	17.4±0.40	13.8±0.37
		200	-	-	-	22.4±0.40	17.8±0.37

- = No. effect

Fungi: *Trichophyton mentagrophytes, Microsporum canis, Asperigellus niger, Asperigellus fumigatus* and *Penicillium* spp.

Yeasts: *Candida albicans* and *Cryptococcus neoformans.*

ese microorganisms were supplied from the Dertment of Microbiology, Faculty of Veterinary dicine, Cairo University, Egypt and King Fahd spital Specialist, Buridah, Saudi Arabia. The ified isolates were examined by different biomical test reactions. *Salmonella* was typed according to the tables of differentiation and identiation proposed by Krieg and Holt (1984), ile *Strptococcus* was typed biochemically according to tables of Sneath et al. (1986).

In vitro Antibacterial Activity:

The sensitivity of the aforementioned bacteria to the alcoholic and aqueous extracts was carried out *in vitro* by the agar diffusion sensitivity test using the bore method as described by Cooper and Woodman (1946). Different concentrations of the tested alcoholic extracts (10, 25, 100 mg/ml) were prepared in 10% aqueous solution of Tween 80 as a vehicle, while the lyophilized aqueous extracts were dissolved in distilled water.

MIC Determination:

For MIC determination, 50 ul of 2- fol dilutions of the active tested plant extracts concentration was added to each well using a microtitration broth-dilution technique according to Jones et al. (1985). One well in each row contained only

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Table (3): Antibacteria activity of medicinal plant extracts against certain Gram-negative bacteria (n=5) (Mean ± S.E.

Plant	Extract	Conc. mg/ml	Diameter of inhibitory zone (mm)					
			Salmonella group C	*E. coli*	*Pr. mirabilis*	*Ps. aeruginosa*	*H. influenza*	*Kl. Pneum*
Peganum harmala	Aq	10	-	-	-	-	10.4±0.25	-
		25	-	-	-	-	14.4±0.25	-
		50	-	-	-	-	19.2±0.37	-
		100	-	-	-	-	23.0±0.45	-
		200	-	-	-	-	27.0±0.00	-
Rhant. epapposum	Aq	100	10.4±0.25	-	-	-	11.6±0.20	-
		200	12.3±0.33	-	-	-	14.7±0.33	-
Cynomorium Coccineum	Aq (No. 5)	25	-	9.8±0.20	11.6±0.25	11.6±0.20	-	-
		50	10.4±0.25	11.4±0.20	13.6±0.40	13.4±0.40	11.6±0.20	-
		100	12.4±0.40	14.2±0.20	14.2±0.20	14.0±0.32	14.6±0.25	10.6±0
		200	15.8±0.37	14.4±0.40	15.0±0.32	15.0±0.32	18.2±0.40	14.2±0
	Alc (No. 6)	25	-	-	-	-	11.4±0.20	-
		50	-	-	-	-	14.8±0.37	-
		100	10.4±0.25	-	-	11.6±0.20	18.2±0.37	12.0±0
		200	15.2±0.20	15.8±0.37	15.2±0.20	15.8±0.37	22.0±0.00	14.8±0
	Alc (No.7)	25	-	-	-	-	10.4±0.25	-
		50	-	-	-	-	13.6±0.40	-
		100	13.4±0.40	11.40±0.20	12.4±0.40	11.4 ±0.23	16.8±0.20	10.6±0
		200	15.8±0.37	15.4±0.25	16.0±0.32	15.2±0.20	21.0±0.00	13.6±0
Withania somnifera	Alc	25	-	-	-	-	11.6±0.20	-
		50	-		-	-	17.0±0.00	-
		100	-	-	-	-	21.0±0.00	-
		200	-	-	-	-	25.8±0.37	-
	Aq.	25	-	-	-	-	11.4±0.23	-
		50	-	-	-	-	14.4±0.25	-
		100	-	-	-	-	19.2±0.37	-
		200	-	-	-	-	24.0±0.00	-
Rhazya stricta	Aq.	10	-	-	-	-	15.2±0.20	-
		25	-	-	-	-	19.2±0.37	-
		50	-	-	-	-	23.0±0.45	-
		100	-	-	-	-	29.2±0.37	-
		200	-	-	-	-	36.0±0.00	-
Centaurea bruguierana	Aq.	10	-	-	-	-	13.6±0.40	-
		25	11.4±0.20	-	10.4±0.25	-	15.0±0.00	-
		50	14.8±0.37	10.8±0.49	14.4±0.25	10.4±0.25	19.4±0.40	10.4±0
		100	18.4±0.40	13.6±0.40	19.2±0.37	14.0±0.45	26.4±0.25	13.6±0
		200	22.4±0.40	16.0±0.45	23.0±0.45	17.2±0.37	30.0±0.00	16.8±0

- = No. effect

Mueller-Hinton broth as an inculation and growth control. Bacteria were grown overnight and then were diluted in fresh Mueller-Hinton broth to a density of approximately 10^8 colony-forming units (CFU)/1 ml, this suspension was further diluted to 10^5 CFU/ml in Mueller-Hinton broth. A semiautomatic inoculator was used deliver 50 ul of the 10^5 CFU/ml suspension to each well containing 50 ul of tested extract dilution or broth. The MIC was calculated for each isolate as the lowest concentration of tested plant extract which no visible growth was observed after hours of incubation at 37°C.

In vitro Antifungal Activity:

This was carried out for revealing the effect coholic and aqueous extracts of some plant the tested fungi and yeasts as explained by

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Table (4): Minimum inhibitory concentration (MIC) of medicinal plant extracts against certain Gram-positive bacteria.

Plant	Extract	Minimum inhibitory concentration (mg/ml) Staph. aureus	Strept group B	Strept. group D	Staph. aureus Meth. Resist.	Bacillus subtilis
Rhanterium epapposum	Aq	-	-	-	-	99.05
	Alc	-	-	-	-	99.24
Cynomorium coccineum	Alc (No. 5)	23.22	23.53	49.86	49.86	99.29
	Alc (No. 6)	99.27	99.17	48.59	23.86	99.31
	Alc (No.7)	98.92	98.75	48.66	23.84	99.99
Withania somnifera	Aq	-	199.99	-	23.81	-
Centaura bruguierana	Aq	-	-	-	23.84	49.05

- = No. effect

Table (5): Minimum inhibitory concentration (MIC) of medicial plant extracts against certain Gram-positive bacteria.

Plant	Extract	Minimum inhibitory concentration (MIC) (mg/ml) *Salmonella* group C	*E. coli*	*Pr. mirabilis*	*Ps. aeruginosa*	*H. influenza*	*Kl. Pneumoniae*
Peganum harmala	Aq	-	-	-	-	8.47	-
Rhant epapposum	Alc.	98.94	-	-	-	99.20	-
Cynomorium coccineum	Alc (No. 5)	48.94	23.32	22.75	22.75	49.99	99.32
	Alc (No. 5)	99.43	199.97	199.95	99.35	23.77	99.12
	Alc (No. 7)	98.93	99.99	99.25	99.31	23.80	99.23
Withania somnifera	Aq	-	-	-	-	23.87	-
	Aq	-	-	-	-	23.83	-
Rhazya stricta	Aq	-	-	-	-	8.42	-
Centaura bruguierana	Aq	23.78	48.78	23.86	49.05	8.36	49.02

- = No. effect

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Table (6): Antifungal activity of some medicinal plant extracts against some fungiand yeasts (n-5) (mean ± S.E).

Plant	Extract	Conc. (mg/ml)	Diameter of inhibitory zone (mm)						
			Candida albicans	*Cryptococcus neoformans*	*Trichophyton mentagrophytes*	*Microsporum canis*	*Aspergillus niger*	*Aspergillus fumigatus*	*Penicillium notatum*
Rhanterium epapposum	Aq	200	-	-	13.4±0.40	-	-	-	-
Astragalus spinosus	Aq	200	-	-	12.00±0.45	11.4±0.25	-	10.8±0.20	-
Tribulus terrestris	Aq	200	-	-	12.4±0.25	12.6±0.25	11.4±0.05	13.6±0.40	12.0±0.4
Heliotropium bacciferum	Aq	100 200	- -	- -	13.6±0.40 15.4±0.25	- -	- -	- -	- -
Chrozophora verbascifolia	Aq	200	-	-	12.8±0.37	-	-	13.6±0.25	-
Calligonum comosum	Aq	200	-	-	14.4±0.40	-	-	-	-
Cynomorium Coccineum	Alc (No. 5)	200	10.6±0.25	11.4±0.25	-	-	-	-	-
Cleome amblyocarpa	Aq	200	-	-	12.6±0.25	-	-	-	-

- = No. effect

and Lamb (1983). Different concentrations of the tested extracts were used as previously mentioned under antibacterial activity test.

RESULTS AND DISCUSSION

The antimicrobial activity of each plant extract was studied at the concentrations of 10, 25, 50, 100 and 200 mg/ml. Only those concentrations, which showed activity are reported in Tables (2, 3, 4, 5 and 6).

The alcoholic extract of peels of flower of *C. coccineum* at concentrations higher than (25 and 50) and (50 and 25) mg/ml inhibit (*Staph. aureus* and *Strept.* groups B and D) and *Salm.* group C and *E. Coli*), respectively (Tables 2 & 3). However, B. Subtilis and Kl. pneumoniae were less sensitive to the exract.

The alcoholic extract of peels of stems & and inner pulp of *C. coccineum* at concentra higher than 25 mg/ml highly inhibit *Staph au* methicillin resistant (Table 2) *H. influe* showed high sensitivity towards the alcoholi tracts of peels of flower, peels of stems & and inner pulp of *C. coccineum* at concentra higher than 50,25 and 25 mg/ml, respecti Moreover *E. coli, Pr. mirabilis* and *Ps. aerug* were moderatly sensitive to the alcoholic e of peels of flower of *C. coccineum* at conc tions higher than 25 mg/ml (Table 3).

The aqueous extract of *W. somnifera* at conc tions higher than 25 mg/ml inhibit *Staph. a* methicillin resistant and *H. influenza* (Tabl 3).

Moreover, the aqueous extract of *C. bruguie* at concentrations higher than 25, 25, 50, 1 25 mg/ml inhibit *Staph. aureus* methicillin

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ant (Table 2), *Salm*, group c, *E. coli*, *H. influenza* and *Pr. mirabilis* (Table 3), respectively. These findings are in agreement with those reported by Al-Yahya et al. (1983), who found that, *Centraurea schimperi* has a higher antibacterial activity against *E. coli*.

The antibacterial activity of *C. conccineum*. *W. somnifera*, *C. bruguierans*, *P. harmala*, *R. epapposum* and *R.stricta* may be due to their main content of (anthocyanins) (alkaloids & withanolids); (flavonoids & sesquiterpene lactones); (alkaloids); (flavonoids & coumarins) and (alkaloids & flavonoids), respectively, Similar results are reported by Zaki et al. (1984), who found that flavones and alkaloids of Arterisia and anthraquinoes of *Calligonum* were the most effective antimicrobial compounds.

The MIC for the active plant extract towards the selected Gram-positive bacteria are showen in Table (4). The values of MIC for *C.bruguierana*. *R. stricta*. *P.harmala*. *C. coccineum* (No. 6) and *W. somnifera* extracts against *H. influenza* were 8.36, 8.42, 8.47, 23.77 and 23.87 mg/ml, respectively (Table 5).

All the tested fungi and yeasts were not sensitive towards all the tested aqueous and alcoholic extrats except *T. mentagrophytes* which was slightly sensitive towards the aqueous extracts of *H. bacciferum*, *R. stricta*, *A. spinosus*, *T. terrestris* and *C. verbascifolia* (Table 6). These results were consistent with those reported by Al-Meshal et al. (1982) and Al-Yahya et al. (1983). They recorded tha, *Capparis spinosa*, *Chrozophora obliqua*, *Cleome africana*, *Artermisia inculta*, *Centaurea schimperi* are not active against *C. albicans*. On the other hand, Salih and Nadir (1984) reported that *C. comosum* was effective against *Candida* spp.

The most interesting findings in this study is the high activity of *C. bruguierana*, *R. stricta*, *P. Harmala*, *C. conccineum* and W. somnifera extracts against *H. influenza*. In addition *C. coccineum* extracts showed moderate antibacterial activity against all tested bacteria.

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