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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (4): 630-632, 2000

Antibacterial and Antifungal Activities of *Launaea nudicaulis* (Roxb.) And *Launaea resedifolia* (Linn.)

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Abstract: Antibacterial and antifungal activities of some extracts of *Launaea nudicaulis* and *L. resedifolia* have been determined by standard methods. Methanol extract of *L. nudicaulis* and *L. resedifolia* showed 18.5 and 20.5 mm zones of inhibition against *B. subtilis*, respectively, as determined by disc diffusion method. Ethanol and DMSO extracts also exhibited antibacterial activities against *E. coli* and *S. aureus* but to lower degrees. When these activities were measured by well-method, all these extracts exhibited high activities against *K. pneumoniae* and low against *E. coli* compared with the control. Antifungal activity was determined by measuring the linear growth in slants on 4th day of incubation against an *Aspergilus* spp. Methanolic extracts of *L. nudicaulis* and *L. resedifolia* at 0.209 mg/ml levels exhibited 45 ± 6 mm and 37 ± 6 mm linear growth which decreased to 22 ± 5 mm and 28 ± 4 mm, respectively, at 0.838 mg/ml concentration.

Key Words: Antibacterial activity, Antifungal activity, *Launaea nudicaulis, Launaea resedifolia*

Introduction

Cholistan desert covers a large area of about 26,000 sq.km. It is situated between 27°, 42′ and 29°, 45′ north latitudes and 69°, 52′ and 75°, 24′ east latitudes. Cholistan desert is bound by Bahawalnagar on north-east, on the west side by Bahawalpur and eastern side by Great Indian desert. Although the vegetation of the area is scarce, some endemic plant species exist which are commonly used by local peoples of Cholistan desert against various diseases (Akram *et al*., 1991).

Launea nudicaulis (Roxb.) locally called Jangli booti is a medicinal plant. It's milky material is taken during the constipation. Leaves are used to relieve fever in children, in the treatment of itches of skin, cuts, ulcers, swellings, bilious fever, eczema eruptions and rheumatism. It's roots are used in toothache. *Launea resedifolia* (Linn) O.Kuntze occurs frequently in the bushes of *Calligonium polygonoides* on the top of sand dunes and inter-dunal areas and has medicinal properties (Bhandari, 1988; Baquar, 1989).

The compounds isolated and characterized from these two plants include taraxasterol and taraxeryl acetate (Prabhu and Venkateswarlu, 1969), glycoside comprised of xylose and a moiety of aglycon (Sharma *et al*., 1980), lupeol acetate, lupeol, e-sitosterol, e-sitosterol glucoside, luteolin, aprgenin-7-O-glucoside and luteolin-7-O-glucoside (Abdel-Salam *et al*., 1982), flavones from *L. nudicaulis* were apigenin-7-glucoside, 7-gentiobiosides, 7-rutinosides, 7,3′- diglucoside, 7,4′-diglucoside and 7-gentiobioside-4′-glucoside (Mansour *et al*., 1983), aesculetin, cichoriin and luteolin-7-O-glucoside from *L. nudicaulis* (Sarg *et al*., 1986), 3,4-dihydrocopoletin, esculetin, cichoriin, luteolin-7-O-glucoside, stigmasterol, beta-sitosterol, friedelin, lupeol and beta-sitosterol glucosides (Sarag *et al*., 1987), apigenin, dihydroxy coumarin, luteolin-7-O-glucoside and epigenin-5-O-glucoside from *L. resedifolia* (Saleh *et al.,* 1988), triterpens, alpha-amyrin, moretenol and lupeol, their acetate derivatives as well as their esters, Δ^{7}-stigmasterol and its 3-O-glucoside from *L. resedifolia* (Abd-el-Fattah *et al*., 1990) and luteolin, luteolin-7-O-glucosides, luteolin-7-O-rhamnoside, apigenin-7-O-glucoside, aesculetin and its 7-O-glucoside (cichoriin), ferulic acid and methyl caffeoate (Giner *et al*., 1992). In continuation of our objectives to explore plants of Cholistan desert for biological activities, the present studies were designed to demonstrate antibacterial and antifungal activities of the extracts of *L. nudicaulis* and *L. resedifolia*. This is a preliminary study and work is ongoing in the isolation and characterization of active components.

Materials and Methods

Young plants were collected from Cholistan desert for these studies. Fresh plants were air dried for more than a week and used for the extraction. Bacterial samples like *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* were obtained from HEJ Research Institute of Chemistry, University of Karachi. *Klebsiella pneumoniae* cultures were a kind gift from C.M.H., Bahawalpur. *Aspergilus* spp. was isolated in the laboratory and used without further characterization. Pre-prepared antibiotics discs were obtained from the manufacturers and used as positive controls.

70 g ground plant sample was taken in a soxhlet apparatus and extracted with methanol for 8-10 hours or was soaked in methanol for 8 days. Solvent was evaporated by rotary evaporator. The residue obtained was re-suspended in various solvents (methanol, ethanol, water and dimethylsulfoxide (DMSO)). Various grades of stock solutions were prepared and their biological activity determined. Antibacterial and antifungal activities were determined by standard methods as compiled by Farhana (1999).

Results and Discussion

Results show that maximal antibacterial activity, as performed by disc diffusion method, is exhibited by methanolic extract of *L. nudicaulis* especially against *B. subtilis*, i.e., 18.5 mm zone of inhibition compared with that of solvent, i.e., <7.0 mm (Table 1). This antibacterial activity is concentration dependent; that is, higher at 1 mg/disc and lower at 0.5mg/disc. Ethanol and DMSO extracts are also effective against the *B. subtilis* (10-14.5 mm zone of inhibition). DMSO at both these concentrations remained effective equally against the three species. The least effective extract proved again methanol extract against the *E. coli* which showed little activity (7.5-8.5 mm) compared with the control (<7 mm). Similar profiles have been recorded for *L. resedifolia* (Table 1).

When the same extracts were tested against *E. coli* and *K. pneumoniae* by well method, similar profiles were demonstrated (Table 2). Maximum zone of inhibition (20-22.5 mm) of ethanol extract was found against *K. pneumoniae* which remained un-tested in the disc diffusion method. For *K. pneumoniae* the control values were upto 25 mm zone of inhibition. Ethanol

Table 1: Antibacterial activity of some extracts of *L. nudicaulis* and *L. resedifolia* after 18-24 hours of incubation by disc diffusion method. Results are mean of 3 independent experiments. (n = 3, S.D.< ±3.5 mm)

Extract		*E. coli* (G-ve)	*B. subtilis* (G-ve)	*S. aureus* (G+ve)
L. nudicaulis				
DMSO extract	(1 mg/disc)	13.0	14.5	13.5
	(0.5 mg/disc)	11.5	12.5	11.5
Ethanol extract	(1 mg/disc)	14.5	14.0	11.5
	(0.5 mg/disc)	11.0	10.5	8.0
Methanol extract	(1 mg/disc)	8.5	18.5	11.5
	(0.5 mg/disc)	7.0	15.5	7.5
L. resedifolia				
DMSO extract	(1 mg/disc)	12.5	20.5	19.5
	(0.5 mg/disc)	9.5	12.5	10.5
Ethanol extract	(1 mg/disc)	10.5	16.5	9.5
	(0.5 mg/disc)	9.0	13.5	7.5
Methanol extract	(1 mg/disc)	14.5	16.5	13.5
	(0.5 mg/disc)	11.0	14.0	10.5
Controls				
Ofloxacin	(10 µg/disc)	19.0	18.5	20.0
Piperacin tazobactum	(10 µg/disc)	23.0	21.5	21.0
Augmentin	(10 µg/disc)	22.0	21.5	21.0
Solvents				
DMSO	(10 µl/disc)	<7.0	<7.0	<7.0
Ethanol	(10 µl/disc)	<7.0	<7.0	<7.0
Methanol	(10 µl/disc)	<7.0	<7.0	<7.0

Table 2: Antibacterial activity of some extracts of *L. nudicaulis* and *L. resedifolia* after 18-24 hours of incubation by well-method. Results are mean of 2 independent experiments. (n = 2, S.D.< ±4.5 mm)

Extract		*E. coli* (G-ve)	*K. pneumoniae* (G-ve)
L. nudicaulis			
1. DMSO extract	(1 mg/well)	14.5	20.0
	(0.5 mg/well)	7.5	17.5
2. Ethanol extract	(1 mg/well)	15.5	22.5
	(0.5 mg/well)	2.0	20.0
3. Methanol extract	(1 mg/well)	15.0	18.0
	(0.5 mg/well)	7.5	13.5
L. resedifolia			
1. DMSO extract	(1 mg/well)	12.5	22.0
	(0.5 mg/well)	10.5	20.5
2. Ethanol extract	(1 mg/well)	15.0	22.5
	(0.5 mg/well)	10.5	19.0
3. Methanol extract	(1 mg/well)	16.0	20.5
	(0.5 mg/well)	13.5	18.5
Control			
1. Ofloxacin	(10 µg/well)	19.0	25.5
2. Piperacin tazobactum	(10 µg/well)	23.0	24.0
3. Augmentin	(10 µg/well)	22.0	25.0
Solvents			
DMSO	(10 µl/well)	<6.5	<12.0
Ethanol	(10 µl/well)	<6.5	<12.0
Methanol	(10 µl/well)	<6.5	<12.0

Table 3: Antifungal activity of methanolic extracts of *L. nudicaulis* and *L. resedifolia* against *Aspergilus* spp. Gravison (known antifungal) dissolved in water acted as positive control(50mg/ml). Results are mean of 2-3 independent determinations. (n=2-3, ±S.D.)

Test sample			Linear Fungal Growth (mm)			
Stock soln. (%)	Dose (µl)	Conc. (mg/ml)	3rd day	4th day	3rd day	4th day
			L. nudicaulis		*L. resedifolia*	
1.25	67	0.209	42±7	45±6	35±6	37±6
	134	0.419	32±6	33±6	30±6	32±5
2.5	67	0.419	30±6	32±5	31±5	34±5
	134	0.838	20±6	22±5	26±6	28±4
Solvent	134	-	50±5	50±5	50±5	50±5
Control (Gravison)	-	50	Nil	Nil	Nil	Nil

extract of *L. nudicaulis* also proved effective against *E. coli* and 12-15.5 mm zone of inhibition was seen, whilst DMSO and methanol extracts remained least effective at lower concentrations. Similar findings have been recorded for *L. resedifolia.* This clearly demonstrated that all the extracts were effective against the tested bacteria. Nevertheless, both these methods presented a clear picture that crude extracts posessed antibacterial activity.

Antifungal activity was determined by the inhibition of linear fungal growth in the slants of *Aspergilus* spp (locally isolated but not further characterized) on 3rd and 4th day of incubation at 37±2°C (Table 3). The standard antifungal drug Gravison at 50mg/ml levels inhibited 100 percent growth. Methanol extracts of *L. nudicaulis* and *L. resedifolia* at different concentrations inhibited fungal growth; 50 percent fungal growth inhibition has been observed by *L. nudicaulis* at 0.209 mg/ml and 0.838 mg/ml concentrations, from 45±6 to 22±5 mm, respectively, compared with the solvent (Table 3). Similar profiles have been observed for *L. resedifolia* wherein these inhibitory values are 37±6 (0.209 mg/ml) and 28±4 mm (0.838 mg/ml).

Similar results have been reported in the literature. Shabana *et al*. (1988) have determined the antibacterial and antifungal activity of 60 desert plants. Extracts of plant species belonging to 23 families were tested on 5 species of bacteria (*Staphylococcus aureus, Streptococus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and three species of fungi (*Microsporum gypseum, Aspergilus niger, Candida albicans*). Of these plants, 43 species were active against one or more microorganisms and 7 species showed a broad spectrum of activity. Extracts of 3 plants showed significant effects on *P. aeroginosa* which was resistant to common antibiotics and 5 plants were effective against *M. gypseum*.

In summary, the present study reveals some useful information about the biological activity of extracts of *L. nudicaulis* and *L. resedifolia*. Further work is suggested on the active components be isolated and their structure determined. Work is in progress on these aspects.

Acknowledgment

Thanks are due to Dr. M. Arshad, CIDS (Cholistan Institute of Desert Studies, Bahawalpur), for plant samples and colleagues in HEJ Research Institute of Chemistry, Karachi, for encouragement and providing training in these assays during the 2nd National Workshop on Bioassays.

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