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ANTIMICROBIAL ACTIVITIES OF SOME MEDICINAL TREE SPECIES OF HANUMANGARH DISTRICT OF RAJASTHAN

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Hanumangarh district is rich in medicinal tree species. Medicinal tree species like *Albizzia lebbeck, Moringa oleifera* and *Pongamia pinnata* were screened for their antimicrobial activities. Ethyl ether and alcoholic extracts of leaves of all these selected tree species showed positive reactions against bacterial pathogens i.e. *Staphylococcus aureus*, and *Escherichia coli* and fungal pathogen *Candida albicans*. The leaves of these selected tree species were analysed for flavonoid contents i.e. Quercetin and Kaempferol. Flavonoid contents like Quercetin and Kaempferol were isolated and identified. As antimicrobial principles they showed antimicrobial activities against all the test pathogens. The maximum total flavonoid contents (4.90 mg./gdw) was found in leaves of *Moringa oleifera* while minimum (2.40mg./gdw) in leaves of *Albizzia lebbeck*.

Keywords: Antimicrobial activities; Flavonoids; Hanumangarh district; Kaempferol; Medicinal tree species; Quercetin.

Hanumangarh district, a part of semi-arid region of northern Rajasthan, is rich in medicinal tree species. This region exhibits a great variety of geological, physiographical, climatic, edaphic and biotic conditions and represents diversity of medicinal tree species, which occur on a wide range of habitat. These medicinal tree species are source of phytochemicals of pharmaceutical interest such as flavonoids, sterols, alkaloids, phenolic compounds, sulphides, isothiocyanates, anthocynins, terpenoids etc. These are the active principles which act as antioxidants, anticarcinogenic, antimicrobials and immunity stimulants. A number of tree species have been screened for their antimicrobial activities and evaluation of antimicrobial principles[1-6].

Tree species from Hanumangarh district like *Albizzia lebbeck, Moringa oleifera* and *Pongamia pinnata* were screened for their antimicrobial properties. Fresh leaves of all the selected medicinal tree species were collected from Junction area and pulverized into a paste. Cold extraction was done by blending the paste with ethyl ether and 50% ethanol in the ratio of 1 : 2, in a Waring Blender at 2500 rpm for 10 min. The mixture was centrifuged at 3000 rpm. The supernatant was evaporated to dryness and the residue was suspended in double distilled water. The micro-organisms used for screening were *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative) and *Candida albicans* (Fungal pathogen). The growth medium used for *Staphylococcus aureus* and *Escherichia coli* was Nutrient broth (10% peptone, 0.5% labanco and 0.5% NaCl, pH adjusted to 7.5) and for *Candida albicans* Sabourands liquid medium (1% peptone, 4% glucose, pH adjusted to 5.8). Paper discs of known concentration of standard antibiotics namely chloramphenicol, penicillin and mycostatin were used for comparison. Blank paper discs were used as control. Control discs dipped in ethyl ether and 50% ethanol, plates (5 each for *Staphylococcus aureus, Escherichia coli* and *Candida albicans*) were employed for each extract. The ratio of inhibition zone of various test samples was compared with the inhibition zone from the high concentration antibiotic reference discs.

Extraction of Flavonoid Contents (Antimicrobial principles)- Dried and powdered leaves of all the selected medicinal tree species were separately soxhlet extracted with 80% hot ethanol[7] on a water bath for 24 hrs. Each of the extracts was concentrated and concentrate re-extracted with petroleum ether (Fraction-I), ether (Fraction-II) and ethyl acetate (Fraction-III) in succession. Fraction-III was dried *in vacuo* and the resultant was hydrolysed with 7% H_2SO_4 for 2 hrs. The mixture was filtered and the filterate extracted with ethyl acetate. Concentrated ether and ethyl acetate fraction were applied on TLC plates along with standard reference compounds and the plates developed with the solvent system n-butanol, acetic acid and water (4:1:5) when kaempferol and quercetin were detected. The compounds were isolated by preparative TLC and crystallized, mp (quercetin 309°-311°C and kaempferol 271°- 273° C). IR spectra compared well with their authentic samples. Quantitative estimation of flavonoid contents was carried out by method of Kariyone *et al*[8]. and Naghski *et al*[9]. for quercetin and Mabry *et al.*[10] for kaempferol.

Antimicrobial activities of all the selected tree species is given in table 1. The present study indicates that ethyl ether and alcoholic extracts of leaves of *Albizzia lebbeck, Moringa oleifera* and *Pongamia pinnata* show

Table 1. Antimicrobial activity of leaf extracts of selected medicinal tree species and reference antibiotics.

Plants	Extract	Test Organisms				
		S. aureus		*E. coli*		*C. albicans*
		I/C[a]	I/P[a]	I/C[a]	I/S[a]	I/M[a]
Albizzia lebbeck	Ether	0.89	0.83	1.09	1.71	0.43
	Alcoholic	0.78	0.69	0.90	1.43	0.58
Moringa oleifera	Ether	0.87	0.46	0.82	1.23	0.87
	Alcoholic	0.48	0.31	0.68	1.30	0.33
Pongamia pinnata	Ether	0.64	0.48	0.53	0.47	0.60
	Alcoholic	0.51	0.43	0.75	0.38	0.35

a= Ratio of diameters of the inhibition zone to leaf extracts (10µg) under observation (I) and diameter of inhibition zone due to standard reference antibiotics; C= Chloramphenicol (30µg) against *S. aureus* = 30 mm and *E. coli* 32 mm; P= Penicillin (10 units) against *S. aureus* =32 mm; S= Streptomycin (10µg) against *E. coli* =20 mm; M = Mycostatin (100 units) against *C. albicans* = 32 mm.

antimicrobial activity against all the test organisms. Thus, the activity of all these test extracts against both bacteria and fungal pathogen indicates that selected tree species are resistant to bacterial and fungal attacks due to the presence of some biologically active secondary products. Maximum antimicrobial activity was exhibited by the leaf extracts (Ethyl ether and alcoholic extract) of *Moringa oleifera* against *Candida albicans* where as leaf extracts of *Albizzia lebbeck* showed maximum activity against *Staphylococcus aureus* and *Escherichia coli*.

Table 2. Flavonoid contents (mg/gdw) from leaves of selected medicinal tree species.

Plants	Quercetin	Kaempferol	Total contents
Albizzia lebbeck	1.10	1.30	2.40
Moringa oleifera	1.90	3.00	4.90
Pongamia pinnata	2.50	2.00	4.50

The present investigation shows (Table 2) that among all the tree samples tested the total flavonoid contents were found to be maximum (4.90mg/gdw) in leaves of *Moringa oleifera* while minimum (2.40mg/gdw) in *Albizzia lebbeck*.

The maximum quercetin (2.50 mg/gdw) was found in leaves of *Pongamia pinnata*, while minimum (1.10 mg/gdw) in *Albizzia lebbeck*. The maximum amount of kaempferol (3.00 mg/gdw) was found in leaves of *Moringa oleifera*, while minimum (1.30 mg/gdw) in *Albizzia lebbeck*.

The medicinal tree species, under study are a potential source of antimicrobial principles. These are resistant to bacterial and fungal attacks due to presence of biologically active substances i.e. antimicrobial principles. These retain potentialities to synthesize the flavonoid contents which are active principles against bacterial as well as fungal pathogens. Due to presence of these secondary products the selected medicinal tree species can be used in drug and pharmaceutical industries.

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