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RESEARCH ARTICLE

Antibacterial and antioxidant activity of medicinal plants

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SUMMARY

In the present study, three plants were screened for potential antibacterial and antioxidant activity.The plants screened were *Rosa indica*, *Azadirachta indica* and *Moringa oleifera* which are traditionally used in India to treat various diseases. In evaluating the antibacterial activity two organic solvents were used *i.e.* acetone and ethanol to extract the antimicrobial components. Antibacterial activity was tested against two Gram positive *i.e. Staphylococcus aureus*, *Bacillus pumulis* and two gram negative *i.e. Klebsiella pneumonia*, *Escherichia coli* bacterial strains. The antibacterial nature of extracts was assessed by agar well diffusion method. The ethanolic extracts of *Rosa indica* petals were found to be most effective against all the pathogens used. The MIC was determined only for ethanolic extracts and found to be ranging between 0.025mg /ml to 33.33mg/ml. In evaluating antioxidant activity, all three plants were screened for total phenols, flavonoids and free radical scavenging activity. Free radical scavenging activity was evaluated using DPPH. Significant differences in DPPH scavenging activity were found between the species investigated ranging from 74.72 per cent to 83.40 per cent. The total phenol content of the investigated species ranged from 74 to 96 mg CE/g extract while flavonoid content ranged from 39 to 52 mg QE/g extract. In addition photosynthetic pigments (Chl A, Chl B, and carotene) were also determined for all three plants under study.

Key Words : Antimicrobial, Antioxidant, Antibacterial, Photosynthetic

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Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25 per cent of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80 per cent. Rose has influenced cultures aesthetically, economically, medically, religiously and spiritually since humankind could smell and appreciate its fragrance. *Moringa oleifera* is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. In developing countries, *Moringa* has potential to improve nutrition, boost food security, foster rural development, and support sustainable landcare. It may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic and possible adjuvant. *Azadirachta indica* is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to India and Pakistan growing in tropical and semi-tropical regions. Its fruits and seeds are the source of neem oil.*Bacillus subtilis*, is a Gram - positive, catalase- positive bacterium*K. pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines. *Staphylococcus aureus*, it is a facultative anaerobic Gram-positive coccal bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated

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that 20 per cent of the human population are long-term carriers. *Escherichia coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.

So, all the above mentioned medicinal plants and microorganisms are taken into consideration for completion of the dissertation.

MATERIALS AND METHODS

The fresh leaves and petals were collected from the Nursery of School of Forestry and Environment, SHIATS, Allahabad which were surface sterilized simply by washing under tap water and distilled water and dried in shed for 20 days. After drying, leaves and petals were grounded in a grinder mixer to powdered form and stored for further use.The four pre isolated bacterial cultures were collected from Microbial Culture Collection Bank, SHIATS. These cultures were sub cultured on NA slants and stored at 4 ^{0}C till use.Plant extracts were prepared using two organic solvents, *viz.*, ethanol (70%) and acetone. Minimum inhibitory concentration (MIC) of ethanolic plant extracts against the four pathogenic bacteria was determined (Busani *et al*., 2012). Photosynthetic pigments *i.e.* chlorophyll A, B and total carotenoids in the leaves and petals of medicinal plants were estimated (Saric *et al*., 1976). Free radical scavenging activity was evaluated using L-ascorbic as standard antioxidant using the stable radical DPPH method (Chan *et al.*, 2007).Total phenolic content(Bray *et al.*, 1954) and total flavonoid content(Morena *et al*., 2000) were determined.

RESULTS AND DISCUSSION

The salient features of the research as observed have been summarized here under:-

The antibacterial activity of *Azadirachta indica* leaves is maximum 28.0 ± 1.0 mm against, *S. aureus* for ethanolic extract, while 25.0 ± 1.0 mm against *Bacillus pumulis* for acetone extract and minimum 14.0 ± 1.0 mm against *K. pneumoniae* both for acetone and ethanolic extract.The antibacterial activity of *Rosa indica*is maximum 21.0 ± 1.0 and 24.0 ± 1.0 mm against *Bacillus pumulis* and minimum 14.0 ± 0.0 and 17.0 ± 0.0 mm against *S. aureus*, both for Acetone and Ethanolic extract, respectively.The antibacterial activity of *Moringa oleifera* leaves is maximum 10.0 ± 1.0 and 15.0 ± 1.0 mm against *K. pneumoniae* both for acetone and ethanolic extract, respectively, while it showed no activity against *E. coli*.Variable concentrations of ethanolic extract of plant samples were effective against various pathogenic bacteria. The least effective concentrations of ethanolic extract of *Rosa indica* were found to be 0.925 mg/ml against *S. aureus* and *K. pneumoniae*,0.154 mg/ml against *E. coli*,0.025 mg/ml against *B. pumulis.* The least effective concentrations of ethanolic extract of *Azadirachtaindica* leaves were found to be 0.925 mg/ml against *S. aureus*,5.55 mg/ml against *E. coli*,0.025 mg/ml against *B. pumulis* and only higher concentration 33.33 mg/ml is effective against *K. pneumoniae*.The least effective concentration of ethanolic extract of *Moringa oleifera* leaves was found to be 5.55 mg/ml against *S. aureus*, while only higher concentrations 33.33 mg/ml are effective against *E. coli, B. pumulis and K. pneumoniae.*Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light. In this study, acetone extracts of *Rosa indica*, *Azadirachta indica* and *Moringaoleifera* leaves were analysed to determine chlorophyll A and B content(Table 1).

Carotenes contribute to photosynthesis by transmitting the light energy they absorb from chlorophyll and also protect plant tissues by helping to absorb the energy from singlet oxygen, an excited form of the oxygen molecule O_2 which is formed during photosynthesis.In this study, acetone extracts of *Rosa indica* petals, *Azadirachta indica* leaves and *Moringa oleifera* leaves were analysed to determine carotene content (Table 2).

Table 1:Comparative analysis of chlorophyll A and B content in the acetone extracts of various plants under study

Plant material	Plant part used	O.D. at 662 nm	O.D. at 644 nm	Value of X		Chlorophyll content (mg/g dry weight)	
				Chl A (X_1)	ChlB (X_2)	Chl A	Chl B
A. indica	Leaves	1.046	0.430	9.808	4.349	0.490	0.217
M. oleifera	Leaves	1.732	1.072	15.884	14.743	0.794	0.737
Rosa indica	Petals	0.128	0.175	1.079	3.154	0.054	0.157

Chl A=Chlorophyll A, Chl B=Chlorophyll B

Table 2 : Comparative analysis of carotene content in the acetone extracts of various plants under study

Plant material	Plant part used	O.D. at 440 nm	Value of X	Carotene content (mg/g dry weight)
A.indica	Leaves	1.333	2.464	0.123
M.oleifera	Leaves	2.686	4.402	0.220
Rosa indica	Petals	0.392	0.706	0.035

Volume of acetone=10 ml, Weight of sample=200 mg

DPPH scavenging activity was found maximum at 1.0 mg/ml concentration of ethanolic extract of *Rosa indica* Petals *i.e.* 83.40 per cent, which was quiet nearer to the standard we used *i.e.* L-Ascorbic acid while at the same concentration it was found to be 74.72 per cent and 75.85 per cent for *Azadirachta indica* leaves and *Moringa oleifera* leaves, respectively (Fig. 1and Table 3).

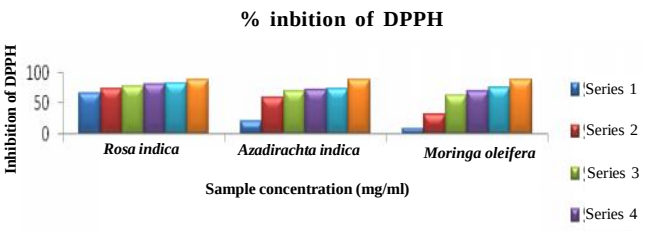

Fig.1: Comparative analysis of free radical scavenging activity of different plant materials at different concentrations

Highest concentrationof total phenols was 96 mg CE/g which was found to be present in the ethanolic extract of *Rosa indica* petals at 1.0 mg/ml concentration where as lowest *i.e.* 74 mg CE/g in ethanolic extract of *Azadirachta indica* leaves at the same concentration. While in case of ethanolic extract of *Moringa oleifera* leaves the total phenolic content was found to be 83 mg CE/g at the same concentration (Table 4).

Highest concentrationof total flavonoids was 52 mg QE/g present in the ethanolic extract of *Moringa oleifera* leaves at 1.0 mg/ml concentration, where as lowest *i.e.* 39 mg QE/g in ethanolic extract of *Rosa indica* petals at the same concentration. While in case of ethanolic extract of *Azadirachta indica* leaves, the total flavonoids content was found to be 42 mg QE/g (Table 5).

Table 3: Comparative analysis of free radical scavenging activity of different plant materials at different concentrations

Plant material	Material used	Concentration(mg/ml)	O.D. at 517nm	% inhibition of DPPH
L-Ascorbic acid	-	1.0	0.221	88.98
Rosa indica	Petals	0.2	0.658	67.19
		0.4	0.514	74.37
		0.6	0.432	78.46
		0.8	0.362	81.95
		1.0	0.333	83.40
Azadirachtaindica	Leaves	0.2	1.591	20.68
		0.4	0.822	59.02
		0.6	0.599	70.14
		0.8	0.545	72.83
		1.0	0.507	74.72
Moringaoleifera	Leaves	0.2	1.819	09.32
		0.4	1.357	32.35
		0.6	0.723	63.95
		0.8	0.600	70.09
		1.0	0.485	75.82

O.D. of control (DPPH in methanol) at 517 nm=2.006

Table 4: Total phenolic content of ethanolic extracts of various plant samples under study

Plant material	Plant part used	Concentration(mg/ml) of plant extract	O.D. at 650 nm	Total phenol (mg CE/g extract)
Rosa indica	Petals	1.0	0.687	96.00
A.indica	Leaves	1.0	0.534	74.00
M. oleifera	Leaves	1.0	0.622	83.00

Table 5: Total flavonoid content of ethanolic extracts of various plant samples under study

Plant material	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm	Total flavonoids (mg QE/g extract)
Rosa indica	Petals	1.0	0.443	39
A.indica	Leaves	1.0	0.468	42
M.oleifera	Leaves	1.0	0.586	52

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