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Antibacterial activity of ethanolic and aqueous crude extracts of *Lippia nodiflora* of Khairpur Mirus, Sindh, Pakistan

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Abstract

The ethanolic and aqueous extracts prepared from fruit, leaves and stem of *Lippia nodiflora* were examined against Gram positive and Gram negative bacteria; *Staphylococcus aureus, Bacillus subtilis,* Staphylacaccus saprophytious, Staphlococcus epidelmidis, Streptococcus faccolis, Streptococcus pyrgenes *Micrococcus luteus, Eschericihia coli and Bordetella pertussis.*

The ethanolic extracts of all parts stem, fruit and leaves were found active against test organisms, but fruit extract showed more activity than leaf and stem extract. The aqueous extract did not show significant activity.

Keyword: Antibacterial activity, *Lippia nodiflora*,t.

1. Introduction

The *Lippia nodiflora* is a perenial cruper of the family verbenaceae (Tyler *et al.,* 1988). It has world wide distribution, including Indo-Pakistan sub continent. It mainly occurs in wet places,specially along the banks of canals and rivers (Saeed 1970). Locally in Sindh it is available in Larkana, Thatta, Mirpurkhas, Hyderabad and Karachi. It has different names in different languages as in (English) Matlippia, (Gujrati) Ratoliya, (Punjabi) Bakan (Sanskrit) Agrijuala, Bakan and in (Sindhi) called Bukan or Waken. It is used as folk medicine. A poultice composed of the fresh plant is a good medicine for boils and cervical glands. sance of leaves and fruit and is eaten and is useful for treating irritation of internal piles. (Kirtikar and Besin 1984). It is also used se for pain of knee and joint (Chopra and Nayar 1986). Its mixed powder with seeds of *Cuminum cyminum* is used in the treatment of gonorrhea (Stanley *et al,* 1969). The stem decoction with *Leucas aspera* and roots of *Ocimum gratissimam* was used for malarial fever (Plus and Atal 1984). The extract of *L. noiflora* leaves, mixed with onion and ginger oil is used for the treatment of Alopecia (Ponnia and Saraja 1989). The ethanol extract is used as antiascariasis against earth worm (Kaleysa, 1975). The petroleum extract of *L. nodiflora* has been reported to lower blood sugar and serum cholesterol level (Ravishankar *et al.*, 1980). The plant infusion in Iran was used for gastric stimulant, diuretic and for healing wounds (Zargaria, 1992). The petroleum extract of whole plant reported to have hypotensive and cardiotonic effects (Farid, 1993). The plant leaves are used as antidote to snake venom and for treatment of snake bite (Nadkarini, 1954). Taking bath with water boiled with leaves gives relief from itching of measles and chicken pox (Manjunath, 1956). It is reported that, it contains sesquiterpens, 4.2%, o-propy l-4-10%, dimethyl bi-saboline 3.6%, candiene 4.2% calamenene 19.9%, Caryophyllene 18.7%, Copaene 8.4% , Monoterpenes 3.2%, Carvacerof 3.2%, 4-iso-propyl-methylcyclohexane 7.8%. Paracymen -8-10.6%, Linolol 13.7%, B-ocimene trace, b-pinene 8.1%.

The bacteria tested for the sensitivity are *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Bordetella pertussis Escherichia coli, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus faecalis and Streptococcus pyogenes.*

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Geneus *Staphylococcus* cell shape is spherical and arranged in bunches, it is non-sporing, aerobic and facultative anaerobic. It is a common pathogen, produces superlative diseases in human beings like boils, carbuncles, osteomylitis, deeper tissue abscesses, wound infection pneumonia, septicemia, food poisoning and gastroentritis (Vol. *et al.,* 1970) Genus Bacillus belongs to the family bacillaceae, it is spore forming, chemoorganotrophic, aerobic, rod shape gram positive, arranged inhain, motile and found in soil. e. g. *Bacillus subtilis*. It shows pellicle growth on surface of the liquid medium and produce ammonia smell, it is capable of producing bacitracin antibiotic (Robisher *et al.,* 1974).

Escherichia coli is Gram negative, rod shaped, non-spore forming bacteria. It is found in soil, water sewage and responsible of causing 80% urinary tract infection, gastroentritis and miscellaneous infection.

Genus Boretella is Gram negative cocco-bacilli,non sporing, inhabitant of respiratory tract. e. g. *Bordetella* pertussis, causing whooping cough (pertussis). Genus *Micrcoccus* is gram-positive, non motile, arranged in cluster, it is either saprophyte or parasite and broadly used in antibiotic assay e.g. Penicillin (Paul and Sainsbury *et al.,* 1981).

2. Material and Methods

Lippia nodiflora was collected from district Khairpur in the month of June. The leaves, fruit and stem were separated and were placed in the dark room at room temperature for 25 days to dry. The components of *Lippia noiflora* were extracted by crushing them separately into powdered form through hamer mill. Later 50g powder of every part (Fruit, leaf and stem) was put into the conical flask separately and 250 ml. of distilled water was added into each flask. These flask were kept 72h for maceration, finally the extract as filtered by Whattmans no.1 filter paper under vacuum evaporation by rotary evaporator at 40°C.The ethanolic extract was prepared in the same way where 250ml of ethanol absolute was poured instead of distilled water. The residual yield in shown in Table.1.

Table –1. The residual yield of leaf, Stem and Fruit

S. No	Part used	Wt. of dried powder	Solvent	Yield
01	leaf	50g	Ethyl alcohol i.Distilled water	3.3g 2.16g
02	Steam	50g	Ethyl alcohol i.Distilled water	3.0g 2.4g
03	Fruit	50g	Ethyl alcohol Distilled water	3.0g 2.4g

3. Preparation of dilutions

All the apparatus used were sterilized in the oven at 200°C for 30 minutes. A series of four dilutions were prepared for each extract and marked as n_1, n_2, n_3 and n_4. The dilutions were prepared in the following scheme.

a) First dilutions n1.was prepared by dissolving 100mg of extract in 10ml of ethyl alcohol.

b) Second dilution was prepared by taking one ml of dilution n^1.and 1ml of ethyl alcohol.

c) Third dilution n_3.was prepared by taking one ml of dilution n_2 and 1ml of ethyl alcohol.

d) Fourth dilution was prepared by using one ml of n_3 and one ml of ethyl alcohol.

After the preparation of extract and dilutions, the laboratory media e.g. Nutrient agar, Nutrient broth, MacConkeys agar and MacConkeys broth were prepared. The fresh inoclm of all organisms viz *Staphylococcus aures, Bacills subtilis, Micrococcs luteus, Escherichia coli* were prepared by inoculating them in fresh media plate an slants, incubated for 24h at 37°C. *Boretella pertussis* was incubated for 48-72h. Later, the slants were washed with 5ml of normal saline and suspension was prepared by inoculating it in to flask containing 100ml of cooled melted nutrient agar. This medium was poured in sterilized petri plates and was allowed to solidify. These were incubated at 37°C for 24h. After incubation antibacterial activity was determined following the method of Koneman *et al.,* (1997).

4. Result and discussion

Around three billion people of under developed countries are affilieted by bacterial infections (Walsh and Warren, 1979). As a result it becomes difficult to treat infectious diseases. Therefore, attempt has been made to study the antibacterial activity of *Lippia nodiflora* over the common pathogenic bacteria. The pathogenic bacteria were tested in vitro for their sensitivity to ethanol extract (Table:2).

According to the antibacterial activity exhibited by extract, it was divided in to four categories, A, B, C, D with E, representing control group. The result demonstrate that the extract from all test dilutions sowed maximum antibacterial activity against the organisms tested. It was observed that the fruit extract were more active against the test organisms relative to the extract of other parts. The present research finding provide a firm experimental evidence that the plant is useful in healing infections wounds, ulcers and scars, because it has shown antibacterial activity against, *Staphylococcus areus,* which common cause of skin infection. Our antibacterial results is an agreement with results of Foretier, *et al*, (1988) and Riaz, *et al* (1995). They showed very effective antibacterial activity results against the pathogenic bacteria. Further antibacterial activity needs more quantitative analysis for the identification of antibacterial chemical constituents.

Table-2. Antibacterial activity of stem, fruit and leaves by Ethanolic extract of *Lippia nodiflora* against pathogenic bacteria along with control.

The values in the table indicates the zone of inhibition in millimeters (mm)

Test microorganism	Average zone of inhibition (mm)				
	Dilution	Fruit	Leaf	Stem	Control
Staphylococcus aureus ATCC No. 6563	n_1	B	B	C	E
	n_2	B	C	C	E
	n_3	C	C	C	E
	n_4	C	D	D	E
Bacillus subtilis ATCC No. 6631	n_1	B	B	B	E
	n_2	B	C	B	E
	n_3	C	C	C	E
	n_4	C	D	C	E
Micrococcus luteus ATCC No. 6461	n_1	B	D	D	E
	n_2	C	D	D	E
	n_3	C	E	E	E
	n_4	C	E	E	E
Bordetella pertussis ATCC No. 4617	n_1	B	D	C	E
	n_2	B	D	C	E
	n_3	C	D	D	E
	n_4	C	D	D	E
Escherichia coli ATCC No. 8739	n_1	C	C	D	E
	n_2	C	D	D	E
	n_3	D	D	D	E
	n_4	D	D	D	E
Staphylococcus saprophyticus ATCC No. 25922	n_1	C	C	D	E
	n_2	C	C	D	E
	n_3	D	C	D	E
	n_4	D	C	D	E
Staphylococcus epidermidis ATTC No.	n_1	A	D	C	E
	n_2	A	C	C	E
	n_3	B	D	D	E
	n_4	B	C	D	E
Streptococcus fawcalis ATTC No. 10449	n_1	A	B	B	E
	n_2	A	B	B	E
	n_3	C	C	D	E
	n_4	B	C	D	E
Streptococcus pyogenes ATTC	n_1	A	C	D	E
	n_2	A	C	D	E
	n_3	A	C	D	E
	n_4	A	C	D	E

Key: A = 2630mm; B = 2225mm; C = 1519mm; D = 9214mm; E = below 99mm

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