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Antimicrobial activity of few regional Plants

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ABSTRACT

*The present work is aimed to screen and test the anti-microbial properties of active principles, extracted from a few of the plants commonly grown in various parts of Koppal locality. Modern scientific medicine has concentrated to a large extent on curative or system regulating medicines, many other medicines that are synthetics also duplicated the plant products chemically, but there is nothing as good and as pure as nature. Some of the medicinal plants are believed to cure practically every human ailment from head to toe; no doubt many mineral and animal products are used as medicines. But it is the plants and their products that contribute to a greater extent in the preparation of medicine (Hill, 1979). Well, the best of these herbal medicines have in fact been incorporated in modern medicines. There is much to explore from the herbs of medicinal value using our knowledge that has been perpetuated from generations. In the present study, the ethonolic extract of *E.officinalis* indicated the strong inhibition against *S.typhi*, *E.coli*, and *P.aeruginosa*. Indicating the antibacterial activity against the pathogenic organisms.*

Keywords— Antimicrobial activity, Medicinal plants, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli*.

1. INTRODUCTION

The knowledge of medicinal plants dates back perhaps to the origin of the human race; the ancient literature has references not only of plants reputed to cure difficult and incurable diseases but also of plants related with many beneficial properties.

In India, the references to the curative properties of some herbs seen in the Rigveda (3500- 1800 BC) seem to be the earliest records of use of plants in medicine, (Jain, 1968). compared to this more detailed account was found in Atharvaveda, after the Vedas, there was a gap of 1000 years. Then appeared work of Charaka and Susruta and since then the story goes on.

It is estimated that in India over 4,000 species of plants are distributed and among them, 2500 to 3000 species are in general use as medicines in some forms or the other. The Himalayas and Nilgiri hills are known to be the natural home for many medicinal plants. A good number of them are now being cultivated in different regions in our country (Dutta, 1982)

It has been estimated that out of about 2000 drugs used in curing human ailments in India, only about 200 are of animal origin and another about 200 are of mineral origin, the rest all about 1,500 drugs are of plant origin (Jain, 1968).

The question of subjecting medicinal herbs to the modern scientific test has been often raised, until about 50 years ago; few of our medicinal plants have been subjected for scientific testing and experimentation.

The Central Drug Research Institute, at Lucknow, is among the pioneer institutes in our country to carry out researches on indigenous medicinal plants and their products.

Some workers have contributed their might in screening and testing the medicinal properties of some of the plants in our country. Ray and Mujumdar (1977) have screened about 105 Indian herbs for the medicinal properties against pathogenic organisms. Ogunlana and Remstad in (1978) used 48 plant extracts against pathogenic bacteria. Dhawan et al. (1979) tested some Indian medicinal plants against bacteria. Similarly, the work is going on and some of the weeds are long been known to be used medicinally (Mukadam et al 1976, Dhawan et al, 1980, Sinha, 1985).

The present work is aimed to screen and test the anti-microbial properties of active principles, extracted from a few of the plants commonly grown in the Koppal locality.

2. SURVEY OF LITERATURE

The use of medicinal plants for antimicrobial screening has been prevalent for many years. Some of the works are quoted below Kalaw and Sacay (1925), Burkill (1719) and George et al (1947) showed alcoholic extracts of leaves and roots of *Phyllanthus niruri*, show antibacterial activity against E-Coli. George et al (1947), Burkill-II (1324) Kirtikar and Basu II (1078-79), showed that alcoholic extracts of henna leaves show mild activity against *Micrococcus pyrogenes*, *V.aureus* and *Ecoli*. Dhar et.at; 1968, have shown that the whole plant of *Hemidesmus indicus* has antimicrobial activity against a few bacteria, fungi and protozoa. Geobios (1978) proved the antifungal activity of plant extract of *Asparagus racemosa*. Similarly, the bark of *A.racemosa* has both anti backs. and antifungal activity (wealth of India 1945, IA, 471). Prasad et al (1983) mentioned that *H. indicus* roots extracted with petroleum ether, chloroform and alcohol have the antibacterial activity against *Staphylococcus aureus*, *Staphylococcus albus*, *Salmonella typhosa*, *Vibrio cholerae*, *Ecoli*, *Shigella shigar*, *Shigella flexneri* and *Shigella sonnei*. V.K. Sharm, 1990, reported tuberculastic activity of henna leaves. M Maharkar and cacus showed the bacterial action of *Gymnema sylvester* in vitro condition.

Perez. C and Anesini, C: 1994, Boiling water extracts of 54 plant families of Argentine folk medicine, were screened for antibacterial activity against *Salmonella typhi*, the agar-well diffusion method was used, 24 species showed anti bact. activity, among them *Cassia occidentalis* roots, *Heimia salicifolia* arial parts, *Punica granatum* fruit pericarp and *Rosa borboniana* flowers produced some of the more effective extracts.

Kim-B.W, et al, 1994; Methonol extracts of 6 plants, *Alchornea cardifolia*, *Bridelia ferrugi inea*, *Eucalyptus citriodora*, *Hymenocardia acids*, *Maprounea Africana* and *Sida rhombifolia*, demonstrated a marked antibacterial activity against the microorganisms *Klebsiella pneumonlae*, *Staphylococcus aureus* and *S. mutans* and extracts of four plants *A. cardifolia*, *E. citriodora*, *M. africana* and *S. rhombifolia* exhibited significant antifungal activity against *Asperaillus niger*, *Candida albicans* and *microsporum gypseum*.

Linuma M. et al 1994; from the root of *Qsmosia monosperma* among 10 Isoflavonoids, 2, 3, - Dihydroauriculatin showed moderate activities against oral microbial organisms *Streptococcus mutans*, *Prophyromonas gingivalis* and *Actinomyces*. Locher C.P. et al 1995, *Aleurites moluccana* extracts showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Pipturus albidus* and *Eugenia malaccensis* extracts showed growth inhibition of *S aureus* and *P. uginosa*, *Psycholria hawaiiensis*, *Solanum niger* inhibited growth of fungi *Microsporum canis*, *Trichophyton rubrum* and *Epidermophyton floccosum*, while *Ipomea species*, *Pipturus albidus*, *Scaevola sericea*, *Eugenia malaccensis*, *Piper methysticum*, *Barringtonia asiatica* and *Adonsonia digitata* extracts showed anti-fungal activity to lesser extent. Manandhar N.P, et al 1969: Methnol extracts of 20 plant species assayed against 11 strains of bacteria and 4 strains of fungi under duplicate assay of with and without exposure to ultra violet radiation. Two plant extracts were effective to treat diarrhoea and dysentery, were the bark from *Terminalia alata* and *Mallotus phillppensis*.

3. MATERIAL AND METHODS

3.1 Collection and Identification of Plants

In any investigation of medicinal plants, their botanical identification forms a very crucial and basic part of the work.

For the present study, *Gymnema sylvester* was collected near, S.G. College area in Koppal, roots of *Asparagus racemosus* and dried good fruits of *Embica officinalis*. Were brought from the market in Gulbarga, and other plants wildly grown were collected from Gulbarga University Campus.

The plants were identified with the help of flora of presidency of Madras by J.S. Gamble (1935), Flora of Karnataka by Cecil, J. Saldanha (1980) and Flora of Hassan District, Karnataka, India by Cecil, J.Saldanha and Dan H. Nicolson (1976). The Voucher specimens of all the ten species are deposited in the Herbarium of Gulbarga University, Gulbarga.

Table 1: Plants used for the study

Plant	Name of the Plant	Family
A	<i>Emblica officinalis</i>	Euphorbiaceae
B	<i>Asparagus racemosus</i>	Liliaceae
C	<i>Gymnema sylvester</i>	Asclepiadaceae
D	<i>Acalypha indica</i>	Euphorbiaceae
E	<i>Euphorbia hirta</i>	Euphorbiaceae
F	<i>Atylosia scarabaeoides</i>	Leguminosae
G	<i>Hemidesmus indicus</i>	Asclepiadaceae
H	<i>Phyllanthus niruri</i>	Euphorbiaceae
I	<i>Lawsonia inermis</i>	Lythraceae
J	<i>Tribulus Terrestris</i>	Zygophyllaceae

3.2 Introduction about the Microorganisms used for testing

The antibacterial activity of all 10 plant extracts was carried out against the pathogenic bacteria namely *Staphylococcus aureus*, *Salmonelatyphi*, *Pseudomonas aeruginosa* and *E.coli*.

Among these selected bacteria only *S.aureus* is gram +Ve and all the three bacteria are gram-Ve. Lt is found experimentally that gram +Ve bacteria are less pathogenic to man, hence gram-Ve are given more preference here in the present study.

3.2.1 Staphylococcus aureus: They are spherical cocci, approximately 1 micrometre in diameter, arranged in grape-like clusters. They may also be found singly, in pairs and in short chains. They are non-motile, non-spore former and non-capsulated and are uniformly gram +Ve. Most strains produce golden yellow pigment though some may be white or yellow.

Pathogenicity in man

Local infections which are limited to the skin and subcutaneous tissue causes boils, syscosis barbae, pustular acne, localized abscesses cellulitis, etc. When generalized they may cause septicemia, acute osteomyelitis and pyaemic abscesses.

3.2.2 Salmonella typhi: These are gram-ve, rod-shaped, non-sporing bacilli 2.5 by 0.5 μ , actively motive with peritrichate flagella. These are aerobes or facultative anaerobes. The colonies are smooth, colourless, translucent and pale. The colonies are round about 2mm is the diameter.

Pathogenicity in man: Typhoid fever. (Found in the blood; bacteremia). Lymphoid follicles of the small intestine in faeces. Mesenteric lump nodes and Bronchopneumonia and empyema.

3.2.3 Pseudomonas aeruginosa: These are gram -ve rods, actively motile, non sporing organisms, 1.5-3 μ by 0.5 μ , with rounded ends and bipolar flagella. They occur singly or in pairs or in short chains. They grow in ordinary media under anaerobic conditions. In the broth abundant growth occurs with uniform turbidity and the liquid is bluish green in colour. They produce bluish green pigments.

Pathogenicity in man

Occurs as commensal in the intestine of man and animals, when the defensive mechanism of the body is poor, produces suppurative wounds, otitis media, peritonitis, cystitis, bronchopneumonia and empyema. In children causes diarrhea and septicemia.

3.2.4 Escherichia coli: It is a gram-ve, straight rod, measuring 1-3 μ m x 0.4-0.7 μ m arranged singly or in pairs. It is motile though some strains may be non-motile, capsules are found in some stains, spores are not formed and colonies are large, thick, greyish white.

Pathogenicity in man

It causes urinary tract infection, Diarrhea or gastroenteritis, pyogenic infection and Septicemia.

3.3 Antibacterial activity

Antibacterial activity of ethol extracts of 10 plant materials mentioned in the earlier parts was assessed against E coil, Salmonella typhi, P.aeruginosa and Staphylococcus aureus, by the cup -plate method. The following materials were used:-Medium A as described in Indian pharmacopoeia. Sterilized petridishes, pipettes of 0.1 to 0.2ml.Cultures and nutrient broth; and Sterilized test tubes containing solutions of extracts of known concentration.

3.3.1 Preparation of media: Medium A of Indian pharmacopoeia was prepared by dissolving bacteriological peptone (6.0 gms). Yeast extract (3.0 gms), beef extract (1.5 gms). Pancreatic digest of casein (4.0 gms), dextrose (1,0 gms)and agar (15.0 gms) in distilled water to produce 1 liter of medium. Then it was sterilized for 30min at 15 lbs pressure. The PH of the solution was adjusted to 7.3-7.4 by using sodium hydroxide and hydrochloric acid.

3.3.2 Preparation of sub-cultures: The microorganisms used in the present study for the testing of antibacterial activity of the extracts were obtained from the laboratory-stock of microbiology department. Before the day of testing, the organisms were sub cultured in sterile nutrient broth, after 12 hours of incubation the same was used as inoculum for the test.

3.3.3 Sterilisation of media: The media used in the present study, Nutrient agar, and nutrient broth, were sterilized in a conical flask or suitable capacity by autoclaving at 15 lbs pressure for 20 minutes. The materials used were sterilized by employing hot air oven at 160 $^{\circ}$ c for 1 hour.

3.3.4 Preparation of solutions of extracts: Solutions of all the extracts of 10 plant materials were prepared in dimethyl floramide (DMF) and tested at the concentration of 400 mcg in 0.1 ml solution.

3.3.5 Method of testing:

Cup-plate method: This method depends on the diffusion of an antibiotic (or test material) from a cavity through the solidified agar layer of a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antibiotic.

The sterile media was poured while hot into sterile petriplates each containing 15 ml medium. The petriplates containing the media were seeded (i.e. lawn cultured) with different test bacteria, ensured that the layers of medium were uniform in thickness by placing the dishes on a leveled surface, and living it for some time. Then with the help of sterile cork borer (8mm in diameter) five wells (cups) were made into the media and each well was for a particular extract of the plant material under study. Then 0.1 ml of the plant extracts was put in each of such cups, and a cup had standard streptomycin.

These petriplates were allowed to stand for a few minutes to permit good diffusion. Petriplates were then kept for incubation at 37 $^{\circ}$ c for 24 hours to obtain good inhibition zone (I.A). Sterile conditions were maintained throughout the experiment. Three plates

were used per organism, with 10 plant material (four plant material in 2 plates and 2 plant materials in 1 plate) totally 12 petriplates for 4 microorganisms. At the end of 24 hours, the plates were observed for the zone of inhibition. The inhibition of growth of the bacteria was recorded by measuring the diameter of the inhibition zone in mm (excluding the diameter of the wells). The so occurred measurements were recorded as in table 2.

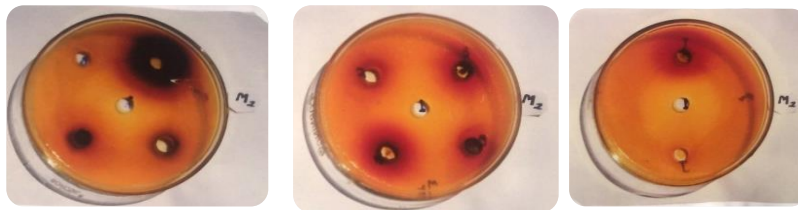


Fig. 1: Antibacterial activity of plant extracts with Microbe 1



Fig. 2: Antibacterial activity of plant extracts with Microbe 2

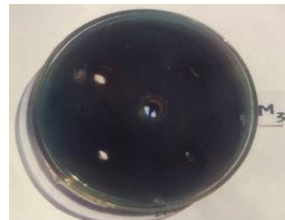


Fig. 3: Antibacterial activity of plant extracts with Microbe 3

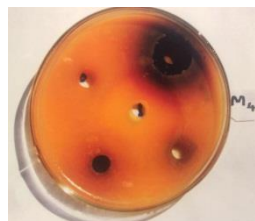


Fig. 4: Antibacterial activity of plant extracts with Microbe 4

Table 2: shows the Antibacterial activity

Drugs / Extracts	Zone of Inhibition (mm)				
	Gram +Ve S.aureus	S.typhi	Gram -Ve P.aeruginosa	E.coli	Mean
Streptomycin	11mm	19mm	13mm	20mm	15.75
Plant A	6mm	20mm	12mm4mm	15mm	13.25
Plant B	1mm	3mm	4mm	-	2.6
Plant C	3mm	4mm	2mm	5mm	3.5
Plant D	1mm	3mm	3mm	6mm	3.25
Plant E	6mm	4mm	7mm	8mm	6.25
Plant F	2mm	4mm	2mm	6mm	3.5
Plant G	2mm	6mm	4mm	7mm	4.75
Plant H	7mm	5mm	6mm	13mm	7.75
Plant I	3mm	4mm	4mm	10mm	5.25
Plant J	1mm	3mm	5mm	8mm	4.25
Bore size=8mm					
DMF=Nil					

4. RESULTS AND DISCUSSIONS

The results of the antibacterial activity were observed for the 10 plant materials against Staphylococcus aureus, S.typhi, P.aeruginosa, E.coli. The results obtained are discussed according to the results obtained in table 2.

- (a) **Emblica officinalis, Gaertn:** The plant extract of *E.officinalis* has shown the maximum effect on *S.typhi* (I.A of 20mm) and moderate activity on *E.coli* and *P.aeruginosa*, and less active against *S.aureus*, compared to streptomycin. $S.typhi > E.coli > P.aeruginosa > S.aureus$.
- (b) **Asparagus racemosus. Willd:** The plant extract had nil effect on *E.coli*; the other bacteria were less affected by this plant extract.
- (c) **Gymnema sylvester. R.Br:** The plant extract showed less activity against all the four bacteria selected for the study.
- (d) **Acalypha indica. L:** The plant extract showed less activity against all the four bacteria selected for the study.
- (e) **Euphorbia hirta L:** The plant extract showed less activity against *E.coli*, *P.aeruginosa* and *S.aureus* and very less activity against *S.typhi* compared to streptomycin.
- (f) **Atylosia scarabaeoides. Benth:** The plant extract had less activity against all the four bacteria under study.
- (g) **Hemidesmus indicus. R. Br:** The plant extract showed less activity against *E.coli* and very less activity against the rest of the bacteria.
- (h) **Phyllanthus niruri. L:** The plant extract showed moderate activity against *E.coli* and very less activity against the rest of the bacteria.
- (i) **Lawsonia inermis. L:** The plant extract showed less activity against *E.coli* and very less activity against the rest of the bacteria.
- (j) **Tribulus terrestris. L:** The plant extract has less activity against all the four bacteria under study. The overall result shows that *E.officinalis* has shown the maximum effect on *S.typhi* and good activity against *P.aeruginosa* and *E.coli* *Phyllanthus niruri* has shown moderate activity against *E.coli*. *Asparagus racemosa* had nil effect on *E.coli*

5. CONCLUSION

Plants have satisfied every human need, one of the important parts is medicine, the natural products of plants are boon to ancient people have suggested the uses of different plants for man, the only thing is he must know how to use them, though the different diseases to be cured. There are many diseases to be cured still and the only way is trying as much as plants on those organisms.

In the present study, the ethonolic extract of *E.officinalis* indicated the strong inhibition against *S.typhi*, *E.coli*, *P. aeruginosa*. It indicates the antibacterial activity against the pathogenic organisms. Whereas the other plant extracts like *P.niruri* has shown inadequate activity against *E.coli*. Other plants have mild to very less activity against all the organisms used.

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