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ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS OF SAURASHTRA REGION

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Abstract: Aqueous crude extracts of 10 traditionally used medicinal plants (*Adhatoda vasica, Ailanthus excelsa, Clerodendron inermi, Lawsonia inermis, Moringa oleifera, Nerium oleander, Punica granatum, Clitoria ternata, Dodonea viscosa, Zea mays, Tinospora cordifolia, Citrus aurantifolia, Bryophylum pinnatum, Aegle marmelos, Vinca rosea, Commiphora wightii, Rosa spps., Raphanus sativus, Aster spps., Lavendula aromatica, Maytenus emarginata and Pedalium murex*) were screened for antibacterial activity against few clinical isolates (isolated from Urine, Blood, Tracheal secretion and Pus). Extracts of certain parts of these plants were tried on 6 pathogenic [*E.coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Salmonella paratyphiB and Staphylococcus aureus.*] and 4 Opportunistic pathogenic [*Staphylococcus epidermidis, Bacillus megaterium, Bacillus subtilis* and *Enterobacter aerogenes*] strains. The antibacterial activity of above plants was evaluated by the Disc Diffusion method and Agar Ditch method. *Punica granatum* showed strong activity against *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis* and *Bacillus megaterium* but showed moderate activity against *Pseudomonas aeruginosa*. Alcoholic extracts of *Punica granatum* was selected for further studies.

Keywords: Medicinal plants, Antibacterial activity, Organic plant extracts

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive numbers of drugs have been isolated from natural sources. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being, and the bio prospecting of new plant-derived drugs. In the quest for new medicines to treat old and emergent diseases such as malaria, cancer and AIDS, attention is now being given to discovering the active ingredients encountered in the treasury of Indian herbs. Medicinal plants thus continue to be an important therapeutic aid for alleviating ailments of human kind and there is an ever-increasing demand for more and more drugs from plant sources.

Pharmaceutical importance of medicinal plants is due to specific constituents of secondary metabolites present in them. It is desirable to have a "need based" approach to research on medicinal plants including screening of plants for biological activity. The effects of herbal compounds and phytochemicals on pathogenic and medically important bacteria have been well studied (Bintu, 1997).

The purpose of this research is to collect and extract plant materials and then to screen them for potential biological activity (anti bacterial activity). India has a rich heritage of knowledge on plant-based drugs both for use in preventive and curative medicines. A country like India is very much suited for development of drugs from medicinal plant. Because of its vast and wide variations in soil and climate, the Indiansubcontinent is suitable for the cultivation of a large number of medicinal and aromatic plants which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavor and food and agrochemical industries. A large number of these plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs, which have high export potential.

Punica granatum is a shrub belonging to the unigeneric family Punicaceae, a native of semitropical Asia widely naturalized pan tropically. Earlier authors included the genus in the family Lythraceae (Lawrence, 1964). *P.*

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granatum or pomegranate as is popularly known is grown in different parts of India but is commercially grown in Maharashtra and Gujarat.

The different parts of the plant commonly used to treat various diseases are, flower, fruit, fruit rind, seed, dried bark of stem and root. As per the Ayurvedic pharmacopoeias the root has good vermicidal effect. It has also been used as an anthelmintic. The bark and seeds are useful in bronchitis. The flowers are used in epistaxis. The unripe fruit is a good appetizer and is useful in nausea and vomiting. The chemical constituents of leaf are betulic, ursolic and tannic acid (Chopra et al., 1956). The leaf powder with sandal wood paste, curd and honey is sometimes used to check miscarriage (Biswas and Ghosh, 1973). The antiviral activity of P. granatum is well documented (Mouhajir et al., 2001).

Considering the aforesaid, the objective of the present study was i) to screen some of the local flora of Saurashtra region for potential antibacterial activity ii) The promising plant to be further extracted in organic solvents and their antibacterial activity evaluated.

MATERIALS AND METHODS

Preparation of the crude extract: Fresh leaves of *Punica granatum* of equal size were plucked; washed thoroughly under running tap water for 1-2 h. Twenty five grams of leaves were gently dried and macerated in 100 ml of distilled water. The resulting slurry was boiled on low flame to concentrate it. When the slurry was reduced to 1/4th of the original volume, it was filtered through muslin cloth and centrifuged at 5000 g for 10 min and the supernatant was collected. For phytochemical analysis, few polar and non-polar solvents (data not shown) were used. Here also 25 g of leaf was selected and macerated in 100ml of solvent i.e. ethanol or methanol. The leaf material was kept in the respective solvent for 24 h for complete extraction of the soluble active compound. The extract was centrifuged and supernatant was collected and was kept in an evaporating dish so that 75% of the solvent gets evaporated. The evaporation time of different solvent varied. The final volume was made approximately to 25ml (±2ml). The final extract is stored in airtight bottles and kept in a refrigerator till further studies.

Antibacterial studies: The strains used for antibacterial study were obtained from a private microbiological laboratory.

Preparation of the plates and microbiological assays: A loop full of the given strain to be tested was inoculated in 10ml of N-broth (Nutrient Broth) and incubated it for 24 h in an incubator at 37 ^{o}C so as to activate the given test bacterial strain. Nutrient Agar (I.P.) plates were prepared by dissolving 37g in 1000ml of distilled water. Inoculation of the test strain was done by the Pour-plate technique. The antibacterial activity of the aqueous extract was determined by Disc diffusion method (Salie et al., 1986). The antibacterial activity of alcoholic extracts was determined by Agar ditch diffusion method (Nair et al., 2003). The inhibition zones produced by these extracts were evaluated by the inhibition zone formed by these extracts against the given test bacterial strain. The pure solvents used were taken as the control and later deduced from the test zones.

RESULTS AND DISCUSSION

A number of plants have been screened for their potential anti bacterial activity and twenty-two most promising plants have been selected and their data is presented here. The plants, their parts used, along with their details are presented in table 1. Initially screening of plants for possible anti microbial activity begins by using crude aqueous extraction followed by various organic extraction methods. The plants used in this study were chosen due to the presence of the compounds that have some interesting biological properties. Similar studies

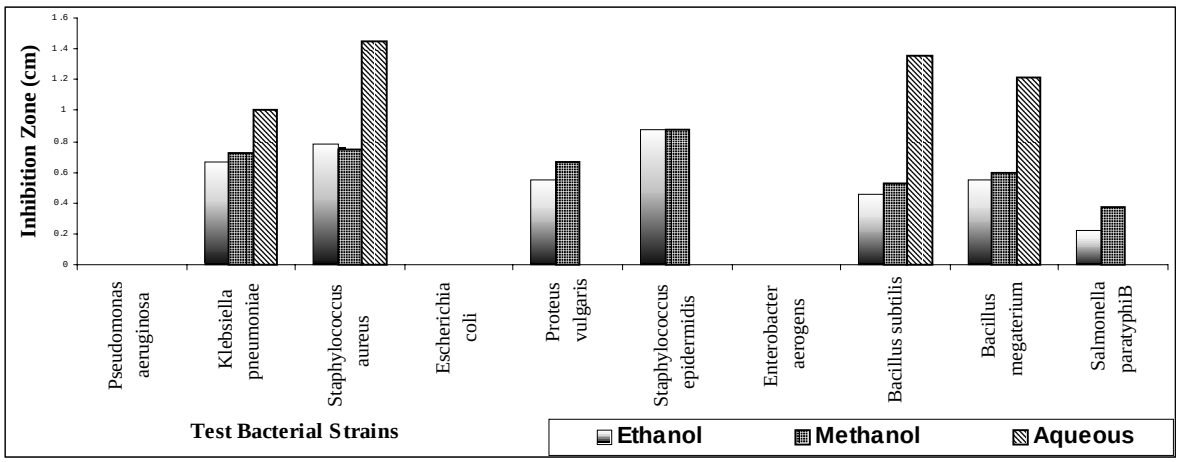

Fig. 1: Comparative study of ethanolic, methanolic and aqueous extracts

where different plants were screened for possible antibacterial activity in various areas have been attempted earlier (Mehmood et al. 1999; Kizil et al., 2002; Rajakaruna et al., 2002). In the present work also, initially aqueous extract of all the plants were studied against the above stated bacteria. The aqueous extracts of some plants were active against gram positive as well as gram-negative bacteria (Table 1). The aqueous extract of *Punica granatum* showed the strongest antibacterial activity; hence this plant was studied in more detail.

The aqueous extract of *Punica granatum* showed antibacterial activity against *S. aureus, K. pneumoniae, B. megaterium* and *B. subtilis*. The maximum inhibition zone was produced against *S. aureus*, while it was not showing any antibacterial activity against *P. aeruginosa, P. vulgaris, E. coli* and *E. aerogenes* (Fig. 1). It is known that the active components derived from plants are mostly aromatic or saturated organic compounds hence the extraction of them in ethanol or methanol is preferred. Here also organic solvent extractions were more effective than aqueous extract against all tested microorganisms. The organic solvents used were ethanol and methanol. Both these are polar solvents. Their dipole moment of ethanol is 1.69 and that of methanol is 1.70.

Table 1: The details of the plants studied and their antibacterial activity

Sr. No.	Name of the Plant	Family	Parts Used	Antibacterial Activity
1	*Adhatoda vasica* V.N. Ardusi	Acanthaceae	Leaf	+
2	*Ailanthus excelsa* V.N. Arduso	Simaroubaceae	Leaf	+
3	*Clerodendron inermi* V.N. Kadvi Mehndi	Verbenaceae	Leaf	+
4	*Lawsonia inermis* V.N. Menhdi	Lytheraceae	Leaf	+ +
5	*Moringa oleifera* V.N. Saragvo	Moringaceae	Leaf	-
6	*Nerium oleander* V.N. Karen	Apocynaceae	Leaf	-
7	*Punica granatum* V.N. Dadum	Punicaceae	Leaf, stem	+ + +
8	*Clitoria ternate* V.N. Batakveil	Papillionaceae	Flower	+ +
9	*Dodnea Viscosa* V.N. Dodonia	Sapindaceae	Leaf	+ +
10	*Zea mays* V.N. Makai	Graminae	Fibres	-
11	*Tinospora cordifolia* V.N. Gado	Menispermaceae	Stem, Leaf, root	-
12	*Citrus aurantifolia* V.N. Limbudi	Rutaceae	Leaf	-
13	*Bryophyllum pinnatum* V.N. Panfutti	Crassulaceae	Leaf	+ +
14	*Aegle marmelos* V.N. Billi	Rutaceae	Leaf	-
15	*Vinca rosea* V.N. Barmaasi	Apocynaceae	Leaf	-
16	*Commiphora wightii* V.N. Guggad	Burseraceae	Leaf	+
17	*Rosa Spps.* V.N. Gulab	Rosaceaea	Flower	-
18	*Raphanus sativus* V.N. Mudo	Brassicaceae	Leaf	-
19	*Aster spps.* V.N. Galgoto	Asteraceae	Flower	-
20	*Lavendula aromatica* V.N. Lavender	Labiatae	Leaf	-
21	*Maytenus emarginata* V.N. Vikdo	Celastraceae	Leaf	+ +
22	*Pedalium murex* V.N. Motu Gokhru	Pedaliaceae	Fruit	-

V.N. – Vernacular Name; (-) – No Antibacterial activity; (+) – Antibacterial Activity present

The anti bacterial activity of both the organic solvents was similar against tested microorganism (Fig. 1). Similar ethanolic extracts were tried for antibacterial activity for plants from Baja California Sur (Mexico) (Murillo-Alvarez et al., 2001) and for Jordian medicinal plants (Nimri et al.,1999). Methanolic extraction was also tried in different plants (Ali et al., 2001; Aziba et al., 2001; Mouhajir et al., 2001).

The comparative study of these extracts is shown in the graph. The ethanolic plant extract produced inhibition zone against *K. pneumoniae, S.aureus, P. vulgaris, S. epidermidis, B. subtilis, B. megaterium* and *S. paratyphi*; maximum being against *S. epidermidis*. It was not effective against *P. aeruginosa, E. coli* and *E. aerogenes*. The methanolic extract produced almost similar inhibition zone against the given test strains. Here also the maximum inhibition zone was produced against S. epidermidis.

It is concluded that *Punica granatum* has a broad spectrum of activity and suggests that it may be useful in the treatment of various microbial infections. It has important pharmacological activities, which may be used as leads in developing novel therapeutic agents. However, the nature and structure needs to be analyzed which can then be used in combating the diseases caused by these microbial species.

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