RESEARCH PAPER

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In-vitro antibacterial activity of some medicinally important plants against plant and human pathogens

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Medicinal plants represents rich source for antimicrobial agents and thus widely used in different countries and are a source of potent and powerful drugs. Considering the vast potentiality of medicinal plants as antibacterial agents, a systematic investigation was undertaken to screen the antibacterial activity of five medicinal plants *viz. Solanum torvum, Adhatoda vasica, Terminalia chebula, Asparagus racemosus and Simarouba glauca* against bacterial pathogens *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The ethanol leaf extracts of five plants showed variable zone of inhibition ranging from 3 to 11 mm against *B. subtilis, S. aureus, E. coli* and *.P aeruginosa*. The methanol extracts of all five plants failed to show zone of inhibition. However, hot water extract of *S. torvum* showed zone of inhibition about 6 mm against *B. subtilis* and *P aeruginosa* and about 7 mm against *S. aureus* and *E. coli.* The highest zone of inhibition was measured about 12 mm for ethanol + methanol extracts of *A. vasica* against *E. coli*. Thus, it reveals that bioactive compounds like alkaloids, flavonoids, terpenoids etc. may be the cause of inhibition and can easily be isolated in presence of ethanol, methanol, ethanol + methanol and hot water.

Key words : Medicinal plants, Antibacterial activity, Zone of inhibition, Bioactive compounds

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INTRODUCTION

The use of plant parts and products as a source of relief from diseases and illness could be traced as back as the beginning of human civilization .The documents written between 4500-1600 B.C. it is Ayurveda, the foundation medicinal science of Hindu culture, in its eight divisions deals with specific properties of drugs and art of healing (Rastogi and Mehrotra, 2005). The potential of higher plants as a source of new drugs is still largely unexplored. Among the estimated 5, 00,000 plant species, only a small percentage has been investigated phytochemically. Medicinal plants represents rich source for antimicrobial and antifungal agents. Plants are used medicinally in different countries and are a source of potent and powerful drugs (Srivastava *et al.,* 1996). India is rich in biodiversity of medicinal plants which are the source of great economic value all over the world. A wide range of various plant parts are used for the preparation of extracts as raw drug possessing vary in medicinal properties.

The different parts used are roots, stem, leaves, fruits, and modified organs. Some of these are collected by local communities for treating illness or wound healing; many other raw drugs are collected in large quantities and traded in the market as raw materials for many herbal industries (Uniyal *et al*., 2006). Although 100 of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Balandrin *et al*., 1985).

Considering the vast potentiality of medicinal plants as antibacterial agents, a systematic investigation was undertaken to screen the antibacterial activity of five medicinal plants *viz., Solanum torvum, Adhatoda vasica, Terminalia chebula, Asparagus racemosus*

and Simarouba glauca against bacterial pathogens *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

Asparagus racemosus (shatavari) is best known as a female rejuvanitive. It is also useful for hyperacidity, stomach ulcers, dysentery and bronchial infections. *Terminalia chebula* (Hirda, *Combretaceae*) useful in ashtama, piles, cough and wound healing. *Solanum torvum* (Ranvangi, *Solanaceae*) shows narcotic, diuretic, antimicrobial properties. *Adhatoda vasica* (Adulsa, *Acanthaceae*) is useful for treating cough and asthma. *Simarouba glauca* (Laxmi taru, *Simaroubaceae*) is a flowering tree shows antimicrobial and insecticidal activity (Joshi and Joshi, 2007).

RESEARCH METHODOLOGY

Collection of plant material:

Fresh leaves of five different plants *viz., A. racemosus, T. chebula, S. torvum, A. vasica* and *S. glauca* free from diseases were collected from Puriya Park, K.K. Wagh college of Agriculture, Nashik, Maharashtra. The leaves were washed thoroughly 2-3 times with water and with autoclaved distilled water and chopped into small pieces and dried under shade.

Solvent extraction:

Thoroughly washed dried leaves of above mentioned five plants were powdered with the help of blender, 5 g dried leaf powder of each plant was mixed in 100 ml of each methanol (M), ethanol (E), 50 ml methanol + 50 ml ethanol (EM) and hot water (HW), respectively. The extraction was successfully done by soxhlet extractor for 48 hrs. The solvent extracts were concentrated and reduced by rotary vaccum evaporator and preserved in airtight bottle at 5^0C until further use.

Growth and maintenance of test microorganisms:

Bacterial cultures of *Bacillus subtillis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from culture collection centre, National Chemical Laboratory, Pune. The bacterial cultures were precultured in Nutrient broth over night in a rotary shaker at 37^0C, centrifuged at 10,000 RPM for 5 min, pellet was suspended in double distilled water and cell density was standardized spectrophotometrically (A_{610} nm) to obtain a final concentration of 10^5 spores / ml.

Antibacterial activity:

The test microorganisms were seeded into respective medium by spread plate technique with 24 hrs old cultures of bacterial growth in Nutrient broth. Six wells of 6 mm diameter were bored in the medium with the help of sterile cork-borer having 6mm diameter and were labeled properly. 50 µl of working suspension of different extracts like E, M, EM and HW of *Asparagus racemosus* and same volume of control was filled in the wells with the help of micropipette. The above process was again repeated with the remaining four plant species. All the Petriplates were incubated at 37^0C for 24 hrs and zone of inhibition were studied and recorded after incubation.

RESULTS AND ANALYSIS

Medicinal plants are important in Indian traditional medicine and most frequently used in Ayurveda. The E, M, EM and HW extracts of dried leaves were recovered by soxhlet apparatus. The tested bacterial strains showed different patterns of inhibition (Table 1). When tested b cork borer method the E extract of *A. racemosus* showed significant activity against *B. subtilis* around 5 mm and *E. coli* around 4 mm. EM extract of same plant showed 4 mm zone of inhibition against *S. aureus*. E and EM extracts of *T. chebula* showed highest zone of inhibition against *S. aureus* measured 7 mm. The broad spectrum antimicrobial activity of *T. chebula* was also reported (Phadake and Kulkarni, 1989). Two possibilities that may account for the higher antibacterial activity of E extracts of *T. chebula* are the nature of biological active components (alkaloids, flavonoids, essential oils, terpenoids, tannins etc.), which may be enhanced in the presence of ethanol; and the sronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity (Ghosh *et al*., 2008). E and HW extracts of *S. torvum* showed variable zone of inhibition against bacterial spp. and highest 7 mm was measured against *S. aureus*. Chloroform and methanol extracts of stem and root part of *S. torvum* showed antibacterial activity and significant zone of inhibition were recorded (Bari *et al*., 2010). The higher antibacterial activity of *S. torvum* was may be due to the presence of steroids, gluco-alkaloid, sapogenins, sitosterol etc. (Yuanyuan *et al*., 2009).

The maximum zone of inhibition was recorded of EM extract of *A. vasica* plant about 12 mm against *E. coli*. EM extract of *S. glauca* also showed significant antibacterial activity and highest 11 mm zone of inhibition was recorded against *E. coli. S. glauca* has been used as febrifuge, antdysentric, antiherpetic and antihelminthic

Table 1: Screening of antibacterial activity of medicinal plants against bacterial pathogens

Medicinal plants	Leaves Extract	Bacterial Species			
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
Asparagus racemosus	E	5±0.50	4±0.57	-	4±0.57
	M	-	-	-	-
	EM	-	4±0.2	-	-
	HW	-	-	-	-
Terminalia chebula	E	5±0.50	7±0.50	4±0.57	5±0.50
	M	-	-	-	-
	EM	-	7±0.10	-	4±1.50
	HW	-	-	-	-
Solanum torvum	E	6±1.33	5±0.51	5±0.50	3±1.00
	M	-	-	-	-
	EM	-	3±1.00	-	3±1.10
	HW	6±1.00	7±0.50	6±0.57	7±0.50
Adhotoda vasica	E	5±0.50	11±2.01	5±0.33	10±0.52
	M	-	-	-	-
	EM	-	3±1.00	-	12±2.00
	HW	-	-	-	-
Simarouba glauca	E	4±1.50	5±1.00	4±0.57	9±2.01
	M	-	-	-	-
	EM	3±1.00	5±0.50	4±0.52	11±0.55
	HW	-	-	-	-
Streptomycin Sulphate	5 µg/ml	8±0.33	7±0.33	7±0.33	9±0.33

Values are zone of inhibition (mm) ± S. D. of three replicates

by Cuban population (Rogi, 1974). Similar properties have been described in other countries, the antiprotozoal (Franssen *et al*., 1997) and antibacterial (Caceres *et al*., 1990) activities. Thus E extracts of five medicinal plants showed zone of inhibition against four bacterial spp. (Fig. 1).

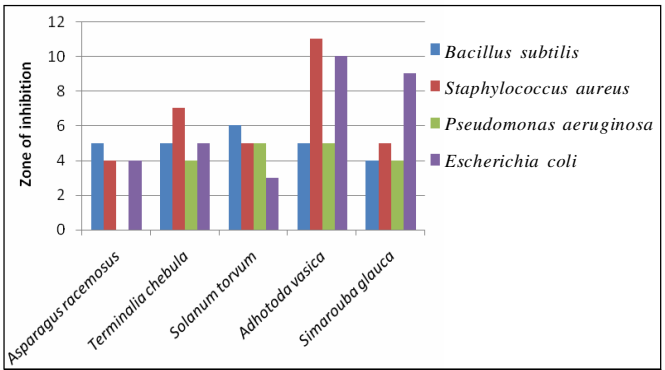

Fig. 1: Ethanolic extract comparison of medicinal plants

Conclusion:

The present investigation revealed that the ethanolic extract of all five plants are highly active against *B. subtilis*, *S. aeureus, P. aeurogenosa* and *E. coli*. The EM extract also shows significant antibacterial activity. The M extract of all plants did not show antibacterial activity. Further studies on these plants are essential for antifungal, insecticidal, identification and isolation of bioactive compound in the extract, their mechanism of action and determination of pharmacological and toxicological effects of extract on microbial growth.

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