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Full Length Research Paper

Antimicrobial screening of selected medicinal plants in Tamilnadu, India

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The present study deals with the antimicrobial activity of the aqueous, acetone and petroleum ether extracts of the leaves, stem and root of *Andrographis ovata* Clarke*, Aristolochia indica* L., *Eclipta prostrata* L. and *Gloriosa superba* L., using agar diffusion method against human pathogens, such as *Escherichia coli, Proteus vulgaris, Psudeomonas aeruginosa* and *Klebsiella pneumoniae*. In the present investigation, all the extracts were found to be effective against four human bacterial species, *E. coli*, *P. vulgaris*, *P. aeruginosa* and *K. pneumoniae*, sensitive to all the plant extracts. The study suggests that the extract of the plant parts possesses potential broad spectrum antimicrobial activity. The antimicrobial activity of acetone extracts was found to be higher than that of distilled water extracts. However, the root extract showed more inhibitory effect than the stem and leaf extracts.

Key words: Medicinal plants, antimicrobial activity, plant extracts, growth inhibition, disc diffusion method.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. According to World Health Organization, medicinal plants would be the source of obtaining a variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/ plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As preventive and curative measures, plants and their products are used in the treatment of infections for many centuries ago. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare (Famsworth, 1985) and India happens to be the largest user of traditional medical cure, using 7000 plant species.

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity (Ritch-Krc et al., 1996; Martins et al., 2001). Antibacterial properties of various plants parts, such as leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades (Leven et al., 1979). Antibiotic principles are distributed widely among angiospermic plants. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial activities (Callow, 1983).

Antimicrobial drugs are used in medicinal practices for treating food-borne diseases (Abramowics, 1990). Use of medicinal plant extracts that are rich in antibacterial compounds could be an alternate way to eliminate these bacteria from palatable items (Santos, 1985; Sai et al., 1997; Minakshi et al., 1998), which has already been proved *in vitro* by Kaushik and Dhiman (2000) and Kaushik (2003). Over the last 25 years, a large number of plant species have been evaluated for their antimicrobial activity. So, the present study attempts to evaluate the antimicrobial activities of the leaves, stem and root of *A. ovata* Clarke (Acanthaceae), *Aristolochia indica* L. (Aristolochiaceae), *E. prostrata* L. (Asteraceae) and *G. superba* L. (Colchicaceae) against selected pathogenic bacteria. Currently, researches are being carried out to investigate the potential applications of medicinal plants, such as *Nerium oleander* (Hussain and Gorsi, 2004), *Adhatoda vasica, Plumbago zeylanica* and many more.

Table 1. Antimicrobial activity of leaf, stem and root extracts of *Andrographis ovata*[a] (µg/100 mg).

Plant parts	Extract	Diameter of inhibition zone (in cm^2)			
		Escherichia coli	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Klebsiella pneumoniae***
Leaf	Acetone	2.4	1.8	2.1	1.8
	Petroleum ether	1.8	2.2	1.7	1.9
	Aqueous	1.5	1.3	1.6	1.4
Stem	Acetone	2.2	2.1	1.9	1.8
	Petroleum ether	1.7	1.8	2.1	1.7
	Aqueous	1.6	1.4	1.5	1.3
Root	Acetone	2.9	2.2	2.1	1.8
	Petroleum ether	1.9	1.8	1.9	1.7
	Aqueous	1.6	1.4	1.5	1.2

[a]Values are mean of triplicate determination on dry weight basis.

Surprisingly, it was found to be effective against many pathogenic micro-organisms. Hence, they can be used for the treatment and cure of various diseases. The present research aimed at screening and evaluating the antimicrobial assay of *A. ovata, A. indica, E. prostrata* and *G. superba* tested against four bacterial strains.

MATERIALS AND METHODS

Collection and identification of plants

The plant materials were collected from the wild population of the Shevaroy Hills (Eastern Ghats) and Salem, Tamilnadu. Plants were identified and confirmed with the authentic herbarium specimen available in the Botany Department, Government Arts College (Autonomous), Salem-7.

Preparation of plant extracts

The fresh plant samples (leaves, stem and root) collected were washed individually under running tap water to remove soil particles and other dirt. The leaves were air dried in the laboratory at room temperature (30 ± 2℃) for 15 days, while the root and stem samples were dried at 60℃ for 2 days in an oven. The dried leaves, stem and root samples were ground well into a fine powder with a mixer grinder. The powder was stored in air sealed polythene bags at room temperature before extraction.

The method of Alade and Irobi (1993) was adopted for preparation of plant extracts. A fixed weight (20 gm) of powdered plant material was soaked separately in 50 ml of distilled water, acetone and petroleum ether for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod. At the end of the extraction, each extract was passed through Whatman No. 1 filter paper (Whatman, England), and the filtrate obtained was concentrated in vaccum using rotator evaporator. Then the extracts were used for antimicrobial activity.

Bacterial inoculum preparation

Bacterial cultures used in this study were obtained from Salem Diagnostic and Reference Laboratory (SDRL), Salem. Bacterial cultures included in this study were *E. coli, P. aeruginosa, Proteus vulgaris* and *Klebsiella pneumoniae*. All the cultures grown in Muller-Hinton agar were prepared and sterilized. The inoculum was used for antimicrobial assay.

Antimicrobial assay

The media and test bacterial cultures were poured into dishes (Muller-Hinton agar media). The test strain (0.2 ml) has an inoculum size (108 cells/ml) when the temperature reached 40 to 42℃. Care was taken to ensure proper homogenization. The plant extracts were tested for antimicrobial activity in the agar well diffusion assay (Perez et al., 1990) against *E. coli, P. vulgaris, P. aeruginosa* and *K. pneumoniae*.

Agar well diffusion method

The antimicrobial activity was tested against (aqueous, acetone and petroleum ether) leaves, stem and root of *A. ovata, A. indica, E. prostrata* and *G. superba*. The inoculation of microorganism was prepared from bacterial culture (Parihar, 2006). About 15 to 20 ml of Muller-Hinton agar medium was poured in the sterilized Petri dishes and allowed to solidify. One drop of bacterial strains was spread over the medium by a rod. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. About 100 ml of the plant extracts were added to the wells. Plates were incubated in air at 37℃ for 24 h. Antimicrobial activities were evaluated by measuring the inhibition zone diameters.

RESULTS AND DISCUSSION

In the present investigation, the antimicrobial activity of 4 plant extracts (leaf, stem and root) against 4 microbial species was recorded. The results obtained in this investigation for the antimicrobial activity of *A. ovata, A. indica, E. prostrata* and *G. superba* using disc diffusion method showed that all the bacterial strains were sensitive to the tested extracts (acetone and petroleum ether) except aqueous extract (Tables 1 to 4).

Tables 1 and 2 show the antimicrobial activity of plant parts' extracts of *A. ovata* and *Eclipta prostrata* against

Table 2. Antimicrobial activity of leaf, stem and root extracts of *Aristolochia indica*[a] (µg/100 mg).

Plant parts	Extract	Diameter of inhibition zone (in cm^2)			
		Escherichia coli	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Klebsiella pneumoniae***
Leaf	Acetone	2.1	1.7	1.9	2.1
	Petroleum ether	1.9	2.1	1.8	1.9
	Aqueous	1.6	1.3	1.4	1.5
Stem	Acetone	1.9	1.8	1.8	1.9
	Petroleum ether	1.7	1.7	1.9	1.8
	Aqueous	1.2	1.4	-	1.3
Root	Acetone	2.4	2.1	1.9	1.8
	Petroleum ether	2.1	1.9	1.7	1.7
	Aqueous	1.4	1.5	-	1.3

[a]Values are mean of triplicate determination on dry weight basis.

Table 3. Antimicrobial activity of leaf, stem and root extracts of *Eclipta prostrata*[a] (µg/100 mg).

Plant parts	Extract	Diameter of inhibition zone (in cm^2)			
		Escherichia coli	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Klebsiella pneumoniae***
Leaf	Acetone	2.1	1.9	1.7	1.8
	Petroleum ether	2.0	1.8	1.9	1.9
	Aqueous	1.5	1.4	1.5	1.4
Stem	Acetone	1.9	2.1	1.9	1.7
	Petroleum ether	1.7	1.9	1.8	1.9
	Aqueous	1.2	1.4	1.5	1.3
Root	Acetone	2.2	2.1	1.8	1.9
	Petroleum ether	1.9	2.0	1.9	1.7
	Aqueous	1.5	1.2	1.4	1.3

[a]Values are mean of triplicate determination on dry weight basis.

Table 4. Antimicrobial activity of leaf, stem and root extracts of *G. superba*[a] (µg/100 mg).

Plant parts	Extract	Diameter of inhibition zone (in cm^2)			
		Escherichia coli	***Psudomonas aeruginosa***	***Proteus vulgaris***	***Klebsiella pneumoniae***
Leaf	Acetone	2.3	2.1	1.9	1.8
	Petroleum ether	1.9	1.8	1.9	1.7
	Aqueous	1.4	1.2	1.5	1.6
Stem	Acetone	1.9	2.1	1.8	1.9
	Petroleum ether	2.1	1.7	1.7	1.8
	Aqueous	1.3	1.5	-	1.3
Root	Acetone	2.5	1.9	2.1	2.1
	Petroleum ether	2.1	1.7	1.9	1.8
	Aqueous	1.2	1.4	-	1.2

[a]Values are mean of triplicate determination on dry weight basis.

E. coli, P. aeruginosa, P. vulgaris and *K. pneumoniae*. It was found that acetone extracts of leaf, stem and root have shown more inhibition than aqueous extracts against *E. coli, P. aeruginosa, P. vulgaris* and *K. pneumoniae*. In *P. vulgaris* leaf, both stem and root extracts showed more or less similar inhibiton zone. Earlier similar investigations were done by Ajaiyeobal et al. (1999) in *Solanum microcarpum* and *Solanum torvum* and Karthikumar et al. (2007) in *E. prostrata*, *E. coli, Salmonella typhimurium* and *Bacillus subtilis*.

Tables 3 and 4 show the antimicrobial activity of plant part extracts of *A. indica and G. superba, E. coli* and *P. aeruginosa* were sensitive to acetone and petroleum ether extracts (leaf, stem and root) of *A. indica* and *G. superba*. With the exception of aqueous stem and root extracts, all other extracts have shown inhibition against *P. vulgaris*, whereas *K. pneumoniae* was sensitive to all the extracts. This was in corroboration with the earlier study done by Srinivasan et al. (2001), Ekwenye and Elegalam (2005) and Karthikumar et al. (2007).

A. ovata is found to be having more antimicrobial activity than *A. indica, E. prostrata* and *G. superba* and its activity is most significant against *E. coli, P. aeruginosa* and *P. vulgaris*. The root extracts showed higher inhibition than the stem and leaf extracts (Tables 1). This indicates that the antimicrobial properties are more concentrated in the root than in the leaves and stem. The volatile components of acetone extracts of *Ocimum sanctum* were highly effective against all the tested microorganisms (namely, *E. coli, S. aureus* and *Klebsiella* species), while various organic extracts of *E. prostrata* were highly effective against all the tested microorganisms (namely, *E. coli, S. aureus, Shigella dysenteriae* and *Salmonella typhi*) and *B. subtilis* and *Cassia alata* leaf extracts were highly effective against *E. coli* and *S. aureus* (Valsaraj et al., 1997; Zavala et al., 1997; Tanira et al., 1994).

The antimicrobial compounds may be found as alkaloids, flavonoids, tannins, phenolic compounds, steroids, saponins and triterpenoids whose presence may be attributed to the medicinal properties of plants (Rabe and Van Stadin, 1997; Ramasamy, 2000; Santhi et al., 2006; Shah et al., 1981). The results of the current study are found to be directly correlated with the observations of earlier workers (Shariff et al., 2006; Castello et al., 2002; Jain et al., 2004). Previous observations on *Mimosa hamata, Nerium oleadaner* and *Baliospermum axillare* leaf and callus extracts showed considerable antibacterial and antimicrobial activity (Suffrendi et al., 2004; Shariff et al., 2006; Singh and Sudharshana, 2003). Many substances may be antimicrobial, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). The findings of the present investigation offer a scientific support to the ethno-medicinal use of the plants by the traditional healers.

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