Antimicrobial and phytochemical studies on twelve medicinal plants against multi-drug resistant *Staphylococcus aureus*

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Abstract

Phosphate Buffer Saline extracts of 12 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against *Staphylococcus aureus* of clinical origin. Of these, 09 plant extracts showed varied levels of antimicrobial activity against one or more test bacterial strains. Qualitative phytochemical tests of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents.

Keywords: Medicinal plants; Phytochemicals, Antimicrobial activity; Multidrug resistance; TLC-bioautography

1. Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1-5]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune-compromised, AIDS and cancer patients [6-7]. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new active compounds for which bacteria may not develop resistance easily and also which should be safe to human use.

2. Materials & Methods

2.1. *Phytochemical analysis*

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by Harborne *et al*. [8a & b]. The plant extracts were screened for

the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

2.2. *Antimicrobial assay*

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Bauer et al. (1966) against a number of antibiotics. All antibiotic discs were purchased from the Hi-Media Pvt. Ltd. (Bombay, India) [9]. The agar well diffusion method [10] with slight modifications was used and accordingly 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on MHA plates. Wells of 6 mm diameter were punched into the agar medium and filled with 40 microlitre (150 mg/ml) of plant extract, solvent blanks and antibiotic (vancomycin, 30 mg/ml conc.) to which the test bacteria were sensitive. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

3. Results and discussion

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The expanding global problem of antibiotic resistance can be controlled and resolved by the use of such natural agents which have great promising antimicrobial potential against MRSA and are safe to use with no side effects that are often associated with synthetic drugs. Fleming's serendipitous discovery of penicillin was the start of exploitation of the most prolific natural source. From plants, the breakthrough in the area of antimicrobials has been realized.

Indian traditional medical systems such as Homeopathy, Ayurved, Unani medicines used by native groups for curative purposes documented the therapeutic efficacy of herbal products. Further support for the suggestion that natural products contain a wealth of biologically active molecules comes from the results obtained by several researchers during antimicrobial testing of these products against different microbes. In the present study phosphate buffer saline extracts of 12 traditionally used Indian medicinal plants have been tested against drug-resistant bacteria. Plant parts used along with their code number are given in Table 1.

Table 1: Name of Plant and its parts used along with their code number

Denotion	Name of Plant	Plant

		parts used
1	*Calendula officinalis*	Flower
2	*Rosa indica* (*Rosa indica*)	Leaves
3	*Chrysanthemum coroneriyum*	Leaves
4	*Calendula officinalis*	Leaves
5	*Polygonum bistorta*	Leaves
6	*Prunus dulcis*	Leaves
7	*Swetia charita*	Leaves
8	*Helianthus annuus*	Leaves
9	*Azadirachta indica*	Leaves
10	*Jasminum sambac*	Leaves
11	*Psidium guajava*	Leaves
12	*Emblica officinalis*	Leaves

Table 2: Antimicrobial activity of recovered isolates against different plant extracts (PBS Extracts)

	Mean Zone Diameter (In mm)											
Samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
N1	**R**	**R**	**16mm**	**R**	**11mm**	**22mm**	**R**	**R**	**15mm**	**14mm**	**R**	**11mm**
N3	**R**	**16mm**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**10mm**
N6	**R**	**R**	**R**	**R**	**R**	**14mm**	**R**	**13mm**	**R**	**R**	**12mm**	**15mm**
S3	**R**	**R**	**15mm**	**14mm**	**R**	**15mm**	**R**	**R**	**20mm**	**R**	**12mm**	**18mm**
E4	**R**	**R**	**R**	**R**	**12mm**	**16mm**	**R**	**R**	**17mm**	**R**	**R**	**R**

P1	**R**	**R**	**15mm**	**R**	**R**	**19mm**	**R**	**R**	**13mm**	**R**	**R**	**R**

R: Resistant i.e. <10mm zone of inhibition

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively as given in Fig. 1. Among the 12 plant extracts, 8 plants were found to have antimicrobial activity as shown in Table 2. *Prunus dulcis* leaves extract showed activity against 5 samples, *Azadirachta indica* and *Emblica officinalis* leaves extract showed activity against 4 samples, *Swetia charita* extract showed

activity against 4 samples, *Chrysanthemum coroneriyum* extract showed activity against 3 samples, *Polygonum bistorta* and *Psidium guajava* leaves extract showed activity against 2 samples, *Rosa indica* extract, *Calendula officinalis* leaves extract and *Helianthus annuus* extract showed activity against 1-1 sample each. During Biochemical characterization, steroids, rasins and glycosides were the most common phytochemicals found in all these plant extracts. So the antimicrobial activity must be due to them.

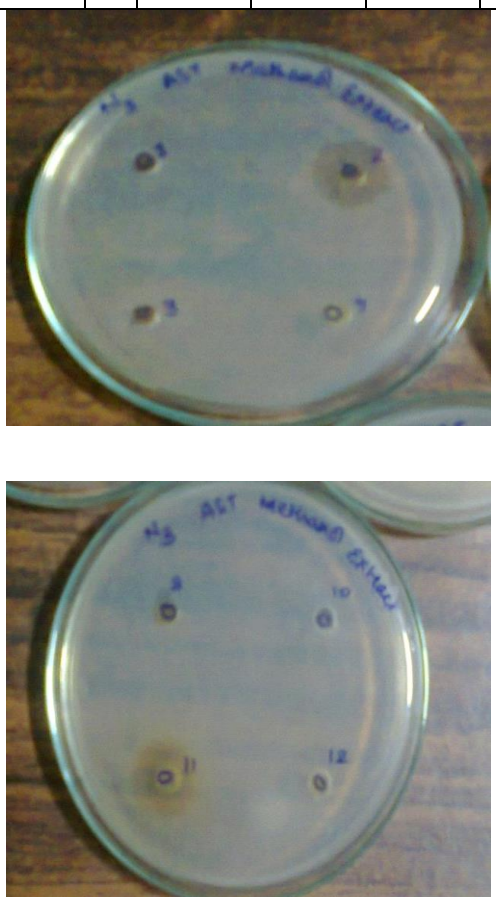

Figure 1 showing results of antimicrobial activity of plant extracts in the form of zone of inhibition of test bacterial isolates growth.

The bioactive compounds are usually distributed as secondary metabolites in every plant cell though leaf is found to be the highest accumulatory plant part and has high Active Pharmaceutical Ingredient (API) [11]. Antimicrobial compounds in plants are reported to be aromatic or saturated organic molecules which are easily solubilized in organic solvents. Therefore, after

preliminary screening for a better solvent, phosphate buffer saline was used for the preparation of twelve plant leaf extracts, which were selected for their antimicrobial spectrum against different microbes as claimed in various ethno-medical studies [12].

The results of screening are encouraging as out of the 12 plants, 09 extracts showed antibacterial activity against one or more test bacteria. Similar reports on antibacterial activities of certain Indian medicinal plants such as *P. granatum*, *Azadirachta indica*, and *Psidium guajava* were also reported by other workers [13-15].

Few plants in this study were also screened previously against other test strains [1, 16] and showed similar results to this study with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction [15].

Phytochemical analysis of 12 active extracts demonstrated the presence of common phytoconstituents like phenols, condensed tannins, glycosides, saponins, flavonoids and alkaloids. These results are in resonance with several workers [17]. The results present here are promising; however, the knowledge of active principles involved in activity and their appropriate medicinal application is still a bit mysterious.

Present study highlights the prevalence of MRSA among various Cancer patients and the antibacterial activity of different medicinal plant extracts against it. During the study we isolated one VRSA sample also which was isolated from Pus sample of a patient who was suffering from Cancer Tumour Burger. The prevalence of VRSA is not as common till now as found in previous studies but screening of one VRSA sample in present study shows the effect of high dose of antibiotics that are given to the cancer patients which makes patients resistant to most of the antibiotics.

The present results offer a scientific basis for the traditional use of PBS extracts of *Azadirachta indica*, *Swetia charita*, *Jasminum sambac*, *Polygonum bistorta*, *Psidium guajava*, *Rosa indica*, *Calendula officinalis*, *Prunus dulcis*, *Thryhanthemum coroneriyum*, *Helianthus annuus*, *Emblica officials.* However, further studies on these medicinal plants are necessary to determine their active constituent-activity relationship. The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

It is therefore concluded that, the plant extract possess antibacterial activity against the test organism i.e. *Staphylococcus aureus.* The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organism. The antibacterial activity of plants may be due to the presence of various active principles in their leaves. Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property. The ethno-botanical study of plant is important for modern day medicine but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. At present, scientists are investigating for plant products of antimicrobial properties. It would be advantageous to standardize methods of extraction and in vitro antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic and interpretation of results would be facilitated. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans. Efficient collaborations with pharmacologists and medical doctors, plant pathologists and micro-biologists are crucial to see the complete development of an interesting lead compound into an exploitable product. However, *in vivo* studies on these medicinal plants are warranting determining toxicity of active constituents, their side effects and pharmacokinetic properties.

The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs. Study of the synergistic interaction of active phytocompounds with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms.

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