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Phytochemical screening and evaluation of antibacterial activity of solvent extracts of *Jasminum arborescens* leaves

K.Bhagath[1]*, T.R.Prashith Kekuda[2], K.R.Venugopala Reddy[3]

[1]Dept. of Chemistry, National Pre-University College, N.E.S Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, (INDIA)

[2]Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, (INDIA)

[3]Dept. of PG Studies and Research in Industrial Chemistry, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, (INDIA)

E-mail: bhagathraok@rediffmail.com

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ABSTRACT

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of micro-organisms. In the present study, Preliminary phytochemical and antibacterial activity of *Jasminum arborescens* leaves was investigated. The powdered leaf material of the plant was extracted using various solvents namely petroleum ether, chloroform, ethanol and water. The solvent extracts were subjected to preliminary phytochemical analysis and tested against Gram positive and Gram negative bacteria. The results revealed the presence of various groups of phytochemicals in different solvent extracts. Marked antibacterial activity was observed in case of aqueous extract followed by ethanol extract, chloroform extract and petroleum ether extract. The antibacterial activity of solvent extracts of the plant tested could be due to active phytoconstituents present in them. Experiments in animal models could reveal the in vivo potential of the plant extracts against disease producing bacteria.

KEYWORDS

Jasminum arborescens;
Phytochemical screening;
Antibacterial activity;
Disc diffusion method;
Solvent extracts.

INTRODUCTION

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms[1]. Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins), however, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported[2]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents[3]. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Me-

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dicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant[4]. In the present study, Preliminary phytochemical and antibacterial activity of leaves of *Jasminum arborescens*, a member belonging to the family Oleaceae (Olive Family) was investigated. Evidently, there are not sufficient scientific studies that confirm the antibacterial property of this plant.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected in December 2007 in and around Hosanagara (Tq), Shivamogga (Dt). The plant samples were identified by specialist and voucher specimen was deposited in the Dept. of Microbiology for future reference. Plants were cleaned off adhering soil/dust in field by shaking properly and using soft brush. Plants were placed in paper bags and brought to the laboratory. Remaining dust particles were removed by quick rinsing with distilled water.

Extraction of plant material using solvents

Leaf material was shade dried and powdered mechanically. About 250g of powdered material was subjected to soxhlet extraction and exhaustively extracted with various solvents namely petroleum ether, chloroform, and ethanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator[5]. About 10g of plant material was transferred to 100 ml of distilled water and boiled for about half an hour. The contents were then filtered and reduced to about 1/3rd of the original volume[6].

Phytochemical screening

Qualitative phytochemical analysis of the crude solvent extracts was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate+2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate+1% HCl+steam, 1 ml filtrate+6 drops of Mayor's reagents/ Wagner's reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate+5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate+1 ml glacial acetic acid+$FeCl_3$+conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate+2 ml acetic anhydride+conc. H_2SO_4. Blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate+conc. HCl+magnesium ribbon pink-tomato red color indicated the presence of flavonoids[7].

Screening for antibacterial activity (Disc diffusion method)

Test bacteria

The test bacteria were obtained from National Chemical Laboratory, Pune. Gram positive bacteria namely *Bacillus subtilis NCIM 2063, Staphylococcus aureus NCIM 2079* and Gram negative bacteria namely *Escherichia coli NCIM 2065, Enterobacter aerogenes NCIM 2340* were used. The pure cultures of test bacteria on Nutrient agar slants were maintained in refrigerator for further use and regularly checked for contamination. Periodic transfers were made aseptically.

Preparation of bacterial inoculum for swab inoculation

Test tubes containing sterile Nutrient broth were aseptically inoculated with the pure cultures of test bacteria maintained on slants and incubated at 37^{o}C for 18 hours to get standard bacterial load. The broth cultures of test bacteria obtained after incubation were used for swab inoculation on agar media.

Method

Disc diffusion method

The antibacterial activity was assessed using the simple disc diffusion method[8]. The test bacterial suspensions were spread over the plates containing Nutrient agar using a sterile cotton swab dipped in broth culture in order to get a uniform bacterial lawn growth. Sterile Whatman filter paper discs of 0.5 cm diameter were impregnated with condensed drug extract (5mg), dried and placed on medium inoculated with test bac-

TABLE 1: Preliminary phytochemical analysis of solvent extracts

Phytochemical group	Aqueous extract	Ethanol extract	Petroleum ether extract	Chloroform extract
Alkaloids	+	N.D	N.D	N.D
Terpenoids	+	+	+	N.D
Flavonoids	+	+	+	N.D
Steroids	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	N.D	N.D	+
Saponins	+	+	N.D	+

+ - Detectable, N.D-not detected

TABLE 2: Antibacterial activity (Disc diffusion method) of ethanol and petroleum ether extracts

Test bacteria	Zone of inhibition in mm				
	Streptomycin (10 mcg/disc)	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Escherichia coli	23	08	13	12	14
Enterobacter aerogenes	25	10	12	11	13
Staphylococcus aureus	18	08	11	10	12
Bacillus subtilis	24	10	12	11	13

Results are average of triplicates; Extract concentration in disc-Appr. 5mg

teria. Streptomycin disc (10 mcg/disc) was used as control. The plates were left for 30 min at room temperature to allow the diffusion of the drug, and then incubated at 37°C for 18 hours. After incubation, the zone of inhibition was measured with a ruler. Experiment was performed in triplicate, and mean value was calculated and compared with standard (Streptomycin).

RESULTS AND DISCUSSION

All the extracts exhibited different kinds of secondary metabolites. Phytochemical screening (TABLE 1) revealed the presence of Terpenoids, Flavonoids, Steroids, Glycosides, Saponins in ethanol extract; Tannins, Saponins, Glycosides and Steroids in chloroform extract; Terpenoids, Flavonoids, Steroids, Glycosides in petroleum ether extract; and Alkaloids, Terpenoids, Flavonoids, Steroids, Glycosides, Tannins, Saponins in aqueous extract. Results of antibacterial activity of various solvent extracts are depicted in TABLE 2. All the solvent extracts showed varying degrees of antibacterial activity on the bacteria tested. Among different extracts tested, more potency was recorded in aqueous extract followed by chloroform extract, ethanol extract and petroleum ether extract.

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness[6]. When people from remote communities get an infectious disease, they are usually treated by traditional healers because of their expertise in making herbal medicines. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. Patients of tribal communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. However, if they are treated in a hospital the chance of contracting a nosocomial infection is increased[9].

CONCLUSION

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. This *in vitro* study demonstrated that the plant can be an effective medicine to combat infectious microorganisms. The antibacterial activity of the solvent extracts could be due to the various phytochemicals present in them. The millenarian use of plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious dis-

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eases. This plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

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