INTERNATIONAL JOURNAL OF CURRENT MULTIDISCIPLINARY STUDIES
Available Online at http://www.journalijcms.com
VOL. 3, ISSUE,09, PP.798-801, SEPTEMBER, 2017
IJCMS

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF AVICENNIA GERMINANS

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ARTICLE INFO

Received 29th June, 2017
Received in revised form 3rd July, 2017 Accepted 14th August, 2017
Published online 28th September, 2017

Keywords:

Antibacterial activity, *Avicennia germinans*, phytochemical analysis.

ABSTRACT

Plants are a rich source of secondary metabolites with interesting biological activities. *Avicennia germinans* is a traditional medicinal plant and the leaves have tremendous medicinal values. The present investigation deals with qualitative screening of secondary metabolite and antimicrobial activity of *A. germinans* leaves extract belongs to family of Acanthaceae. Plant metabolites screenings were performed by using various solvents systems of varying polarity of acetone, ethanol and aqueous extracts. In this examination the crude extracts showed the presence of flavonoids, carbohydrates, saponins, phlobatannins, and volatile oil while Phenol, steroids and terpenoids were absent in all the solvents. Alkaloids present in acetone and ethanolic extracts. On the other hand tannins were absent only in acetone extracted. Anthroquinone was present in ethanolic and acetone extract. The ethanolic leaf extract was tested against Gram positive and Gram negative bacterial pathogens. Plant extract showed antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis* while no inhibitory activity against *Klepseilla pneumoniae.* The medicinal property of *A. germinans* may be attributed to the presence of flavonoids and phenolic compounds with rich antioxidant potential. The therapeutic effect of this plant may be accounted for its counteracting action on free radicals *in vivo*.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry[1].

Natural products has a vital role in pharmacological and commercial industries, produce a lot of health care and medicinal products such as antimicrobial, anti-tumour agent, anti-hepatotoxic, cardiotonic, CNS stimulant, nutraceuticals, sweeteners, food additives and animal feed[2]. In addition, plants contain important bioactive clusters such as alkaloids, flavonoids, saponins, steroids, terpenoids and tannins that are largely contributing to various biological activities in traditional and modern therapeutic principles[3-4].

Avicennia germinans L. is a mangrove plants belongs to the family Acanthaceae. Mangrove forest can decay into peat deposits because of fungal and bacterial processes as well as by the action of termites. It becomes peat in good geochemical, sedimentary and tectonic conditions. *Avicennia germinans* or black mangroves occupy different ecological niches and have slightly different chemical compositions so the carbon content various between the species as well as different tissues of the plant leaves and roots. Mangrove forest can decay into peat deposits because of fungal and bacterial processes as well as by the action of termites. It becomes peat in good geochemical,

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sedimentary and tectonic conditions. *Avicennia germinans* or black mangroves occupy different ecological niches and have slightly different chemical compositions so the carbon content various between the species as well as different tissues of the plant leaves and roots. The plant useful to diuretic, febrigual and anti-inflammatory effects, cure swellings of the skin, leprosy, laxative, sore eyes, sore throats leaves are used as human food, as medicine for infected wounds[5].

A broad range of phytochemicals present in plants are known to inhibit bacterial pathogens[6]. The determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds. To maximize up take the recovery of plant antimicrobials for human consumption, establishing optimal and specific extraction condition using various solvent system. Hence, the objective of this study was to determine qualitative investigation was carried out to evaluate the presence of phytochemicals. Furthermore, the ethanolic leaf extract as a good source for the determination of the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Collection and Authentication of Experimental Plant: The mangrove plant of *Avicennia germinans* leaves were collected from Muthupet mangrove, Tamil Nadu, South India. The leaves were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora[7-8] and standard references[9].

Preparation of Extract: The dried and powdered leaves of *Avicennia germinans* (500 g) were extracted using soxhlet extractor by evaporating with 75% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55°C.

Phytochemical Analysis: The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described[10-12]. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Microorganisms: Bacterial strains were obtained from Department of Microbiology, Pathology section, Thanjavur Tamil Nadu and were used for assay of antibacterial activity; Microorganisms were maintained at 4°C on nutrient agar slants. The studied bacterial strains comprised: *Bacillus subtilis, Staphylococcus aureus, enterococcus faecalis, Escherichia coli, Klebsiella pneumonia.*

Antibacterial Assay: The antibacterial assay was performed by agar well diffusion method[13] for solvent extract. The Muller Hinton Agar media was inoculated with the 100 µl of the inoculum ($1x10^8$ Cfu) and poured in to petriplates. In this method a well was prepared in the plate using a cork-borer (0.85) 50,100µg of test sample was introduced in to the well. The plates were incubated overnight at 37^0C and microbial growth was determined by measuring the diameter of zone of inhibition. The controls were maintained where pure solvent was used instead of the extract for each strain.

RESULTS AND DISCUSSION

The result of phytochemical screening of the alcoholic extracts of *Avicennia germinans* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The plant extract of *Avicennia germinans* used for the present work was choosing on the basis of their medicinal values. Previous study in the naturally the ethanolic extracts of *Avicennia* spp. were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results[14].

Table 1 Qualitative Phytochemical screening on extracts of *Avicennia germinans*

S. No	Name of Test	Test applied / Reagent used	Leaves extract
1	Alkaloids	A. Mayer's	+
		B. Wagner's	+
		C. Hagner's	+
		D. Dragndorff's test	+
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A. 10% Lead acetate	+
		B. $Fecl_3$	+
5	Test for Steroids	A. Salkowski's Test	+
		B. Libermann-Burchard's Test	+
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	-
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A. Biuret test	+
		B. Ninhydrin test	+
		C. Xanthoprotein test	+

-, absents; +, present;

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers[10]. In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *A. germinans*. Boominathan and Ramamurthy[1] reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

The presence of some of these secondary metabolites suggests that the plant might be of medicinal significance and supports the origin for some of the ethno-uses. For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-

inflammatory, anti- microbial antioxidant, anti- cancer activity[15]. It also suggests that the plant might have diuretic properties[16]. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns[17]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions[18]. Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties[15,19]. Volatile oils are used in the industries for various purposes, both as a pharmaceutical/cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections. They are also used as flavouring agents, in aromatherapy, perfumery etc. egs are eucalyptus oil, lemon oil and peppermint.

In the present study plant extract of *A. germinans* showed higher antibacterial activity against *Escherichia coli* than *Bacillus subtilis, Staphylococcus aureus Enterococcus faecalis* while no inhibitory activity against *Klebsiella pneumonia* (Fig.1).

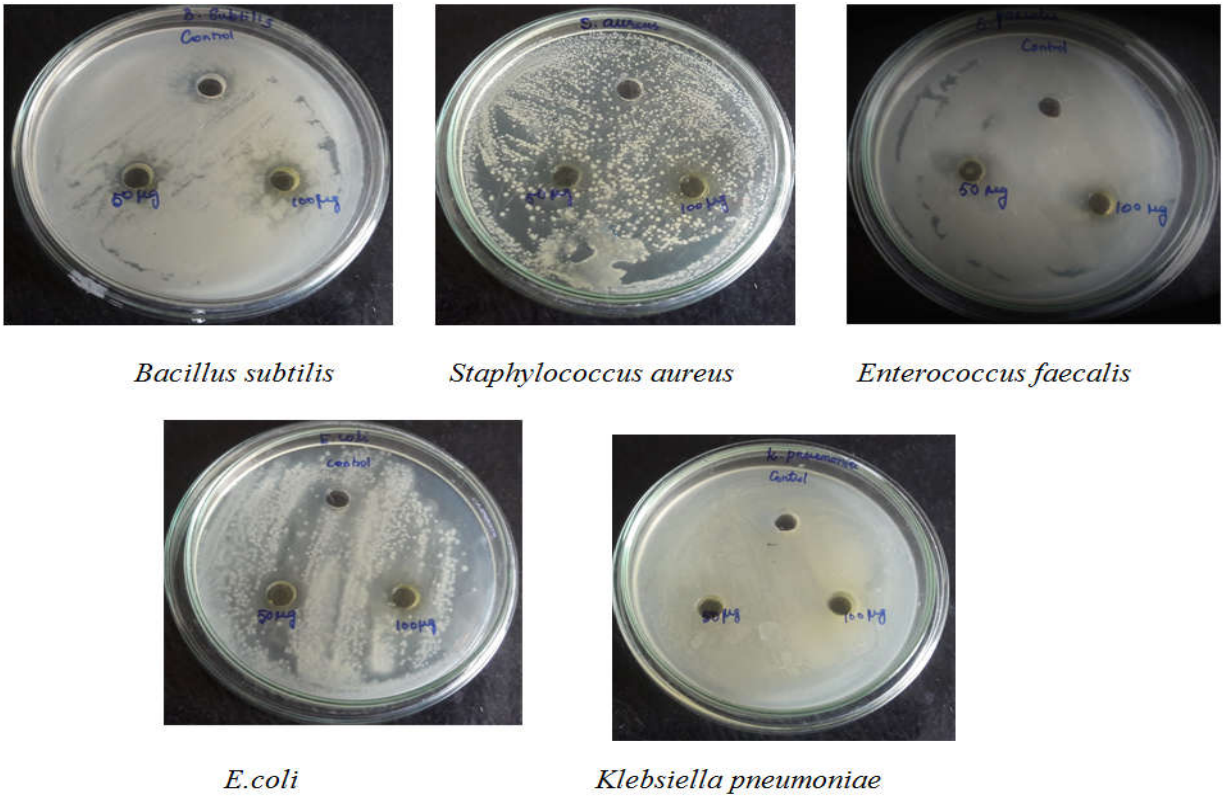

Fig 1 Photograph of Antibacterial activity

This study also shows the presence of different phytochemicals with biological activity that can be valuable therapeutic index. From the result, it is concluded that *A. germinans* have great potential use as phytomedicine and have pharmacological activities. Development of phytomedicine is inexpensive and less time consuming and suitable to our economic conditions.

In overall conclusion the medicinal plants have the great therapeutic and economic values in all over the world. The present results offer a scientific basis for traditional use of *A. germinans* against various ailments. Further studies are required for this plant to validate their medicinal importance. In addition, isolation, characterization and elucidation of the structures of the bioactive compounds which may be responsible for their antimicrobial activity and other medicinal values of this widely available weed *A. germinans*.

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