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ANTIBACTERIAL ACTIVITY IN THE LEAVES OF SEVEN BITTER MEDICINAL PLANTS OF BANGLADESH

M T H Molla¹, M S Ahsan, M T Alam, M E Haque^{*}

¹Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Bangladesh.

Abstract

Context: Development of resistance in human pathogens against conventional antibiotic necessitates searching indigenous medicinal plants having antibacterial property. Seven medicinal plants used activity in folklore, ayurvedic and traditional system of medicine were selected for the evaluation of their antimicrobial activity for this study.

Objectives: Evaluation of the effectiveness of some medicinal plant extracts against four Gram positive and five Gram negative bacteria.

Materials and Methods: The antibacterial activity of the crude ethanolic extracts obtained from the leaves of seven medicinal plants; viz., *Andropogon paniculata*, *Centranthus roseus*, *Adhatoda vesica*, *Vitex negundo*, *Albizia vera*, *Placotria ramonchii* and *Nyctanthes arbor-tristis* were tested against nine bacteria at concentrations of 300, 400 and 500 µg/ml. Standard antibiotic disc kanamycin (30µg/ml) was used for comparison. The minimum inhibitory concentration (MIC) of ethanolic extracts of the leaves of these medicinal plants were determined by testing the extracts on four Gram positive and five Gram negative bacteria by serial tube dilution method.

Results: All the extracts have notable antimicrobial activities against the test organisms. The ethanolic extracts of the leaves showed the highest antimicrobial activities against *Bacillus megaterium* and *Shigella dysenteriae* for *Az. paniculata*, *Al. vera* and *Al. vera*; *Bacillus subtilis* and *Salmonella typhi* for *C. roseus* and *N. arbor-tristis*; *Staphylococcus aureus* and *Salmonella typhi* for *V. negundo* and *Bacillus subtilis* and *Shigella sonnei* for *P. ramonchii* respectively. The extract of the plants had MIC values ranging from 32 to 128 µg/ml. All plant extracts showed no MIC against *Shigella dysenteriae* and against *Shigella flexneri* only *C. nitida* showed MIC 128 µg/ml.

Conclusion: The results revealed that the ethanolic extracts of the plants under present investigation have notable antimicrobial activities.

Keywords: medicinal plants, antimicrobial screening, MIC, bacteria.

Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava et al. 1996).

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel 1991). Although a significant number of studies have been used to obtain purified phytochemicals, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytochemicals present in the plants (Veeramuthu et al. 2006). The greater susceptibility of gram positive bacteria to plant extracts has been previously reported in South American (Paz et al. 1995), African (Kudi et al. 1999, Vlietinck et al. 1995) and Australian (Palombo and Semple 2001) medicinal plant extracts. Susceptibility differences between gram positive and gram negative bacteria may be due to cell wall structural differences between these classes of bacteria. The gram negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tontora et al. 2001).

^{*} Department of Pharmacy, University of Rajshahi, Bangladesh. *Corresponding author: tanzil_hossain@yahoo.com

RESEARCH ARTICLE



Phytochemical Screening and Antibacterial Activity of *Adhatoda vesica*

Sharav Desai^{1*}, Dhara Patel¹

Abstract: Aim of the present study was to perform preliminary phytochemical screening and evaluate the anti bacterial activity for three extracts ethanolic, petroleum ether and aqueous of *Adhatoda vesica* belonging to family *Acanthaceae*. *Adhatoda vesica* is an important herb in the Indian system of ayurvedic medicine. It has been traditionally used to treat cough, whooping cough, cold and lung phlegm in mouth, throat, chest or breast. It is also effective against Tuberculosis, fever, leukaemia and skin diseases. Study was conducted using aqueous, pet. ether and ethanolic extract leaves. A preliminary phytochemical screening was also performed for qualitative determination of the phytoconstituents. Antibacterial assay was carried out using different strains of bacteria like *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041) and *Micrococcus luteus* (MTCC No.106), using cup plate method. Phytochemical screening indicated the presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavonoids, amino acids and reducing sugars. From anti microbial assay was found that ethanolic extract was effective against all the bacterial strains, where as mixed effect observed in remaining two extracts.

INTRODUCTION
Adhatoda vesica belongs to the family of *Acanthaceae*. It is an erect, terrestrial, perennial shrub. The leaves are dark green above and pale yellow below. The flowers are typical, white, arranged in a pedunculated spike. It is a primary herb of the ayurvedic system used in the treatment of cough, bronchitis, asthma and symptoms of common cold. A yogic practice is to chew the leaf buds alone, or with a little ginger root, to clear the respiratory passages in preparation for the vigorous breathing exercises. It is used as an ingredient in numerous popular formulations, including cough syrups, in which it is frequently combined with tulsi (holy basil) and ginger. Its main action is as an expectorant and antispasmodic (bronchodilator). The important active components include alkaloids vasicine (aka peganine) and vasicinone. The former is under development as herbal drug in India, as are the semi-synthetic derivatives of alkaloids, bromhexine and ambroxol. A secondary property of the herb is that it helps to stop bleeding. The roots, leaves and flowers of the plant are used for the extraction of volatile oils (heptanone) and alkaloids, which have great medicinal importance.^[1-3]

MATERIALS AND METHODS

Plant Collection and Sample Preparations

The leaves of the plant *Adhatoda vesica* were collected from the open fields of Vadodra District, Gujarat, India. The plants were identified and the fresh leaves were dried for 7 hours at 50-60°C. The dried samples were then crushed into powder using an electronic blender. The powdered sample was stored in a bottle at room temperature, prior to analysis.

Microorganisms

In this present study the test microorganisms used (bacteria: *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis*

(MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041) and *Micrococcus luteus* (MTCC No.106), were purchased from MTCC Chandigarh. All the bacterial strains were maintained on nutrient agar (NA, Hi-Media) at 37°C. Bacteria were inoculated in nutrient broth (NB, Hi-Media) and incubated at 37°C for 24 hours for prior the test. Mueller-Hinton Agar (MHA, Hi-Media) used for testing the antibacterial activity.^[3-5]

Preparation of Extracts

A powdered sample of 100 gm was weighed and soaked in 200 ml of 95% ethanol in a separating funnel for 36 hours, with occasional shaking. The plant extract was then collected and filtered through Whatman filter paper No.1. The extract was concentrated at 55°C using a rotary evaporator and then air-dried. The dried powder was stored at 40°C in an airtight bottle. Similarly, the procedure was repeated with petroleum ether and water as solvents, using 100 gm of the fresh ground sample, for each extraction. All the extracts were cooled at room temperature.^[6]

Phytochemical Analysis

The extracts were analyzed for the presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavonoids, amino acids and reducing sugars, using the standard procedure.^[7-9]

Anti Microbial Assay

Approximately 20 ml of sterile Muller-Hinton Agar (MHA) was poured into sterile Petri plates and allowed to set. Plates were then seeded with 0.5 ml of a 24 h old bacterial culture and using a sterile glass (L) rod made a lawn culture.^[10] The plates were allowed to dry. For doing agar well diffusion method, wells are made on the plate with the aid of a sterile hole puncture (8.0 mm diameter). Two hundred micro litres of the crude plant extract was poured into the respective wells.^[11-13] The plates thus prepared were left at room temperature for ten minutes, allowing the diffusion of the extracts into the agar. Then the plates with bacterial culture were placed in the incubator at 37°C for

ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC FUNGUS *FUSARIUM* SP. ISOLATED FROM MEDICINAL HONEYSUCKLE PLANT

Huiru Zhang¹, Xinchun Sun² and Chungping Xu^{2*}

¹ College of Bioengineering, Henan University of Technology, Zhengzhou, Henan, 450001, P.R. China

² College of Food and Biological Engineering, Zhengzhou University of Light Industry, Zhengzhou, Henan, 450002, P.R. China

*Corresponding author: c.p.xu@zzuli.edu.cn; xuchunping05@163.com

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Abstract: Endophytes of plants have a wide range of strains comprising important sources of various bioactive metabolites. An endophytic fungus was isolated from honeysuckle, an important Chinese medicinal plant. The phylogenetic and physiological characterization indicated that the isolated strain JY2 corresponded to *Fusarium* sp. The culture filtrate of JY2 displayed antagonism activity against some pathogenic bacteria owing to the existence of antimicrobial compounds. The filtrate revealed the strongest *in vitro* antimicrobial activity on *Pseudomonas aeruginosa* by increasing the permeability of cell membranes. The antibacterial extract was fractionated and purified using silica gel chromatography. Five different bioactive compounds were isolated by bioactivity-guided fractionation from the culture extracts of JY2, and preliminarily identified by HPLC-MS spectral data. These results suggest that *Fusarium* sp. features a potentially remarkable antimicrobial activity and could be valuable to discover the new drugs or agents for antimicrobial purposes.

Key words: Endophyte; antimicrobial activity; *Fusarium* sp.; culture broth

INTRODUCTION

Endophytes are endosymbiotic microorganisms (e.g. bacteria or fungi) that live within plants for at least part of their life without causing apparent disease. Endophytes ubiquitously existing in plants have a wide range of antimicrobial strains comprising important sources of various bioactive metabolites, including antimicrobial, antiviral, anticancer and antidiabetic compounds (Strobel and Daisy, 2003). Bioactive natural compounds produced by endophytes have been showing some promising potential as remedies for human health concerns. The antimicrobial compounds are involved in defense against pathogens (Aly et al., 2011). In addition, antimicrobial compounds may also reduce cell toxicity towards higher organisms because the plant itself serves as a natural selection system (Yang et al., 1977). Plants are generally hosts to one or more endophytes (Schulz et al., 1995). Hence, endophytes have a remarkable potential as a source of

novel, highly active antimicrobial compounds with low toxicity.

The dried flower buds of honeysuckle (*Lonicera japonica* Thumb.) have been utilized in traditional Chinese medicine for over 1000 years (Chen et al., 2005; Li et al., 2003). This plant is commonly cultivated as a highly valued medicinal and garden plant in eastern Asia, particularly China (Chai et al., 2005). Interestingly, the available literature provides no information on the isolation of any endophytes from honeysuckle or identification/proof of bioactive substances with any biological activities.

In the light of this, the purpose of our study was to isolate, select and identify the endophytic fungus *Fusarium* sp. JY2 from honeysuckle. Gram-negative *Pseudomonas aeruginosa* was selected as a test bacterium to investigate the bacterial lytic activity on the culture filtrate of strain JY2.

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ORIGINAL PAPER

Enhanced Antibacterial, Anti-biofilm and Antioxidant (ROS) Activities of Biomolecules Engineered Silver Nanoparticles Against Clinically Isolated Gram Positive and Gram Negative Microbial Pathogens

G. Prasannaraj^{1,2} · P. Venkatachalam^{1,2}

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Abstract Biomolecule-coated silver nanoparticles (AgNPs) were synthesized using ten different medicinal plants and studied their antibacterial and anti-biofilm activity against selected clinically isolated microbial pathogens. Though the bioengineered AgNPs showed effective antibacterial activity, the maximum rate of growth inhibition was recorded in liquid medium. A potential anti-biofilm activity was determined using biomolecule-coated AgNPs derived from *Centella asiatica*, *Plumbago zeylanica* and *Semecarpus anacardium* against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Results strongly suggest that bioengineered AgNPs at lower doses can be used for the treatment of multidrug-resistant bacterial infections with biofilm formation. Further, the XTT assay exhibited an increased level of reactive oxygen species production in bacterial strains treated with bioengineered AgNPs using 10 plant species. Present results clearly indicate that biomolecule-coated AgNPs could be used as effective nanodrug for treatment of infectious diseases caused by multidrug resistant bacterial strains in the near future.

Keywords Silver nanoparticles · Antibacterial activity · Anti-biofilm activity · ROS activity · Clinical pathogens

Introduction

Currently, the nanomaterials are being considered as leading edge in the field of nanotechnology. Their unique properties make them superior and indispensable. Though nanoparticles are synthesized successfully using chemical and physical

✉ P. Venkatachalam
pvenkat67@yahoo.com; pvenkatlab@yahoo.in

¹ Plant Genetic Engineering and Molecular Biology Lab, Department of Biotechnology, School of Biosciences, Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu 636 011, India

² Centre for Nanoscience and Nanotechnology, Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu 636 011, India

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Antibacterial and antifungal activity of medicinal plants. Antibacterial activity of medicinal plants pdf. Antibacterial and antioxidant activity of medicinal plants. Antibacterial activity of medicinal plants 2019. Antibacterial activity of extracts of twelve common medicinal plants from the philippines. Antibacterial activity of medicinal plants from the philippines. Antibacterial activity of some indian medicinal plants. Antibacterial activity of medicinal plants review.

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[Google Scholar]17. The ethnobotAonic study of these plants was studied and confirmed by a botÄnica expert from the Herbario Universitario Hazara, Mansehra. aureus. After plant 3, the antibacterial activity of these extracts against B. coli (17 mm), S. BMC Complement Altern Med. Peirano G. 2000 Dec;66 (8):687Äe A'693. [Free article PMC] [PubMed] [Google Scholar]4. FrÄa water extract from B. Oxford, United Kingdom: Oxford. Publishers: 1984. aureus were inhibited in the water extracts frÄa while in the hot water extract sÄ 3 lo P. ciliata (raÄees). J. Antibacterial and antifungal effects of alcohÄ 3 licos extracts from 41 medicinal plants growing in TuruÄa. officinale and S. The extracts were sieved through a pair of muslin and centrifuged at 4400 rpm for 7 min. Antimicrobial activity of ethanÄÄ 3 aqueous extracts of medicinal plants against patÄ 3 waste water genus. Int J Environ Res Public Health. ciliata extract has potential inhibitory effects on all bacteria tested in both frÄa and hot water; while J. doi extracts: 10.1086/590 062. subtilis, which is comparable with an 3 inhibitory zone exhibited by ceftriaxone and erythromycin. 2007 May 4;111 (2):409Äe I 412.gar is followed by B. album. Ä'Antibacterial Activity of Medicinal Plants and their Constituents in the Context of Skin and Wound Infections, Considering European Legislation and Folk MedicineÄ' International Journal of Molecular Sciences 22, no. Antibacterial treatment 3 a preferred option for treating bacterial infections; however, the emergence 3 antimicrobial resistance and problems toxicity decrease the use of anti-bacterial agents [2, 3]. Plant extracts dissolved in hot and cold water showed variable antibacterial activity against selected bacteria The extract of B. Centrifugation and filtration process were repeated three times. S. [Free article from PMC] [PubMed] [Google Scholar] Pak J Pharm Sci. [PubMed] [Google Scholar] 5. CRUZ MC, SANTOS PO, BARBOSA JR AM, DE MÄeLO DL, ALVIANO CS, Antonioli AR, Alviano DS, Trindade RC. Coli, S.Aureus, and P. coli (0.6 mm), S. OFFICIAL EXTRACTS. Aureus, and P. aeruginosa. aeruginosa (1.0 mm). This trend encourages researchers to search for new effective and safe therapeutic agents. Vulgaris showed a minimal inhibition zone, while other bacteria were resistant to plant extract dissolved in hot water. They observed the imminent antimicrobial activity of J. The source of information about these plant products in the review is represented by research articles listed in scientific databases (Science Direct, Pubmed, Scopus, Web of Science, etc.) published in the last few years. Antibiotic Annu. Aureus, who showed resistance. Aeruginosa and S. Antimicrobial activity of aqueous leaf extract and stem extract from the SANTALUM album. Talibh, Mahasneh am. Antioxidant and antimicrobial activity of feniculum vulgare and crinum maritimas essential oils [PubMed] [Google Scholar] 2. Officinale (leaves) showed a 16 mm inhibition zone against P. The European legislation is in the field of natural products, medicinal use formed by the European Medicines Agency (EMA), based on the scientific work of its Commission on Herbal Medicines (HMPC). aeruginosa (17 mm), P. officinale (leaves), and S. 2009 Jan; 10 (1): 106-121. Traditionally, raw plant extracts are used as herbal medicine for the treatment of human infectious diseases [1, 3]. J Appl Microbiol. [Google Scholar] 10. 32-33. 2011. June 2011; 11: 52. Multiple Enterobacteria enterobacteriaceae new threat to an old problem; review 3 anti-infective therapy. album (wood) showed an inhibition zone 3 n (15 mm) against P. subtilis (14 mm), E. 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