In vitro antimicrobial activity of Achyranthes coynei Sant.

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Objective: To validate the traditional use of Achyranthes coynei (A. coynei) Sant. as an antimicrobial in treatment of various infectious diseases. Methods: Leaf extracts of A. coynei obtained through successive solvent extraction using petroleum ether, dichloromethane, chloroform and methanol were used to screen the antimicrobial activity on five Gram positive, five Gram negative bacteria and two fungi. Minimum inhibitory concentration (MIC) was determined by two fold tube–dilution method. Results: Methanolic leaf extract was more effective than other three extracts on the tested bacteria. Methanolic extract was efficient on Staphylococcus epidermis, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa with MIC values (0.62±0.00) mg/mL. The fungal organisms were less susceptible against extracts tested. Conclusions: These results support the traditional use of leaf extracts of A. coynei as they have antimicrobial potential. Further studies are needed for establishing safety, toxicity and pharmacological activity with phytochemical investigation.

1. Introduction

Art of prevention and treatment of ailments by human using plant and plant parts is time immemorial. Documentary evidences for the usage can be traced back over five to six millennia of the civilization from India. However, over the years, traditional knowledge has given way to the advent of modern medicine and much of the knowledge is lost after generations of unuse. Inability of modern medicine system in treating several communicable and chronic diseases along with the advent of highly resistant microorganisms has resulted in renewed and rejuvenated efforts in the quest for plants with medicinal properties[1]. Plants especially with ethnomedicinal use have attracted the scientific community to evaluate their complete range of biological activities starting from antibiotic to antitumor activities. The potential of these plants as source for new drugs largely remain unexplored even today. Among as estimated 500 000 plant species, only a small percentage have been investigated for their biological or pharmacological activity[2]. Plants screened for antibacterial activities have provided modern medicine with an abundance of drugs and treatments against the infections.

Achyranthes coynei Sant. (A. coynei) belongs to family Amaranthaceae, locally known as Kempu Uttarani in Kannada and Lal Agadha in Marathi. It is a perennial, profusely branching shrub grows up to 2.0-4.5 m high. A. coynei is rated as rare and endemic to India reported from Karnataka and Maharashtra states[3]. The plant is used in the similar lines...
as that of Achyranthes aspera (A. aspera) to treat a range of ailments like leprosy, leucoderma, malaria, etc., by traditional and Ayurvedic practitioners of the region [3,4]. Due to the high medicinal value and usage, A. aspera has been identified as one of the medicinal plant species in high trade sourced by National Medicinal Plant Board of India, and hence there is ever increasing demand for the plant [4,5]. On the contrary, due to endemic status, restricted distribution and similarity with A. aspera, no scientific research has been carried out on medicinal properties of A. coynei. The present investigation aims to screen antimicrobial efficacy of the leaf extracts of A. coynei against ten bacterial and two fungal pathogenic strains.

2. Materials and methods

2.1. Collection of plant material

A. coynei was collected from a single population at Pachhapur, from Belgaum district of Karnataka state, India. Specimen was identified, authenticated and deposited at the Herbaria of Regional Medical Research Centre, Belgaum (voucher specimen number: RMRC 784).

2.2. Preparation of extract

Collected plant material was washed in running water to remove dust particles. The dried leaves were coarsely powdered, subjected to successive extraction with different solvents in increasing order of polarity viz. petroleum ether, dichloro methane, chloroform and methanol. Each time before extracting with next solvent, the material was dried in hot air oven below 50 °C. Extracts obtained were concentrated under reduced pressure at 40 °C using rotary evaporator.

2.3. Antimicrobial activity

2.3.1. Microbial strains

Bacterial strain: Micrococcus luteus (M. luteus, NCIM 2871), Staphylococcus epidermis (S. epidermis, NCIM 2493), Bacillus subtilis (B. subtilis, NCIM 2063), Micrococcus flavus (M. flavus, NCIM 2376), Staphylococcus aureus (S. aureus, NCIM 2671), Escherichia coli (E. coli, NCIM 2574), Klebsiella noclumus (K. noclumus, NCIM 2957), Pseudomonas aeruginosa (P. aeruginosa, NCIM 5029), Salmonella typhimurium (S. typhimurium, NCIM 2501), Enterobacter aerogenes (E. aerogenes, NCIM 5139) and fungal strains: Aspergillus noclaius (A. noclaius, NCIM 902), Aspergillus niger (A. niger, NCIM 620) were procured from National Collection of Industrial Microorganisms, Pune, India.

2.3.2. Preparation of test sample

The leaf extracts of A. coynei were dissolved in 10% dimethyl sulfoxide. Streptomycin and fluconazole were used as standard antibacterial and antifungal drugs, respectively.

2.3.3. Preparation of inoculums

The inocula were prepared in Nutrient broth media after incubation for 18 to 24 h at 37 °C. The suspensions were adjusted to 0.5 McFarland standard turbidity [6].

2.4. Tube dilution method

Two-fold tube dilution method was followed to determine the minimum inhibitory concentration (MIC) of different extracts against the test microorganisms [7]. The concentration of extracts were made ranging from 10.000 to 0.019 mg/mL. Serial two-fold dilutions were prepared ranging from 5.000 to 0.009 mg/mL. Tubes were incubated for 24 and 48 h at 37 °C for bacteria and fungi, respectively. Experiments were replicated triple (n=3) with mean±SD.

Antimicrobial activity of four different leaf extracts were studied against five Gram positive, five Gram negative and two fungal organisms using serial dilution method. The activity was compared against standards (streptomycin for antibacterial and fluconazole for antifungal).

Means between treatments groups were compared against control group for significance using Duncan’s new multiple range post test. MIC was determined as the lowest concentration that inhibits visible growth of microorganisms.

3. Results

The results are presented in Table 1 and activity was expressed as mg/mL. All extracts inhibited growth of organisms confirming antimicrobial property of A. coynei. Varying degrees of MIC for each extract against given organisms are showed. Out of ten microbial organisms tested, A. niger and A. fumigatus (fungal strains) had greater MIC values whereas; B. subtilis and S. aureus were the most susceptible bacterial organisms.

Among the Gram positive bacteria, all leaf extracts were effective on B. subtilis and S. aureus with MIC values less than 1.00 mg/mL. Petroleum ether and dichloromethane leaf extracts showed inhibition at 0.83 mg/mL, whereas chloroform and methanol extracts showed inhibition at 0.62 mg/mL. The other Gram positive organisms M. luteus, S. epidermis and M. flavus were not susceptible, compared to the above organisms, except methanolic extract (MIC<1 mg/mL).

Similarly, among five Gram negative bacteria, P. aeruginosa and E. aerogenes were inhibited more effectively by A. coynei leaf extracts than the others. Escherichia coli, K. pneumonia and S. typhimurium had MIC values ranging from 2.50 to 1.25 mg/mL representing greater resistance to the extracts tested. The antibacterial activity was more prominent on Gram positive bacteria than Gram negative bacteria.

Methanolic leaf extract of A. coynei exhibited considerable antimicrobial activity over all other extracts against pathogens tested. Methanolic extract had higher amount of antibacterial activity in Gram positive bacteria than in Gram negative
bacteria (1.25 to 0.62 mg/mL). In fungal organisms, methanolic extract showed half the MIC than the other three extracts. However the standard streptomycin was more potent antibacterial than all extracts with the MIC values ranging from 0.009 to 0.019 mg/mL and 0.025 mg/mL for fluconazole and streptomycin, respectively. In bacterial organisms, methanolic extract from leaf of A. coynei showed significant antimicrobial activity. Similar results were demonstrated using Achyranthes bidentata extracts on B. subtilis, S. aureus, E. coli, P. aeruginosa and others in A. aspera which proved its antibacterial and antifungal activity both in crude extract as well as by isolated compounds.

The study also revealed that petroleum ether extract showed minimum antimicrobial activity. However, Murugesan et al., showed that petroleum ether extract of Memecylon umbellatum shows significant antimicrobial activity. Furthermore, A. aspera essential oils also showed little fungicidal activity which is corroborated by our study[22,23]. Present study suggests further need for detailed phytochemical investigation and pharmacological studies to support use of this plant by traditional practitioners.

In conclusion, A. coynei contains potential antimicrobial components that may be of practical use for therapy against various infectious diseases. The methanolic leaf extract of A. coynei possesses significant inhibitory effect against tested microorganisms.

4. Discussions

The preliminary investigation presented is based on anecdotal evidence and traditional use of the plant as medicine. The study showed that all the four solvent extracts from leaf of A. coynei were active against potentially pathogenic microbes. This analysis of using several extracts to study efficacy of plants for antimicrobial activity is realized by many studies in several common medicinal plant species[8-11]. Out of the four solvents used for extraction, methanolic extract of plant showed significant antimicrobial activity against microorganisms, followed by chloroform, dichloromethane and petroleum ether extracts. Though, the mechanism of action of these plant constituents is not yet fully known, it is clear that the effectiveness of extracts largely depends on type of solvent used. Different solvents have been reported to have capacity to extract different microbial phytoconstituents, showing highest relative antimicrobial activity. Similar results were demonstrated using Achyranthes bidentata extracts on B. subtilis, S. aureus, E. coli, P. aeruginosa and others in A. aspera which proved its antibacterial and antifungal activity both in crude

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PE: Petroleum ether; DCM: Di–chloromethane; CHL: Chloroform; MeOH: Methanol; G+: Gram positive bacteria; G−: Gram negative bacteria; Standard*: Streptomycin for bacterial and fluconazole for fungal strains; a: Significant P<0.01; b: Fairly significant P<0.05; c: not significant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

A. coynei (Amaranthaceae) a perennial shrub, is endemic to Maharashtra state, India. The authors have recently reported its occurrence to Karnataka State, India. Reports suggest its uses are in lines of A. aspera to treat a range of ailments

Table 1

MIC values (mg/mL) of A. coynei leaf extracts of different solvents.

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by TP. As per literature survey it is seen that, nothing is known about medicinal properties of A. coynei. Present investigation adds to the scientific information in this plant species.

Research frontiers
The main cutting edge in the field of research in this paper is the first report of antimicrobial activity in A. coynei.

Related reports
Antimicrobial studies have been performed in some species of the genus Achyranthes by Balakrishnan et al., 2003 in A. bidentata and many others in A. aspera by Gupta et al., 2004 and Parmar et al., 2012. Authors have taken note of earlier studies to carry out the experiments in the species.

Innovations & breakthroughs
The article is the first report of antimicrobial activity of this endemic and Rare medicinal plant from India.

Applications
It is important to study this plant in detail as the plant carry endemic status. In the present scenario of microbial strains developing resistance against many drugs, it has been important field of research to find out new sources. It may act as substitute for A. aspera and because its endemic status may contain novel phytoconstituent. This study may lead to identification of potent drugs from plant sources.

Peer review
This is a good study in which the authors have evaluated the antimicrobial activity of A. coynei leaves using bacterial and fungal pathogenic strains. The results have demonstrated methanolic leaf extract of A. coynei to be highly effective on four bacterial stains including two Staphylococcus spp.

References