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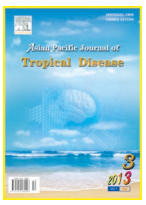

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In vitro antimicrobial activity of *Achyranthes coynei* Sant.

Gireesh Ankad, Vinayak Upadhya*, Sandeep R. Pai, Harsha V. Hegde, Subarna Roy

Regional Medical Research Centre, ICMR, Nehru Nagar, Belgaum-590010, India

PEER REVIEW

Peer reviewer

Dr. Mansingraj S. Nimbalkar, UGC-DSK-PDF, Department of Biotechnology, Shivaji University, Kolhapur, Maharashtra, India.
Tel: +912312609155
Fax: +912312692333
E-mail: mansingrajsu@gmail.com

Comments

This is a good study in which the authors evaluated the antimicrobial activity of *A. coynei* leaves using bacterial and fungal pathogenic strains. The results demonstrated methanolic extract of *A. coynei* highly effective on four bacterial stains including two *Staphylococcus* spp.
(Details on Page 194)

ABSTRACT

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, dichrloromethane, 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.

KEYWORDS

Achyranthes coynei, Endemic, Antimicrobial, Tube-dilution, Minimum inhibitory concentration

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 500000 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 Maharasthra states[3]. The plant is used in the similar lines

*Corresponding author: Vinayak Upadhya, Regional Medical Research Centre, ICMR, Nehru Nagar, Belgaum-590 010, India.
Tel: +918312475477/78
Fax: +918312475479
E-mail: sirsivinayak@yahoo.com
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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 2871), *Escherichia coli* (*E. coli*, NCIM 2574), *Klebsiella noculums* (*K. noculums*, NCIM 2957), *Pseudomonas aeruginosa* (*P. aeruginosa*, NCIM 5029), *Salmonella typhimurium* (*S. typhimurium*, NCIM 2501), *Enterobacter aerogenes* (*E. aerogenes*, NCIM 5139) and fungal strains: *Aspergillus noculums* (*A. noculums*, 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 fluconozole 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

Table 1

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

Organisms	Gram stain	*A. coynei* (mg/mL)				Standard* (mg/mL)
		PE	DCM	CHL	MeOH	
M. luteus	G_+	1.66±0.58[a]	1.25±0.00[a]	1.04±0.29[a]	0.83±0.29[b]	0.012±0.005
S. epidermis	G_+	2.08±0.58[a]	1.66±0.58[a]	0.83±0.29[c]	0.62±0.00[c]	0.012±0.005
B. subtilis	G_+	0.83±0.29[a]	0.83±0.29[a]	0.62±0.00[a]	0.62±0.00[a]	0.015±0.005
M. flavus	G_+	1.25±0.00[a]	1.66±0.58[a]	1.25±0.00[a]	0.83±0.29[b]	0.009±0.000
S. aureus	G_+	0.83±0.29[a]	0.83±0.29[a]	0.62±0.00[a]	0.62±0.00[a]	0.015±0.005
E. coli	G−	2.50±0.00[a]	2.08±0.58[a]	1.66±0.58[a]	1.25±0.00[a]	0.012±0.005
K. pneumoniae	G−	2.50±0.00	2.50±0.00	1.25±0.00	1.25±0.00	0.019±0.000
P. aeruginosa	G−	1.66±0.58[a]	1.25±0.00[a]	0.83±0.29[a]	0.62±0.00[c]	0.009±0.000
S. typhimurium	G−	2.50±0.00[a]	2.50±0.00[a]	2.08±0.58[a]	1.25±0.00[a]	0.019±0.000
E. aerogenes	G−	1.25±0.00[a]	1.66±0.58[a]	0.87±0.29[b]	0.62±0.00[c]	0.012±0.005
A. fumigatus		4.46±1.17[a]	4.46±1.17[a]	3.33±1.17[a]	2.5±0.00[b]	0.025±0.009
A. niger		4.46±1.17[a]	4.46±1.17[a]	4.46±1.17[a]	2.08±0.58[c]	0.025±0.009

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.

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 fluconozole as antifungal control. Results of MIC obtained showed statistical significance at P value <0.05.

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, dichloromoethane 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 phytoconstituents depending on their solubility and/or polarity[12]. Cowan mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily be solubilized in organic solvents. In present study methanolic extract might have higher solubility for more of active antimicrobial 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 extract as well as by isolated compounds[13-20].

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[21]. 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

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

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 phytoconstitutent. 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

[1] Abraham J, Dennis TT. Antibacterial activity of medicinal plant *Cyclea peltata* (Lam) Hooks & Thoms. *Asian Pac J Trop Dis* 2012; **2**(4): 280-284.

[2] Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World J Agric Sci* 2008; **4**: 839-843.

[3] Pai SR, Upadhya V, Hegde HV, Kholkute SD. *Achyranthes coynei* Santapau, 1949 (Amaranthaceae)-an addition to the flora of Karnataka India. *J Threatened Taxa* 2011; **3**(6): 1875-1879.

[4] Gupta AK, Tandon N. *Reviews on Indian medicinal plants*. New Delhi: Indian Council of Medical Research; 2004, p. 140-164.

[5] Parmar N, Rawat M. Medicinal plants used as antimicrobial agents: A review. *Int Res J Pharm* 2012; **3**(1): 31-40.

[6] McFarland J. Standardization of bacterial culture for disc diffusion assay. *J Am Med Assoc* 1987; **49**: 1176-1178.

[7] Joshi RK, Mujawar MHK, Kholkute SD. Antimicrobial activity of the extracts of *Craniotome furcata* (Lamiaceae). *J Ethnopharmacol* 2010; **128**: 703-704.

[8] Rahman MS, Salehin MF, Jamal MA, Pravin HM, Alam A. Antibacterial activity of *Argemone mexicana* L. against water borne microbes. *Res J Med Plant* 2011; **5**(5): 621-626.

[9] Upadhyay RK, Tripathi R, Ahmad S. Antimicrobial activity of two Indian medicinal plants *Tinospora cordifolia* (Family: Menispermaceae) and *Cassia fistula* (Family: Caesalpinaceae) against human pathogenic bacteria. *J Pharm Res* 2011; **4**(1):167-170.

[10] Geethalakshmi R, Sarada DVL, Marimuthu P. Evaluation of antimicrobial and antioxidant potentials of *Trianthema decandra* L. *Asian J Biotech* 2010; **2**(4): 225-231.

[11] Ilango K, Chitra V, Kanimozhi P, Balaji G. Anti diabetic, antioxidant and antibacterial activities of leaf extracts of *Adhatoda zeylanica*. Medic (Acanthaceae). *J Pharm Sci Res* 2009; **2**: 67-73.

[12] Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564-582.

[13] Balakrishnan BR, Sangameswaran B, Arul B, Bhaskar VH. Antibacterial activity of aerial part extracts of *Achyranthes bidentata* Blume. *Indian J Pharm Sci* 2003; **65**: 186-188.

[14] Beaulah AG, Sadiq MA, Santhi JR. Antioxidant and antibacterial activity of *Achyranthes aspera*: an *in vitro* study. *Ann Biol Res* 2011; **2**(5): 662-670.

[15] Khan MTJ, Ahmad K, Alvi MN, Noor-Ul-Amin, Mansoor B, Asif Saeed M, et al. Antibacterial and irritant activities of organic solvent extracts of *Agave americana* Linn., *Albizzia lebbek* Benth. *Achyranthes aspera* Linn., and *Abutilon indicum* Linn-A preliminary investigation. *Pakistan J Zool* 2010; **42**(1): 93-97.

[16] Manjula M, Indira V, Dhasarathan P. Phytochemical characterization and antibacterial activity screening of *Achyranthes aspera*. *Asian J Microbiol Biotech Environ Sci* 2009; **11**(2): 365-368.

[17] Prasad SHKR, Swapna NL, Anthonamma K, Madanprasad RD. Antimicrobial activity of *Achyranthes aspera* and *Aerva lanata* leaf and callus extracts. *Biosci Biotech Res Asian* 2009; **6**(2): 887-891.

[18] Saravanan P, Ramasamy V, Shivakumar T. Antimicrobial activity of leaf extracts of *Achyranthes aspera* Linn. *Asian J Chem* 2008; **20**(1): 823-825.

[19] Sharma S, Shrivastava PN, Saxena RC. Antimicrobial activity of saponins isolated from *Achyranthes aspera* against *Staphylococcus aureus*. *Asian J Chem* 2006; **18**(4): 2766-2770.

[20] Misra TN, Singh RS, Pandey HS, Prasad C, Singh BP. Antifungal essential oil and long chain alcohol from *Achyranthes aspera*. *Phytochem* 1992; **31**(5): 1811-1812.

[21] Murugesan S, Pannerselvam A, Chanemougame T. A phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* Burm. F. *J App Pharma Sci* 2011; **1**(1): 42-45.

[22] Pandey VN, Dubey NK. Effect of essential oils from some higher plants against fungi causing damping off disease. *Biol Plant* 1992; **34**: 143-147.

[23] Ganesan T, Krishnaraju J. Antifungal properties of wild plants. *II Adv Plant Sci* 1995; **8**: 194-196.