Antibacterial Properties of Anthocephalus Cadamba Fruits.

Author(s): Mr. Ram P Mishra

Corresponding Author:
Mr. Ram P Mishra,
Sr. Research Scientist, Department of Microbiology, MRD LifeSciences, B-3/46 & 47, Vibhuti Khand, Gomti Nagar, 226010 - India

Submitting Author:
Mr. Ram P Mishra,
Sr. Research Scientist, Department of Microbiology, MRD LifeSciences, B-3/46 & 47, Vibhuti Khand, Gomti Nagar, 226010 - India

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Abstract

Anthocephalus cadamba is ethnomedically widely used in the form of paste by tribe in Western Ghats for treating skin diseases. In this context, antibacterial properties of Anthocephalus cadamba against a wide range of pathogens were studied. The alcoholic and aqueous extracts of fruits (ripened and un-ripened) of this plant showed significant antibacterial activity against almost all the organisms: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, with zone of inhibition of the maximum 24.0 cm and 22.0 cm against E. coli, P. aeruginosa respectively. The minimum MIC determined, was as low as 1.00 mg/ml for methanolic extracts of green fruit of A. cadamba against P. aeruginosa and S. aureus, respectively.

Introduction

Anthocephalus cadamba (Roxb.) Miq. Syn A. chinensis (Lamk) A. Rich (Rubiaceae) is widely distributed throughout the greater part of India and is used as a folk medicine in the treatment of fever, anaemia, uterine complaints, blood diseases, skin diseases, leprosy, dysentery, and for improvement of semen quality. The leaves are recommended as a gargle in cases of stomatitis (Slkar et al., 1996). Some scientific studies have been carried out to reveal its antimalarial (Stanne and Fanie 2002) and antihepatotoxic activities (Kapil, et al., 1995). The major constituents of bark are triterpenes, tripernoid glycosides, saponins, indole alkaloids cadamine, 3 a-dihydrocadamine, cadamine, isocadamine and isodihydrocadamine (Niranjan et al., 2000; Kitagawa et al., 1996; Mahato and Garai 1998; Brown and Chapple, 1976). In recent years, many possible sources of natural antibiotics are used for several infectious diseases, mostly bacterial and fungal infections. Phytochemistry of A. cadamba and its application in the treatment of various ailments like diabetes mellitus, diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial activity.

Materials and Methods

Sample Collection and Identification of the Plant: The ripened, un-ripened fruits of actively growing A. cadamba from roadside tree were collected at Vibhuti Khand near MRD LifeSciences, Gomti Nagar, Lucknow. Fruits were thoroughly washed under running tap water, rinsed with distilled water and finally air dried and preserved at -20o C till use.

Extraction of bioactive compounds: For extraction of bioactive compounds, 5.0 gm of
powdered plant material was in 50ml of methanol (v/v; 80%); ethanol (v/v; 70%) and ethyl acetate (absolute) and kept in dark for a week, filtered it by Whatman filter paper No.1, filtrate is air dried and solid crystals of the plants extracts was recovered. For hot water extract, plant material was mixed with distilled water (1:10) and kept in boiling water for 2 hrs., followed by filtration by Whatman filter paper No.1 and concentration before use.

**Test Organisms:**
Clinical isolates of bacteria and fungi were used for bioassay studies. The test organism includes Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus. The isolates were maintained on freshly prepared nutrient agar plates and slants and keep in a refrigerator at 40 C until required for use. Single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 oC in shaker incubator at 140 rpm for 14 hrs. Bacterial cells were recovered by centrifugation and were suspended in sterile distilled water; concentration of bacterial cells was optimized to OD 0.1 at 600 nm before use.

**Screening of bioactive compounds against various pathogens:**
20.0 ml nutrient agar media was poured in a sterile Petri dish, 100 ?l of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 30?l plant extracts with positive (Tetracycline; 50?g/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 0C for 14 hrs. The diameters of zones of inhibition were measured.

**Determination of Minimum Inhibitory Concentration (MIC) of extracts:**
This is carried out by double agar gradient plate method. Nutrient agar (10.0 ml) was poured into sterilized Petri dishes, leaving the plate in slanted position. After setting the media, another 10.0 ml of nutrient agar (along with plant extract; 4.0 mg/ml) was added to the plates to make the level unity; thus the plate contained an increasing concentration of plant extract along the diameter of the plate. Now the prepared inoculums cultures were spread. Incubate the plate in upright position at 37 oC for 14 days. Concentration gradient along with the diameter was calculated for each mm. visible colonies were observed, distance was measured from top end and concentration of the compound was calculated as MIC.

**Discussions**

The disc diffusion method was used to determine the inhibition zones of A. cadamba extracts (organic and aqueous). The plant fruits showed significant antibacterial activity against almost all the organisms (Fig. 1-4) and especially good result against E. coli (Fig.1). The methanolic extract of un-ripened fruit was best among all the extracts prepared and tested in the study. Among the test pathogens selected in the study, P. aeruginosa was found to be most sensitive, followed by E. coli and S. aureus, respectively. Some of the extracts like methanolic extracts of un-ripened fruits of A. cadamba gave very low MIC value and inhibited the growth of P. aeruginosa and S. aureus with MIC as low as 1.00 mg/ml.

**References**

Illustrations

Illustration 1

Screening of the methanolic extracts of plant metabolites against various pathogens was performed and data shown below (Fig. 1).

![Graph](image-url)  
*Fig. 1. Zone of inhibition of methanolic extracts of A. cadusoides fruit against various bacterial pathogens.*
Illustration 2

Screening of the ethanolic extracts of plant metabolites against various pathogens was performed and data shown below (Fig. 2).

![Graph showing inhibition zones for different bacteria with and without exposure to the ethanolic extract.](image-url)

Fig. 2: Zones of inhibition of ethanolic extracts of A. cadamba fruits against various bacterial pathogens.
Illustration 3

Screening of the ethyl acetate extracts of plant metabolites against various pathogens was performed and data shown below (Fig. 3).

![Graph showing zone of inhibition for various bacterial pathogens](image-url)
Illustration 4

Screening of the hot water extracts of plant metabolites against various pathogens was performed and data shown below (Fig. 4).

![Graph showing inhibition zones of bacteria by hot water extracts of plant metabolites.](image)
Illustration 5

MIC determined by double agar plate method showed a very good response against pathogens. MIC of the methanolic extracts of plant metabolites against various pathogens was calculated and data shown below (Fig. 5).

![Fig. 5: MIC of methanolic extracts of A. caudatus fruits against various bacterial pathogens.](image-url)
Illustration 6

MIC of the ethanolic extracts of plant metabolites against various pathogens was calculated and data shown below (Fig. 6).

![Graph showing MIC of ethanolic extracts of plant metabolites against various bacterial pathogens.](image-url)
Illustration 7

MIC of the ethyl acetate extracts of plant metabolites against various pathogens was calculated and data shown below (Fig. 7).
Illustration 8

MIC of the hot water extracts of plant metabolites against various pathogens was calculated and data shown below (Fig. 8).

![Graph showing MIC values for different pathogens](image-url)
Reviews

Review 1

Review Title: Antibacterial Properties of Anthocephalus Cadamba Fruits

Posted by Dr. Muniappan Ayyanar on 12 Aug 2011 11:55:01 AM GMT

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<td>Are the interpretations / conclusions sound and justified by the data?</td>
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<td>Are the keywords and abstract or summary informative?</td>
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Rating: 4

Comment:
There is no Results section in the paper. Also the discussion part is poorly written.

Competing interests: No

Invited by the author to make a review on this article?: No

Experience and credentials in the specific area of science: Yes

Publications in the same or a related area of science: Yes


Review 2

Review Title: Antimicrobial activity of the various extracts of the Fruits of Anthocephalus cadamba

Posted by Dr. Avnish Kumar on 12 Aug 2011 03:10:43 AM GMT

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Rating: 4

Comment:
1 herbal medicine or alternative complimentary medicine is more better subject category. 2. interpretation and discussion is missing the literature written under heading 'Discussion' is just mentioning the results. 3. fruit extract prepared in various solvent against group of microbes has been checked. 6. The authors must state this a preliminary study to chosing a better extract for evaluating medicinal value of the plant. More and latest references must be included, importance and limitations of the study should be mentioned in discussion, English must be proper. Introduction part has also needs to be rewritten. 9. Table and figures of MIC are missing. the given graph have poor pixels. 10. newest and more number of references are required

NOTE: paper is acdpetable with new proper headings, introduction and discussion with conclusion. The paper also need detailed material and methods with proper headings.

The heading may be "Evaluation of various extracts of the Fruits of Anthocephalus cadamba for their Antimicrobial activity"

Competing interests: None

Invited by the author to make a review on this article? : No

Experience and credentials in the specific area of science:
about one year

Publications in the same or a related area of science: Yes

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