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A Comparative Study and Extract Optimization for Antimicrobial Properties of Different Parts of Anthocephalus Cadamba.

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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 leaves, bark, ripened fruits and un-ripened fruits of this plant showed significant antibacterial activity against almost all the organisms: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Trichophyton rubrum, Candida albicans, Microsporum, Aspergillus niger, #MRSRA 1101 MH, #MRSRA 1102 MH, #MRSRA 1103 MH and #MRA 1001 with zone of inhibition of the maximum 24.0 mm and 22.0 mm 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.

Keywords: Anthocephalus cadamba, Antimicrobial properties, extract optimization, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Trichophyton rubrum, Candida albicans, Microsporum, Aspergillus niger, MRSRA 1101 MH, MRSRA 1102 MH, MRSRA 1103 MH.

#MRSRA 1101 MH, MRSRA1102 MH and MRSRA1103 MH strains were provided by MRD LifeSciences, isolated in lab previously. These strains were isolated from highly contaminated soil taken from Ram Manohar Lohia Hospital, Gomti Nagar Lucknow and Vivekanand Polyclinic, Nirala Nagar Lucknow.

All the three isolates possesses very high level of drug resistivity (upto 1500?g/ml) against various drug (11 drugs) and having too much inhibitory property for different bacteria (E. coli, S. aureus, P. aeruginosa) and fungi (A. niger, Microsporum, C. albicans, T. rubrum). MRSRA 1102 MH have the capability to produce bluish greenish pigmentation.

#MRA 1001 is a fungal isolate in the lab and maintained at MRD LifeSciences, Lucknow. MRA 1001 has been identified a species belonging to Aspergillus niger group so far. It has capacity of growing in 1% solution of different antibiotics without any media supplement; at almost every pH range studied, temperature range from -20 0C to 100 0C and produce large amount of green spores.

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 (Sianne and Fanie 2002) and antihepatotoxic activities (Kapil, et. al., 1995). The major constituents of bark are triterpenes, tripernoid glycosides, saponins, indole alkaloids cadambine, 3 a-dihydrocadambine, cadamine, isocadamine and isodihydrocadambine (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. In this respect, the most investigated taxa are from angiosperms whereas very little data is currently available about other groups of plants, especially bryophytes (Madsen and Pates, 1952; McCleary et. al., 1960; Banerjee and Sen, 1978; Basile et. al., 1999). Now as the urbanization is increasing and flat culture is establishing deep roots in society, the place for Kadam is decreasing. The young generation, unaware of its importance, is not planting it nearer to home and public parks. This is really surprising that the natives and traditional healers do not have much knowledge about medicinal properties and uses of Kadam the Chhattisgarh forest officials are also not promoting commercial plantation of Kadam. As result, its natural population is decreasing and in near future, one can see it only in old pictures.

The traditional healers of Chhattisgarh use the Kadam bark in treatment of hoarseness of throat. After mixing the bark in cold water, honey and cumin (Zeera), it is given to the patients internally. It is considered as one of the promising remedies. The natives of Chhattisgarh dip the bark in water used for bath. According to them this herbal bath makes the skin soft and free from all infections. The traditional healers of Bastar region use Kadam bark in treatment of eye diseases. It is also used in case of stomatitis. The traditional healers of Chhattisgarh Plains prefer the decoction of leaves in place of bark for same purpose. The fruit juice is given to children to treat gastric irritability. A decoction of the leaves is good for ulcers and wounds. The fruits are edible. The timber is used for making pulp and paper, boxes, crates and furniture. The wood is also used as fuel.

Materials and Methods

Sample Collection and Identification of the Plant: The leaves, bark, ripened and un-ripened fruits of actively growing A. cadamba from roadside tree were collected at Vibhuti Khand near MRD LifeSciences, Gomti Nagar, Lucknow. Fruits were preserved and stored in -20o C; bark and leaves were dried, powdered and stored at room temperature.

Extraction of bioactive compounds: For extraction of bioactive compounds, 5.0 gm of plant material was mixed with 50ml of methanol (v/v; 80%); ethanol (v/v; 70%) and ethyl acetate (absolute) and kept in dark for a week, filtered it by Whattman 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 Whattman 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, Trichophyton rubrum, Candida albicans, Microsporum, Aspergillus niger, MRSRA 1101 MH, MRSRA 1102 MH, MRSRA 1103 MH and MRA 1001. The isolates were maintained on freshly prepared nutrient agar plates and slants, and kept in a refrigerator at 40 C until required for use. Single colony was transferred in sterile 50 ml of nutrient broth (for bacteria), potato dextrose broth (for fungi) and incubated at 37 oC (for bacteria), 28 oC (for fungi) in shaker incubator at 140 rpm for 14 hrs. Bacterial and fungal cells were recovered by centrifugation and were suspended in sterile distilled water; concentration of pathogens was optimized to OD 0.1 at 600 nm before use.

Screening of bioactive compounds against various pathogens: 10.0 ml nutrient agar/ potato dextrose agar media was poured in a sterile Petri dish, 70 ?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 (antibacterial and antifungal compounds, respectively; 50?g/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 oC (for bacteria), 28 oC (for fungi) 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/ potato dextrose agar (5.0 ml) was poured into sterilized Petri dishes, leaving the plate in slanted position. After setting the media, another 5.0 ml of nutrient agar/ potato dextrose 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 of cultures were spread. Plates were incubated in upright position at 37 oC (for bacteria), 28 oC (for fungi) for 14 hrs. 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.

Quantification of plant metabolites: Test for reducing sugar: Take 1.00 gm of plant sample in a test tube and add 10.0 ml of de-ionized water then add few drop of Fehling solution and heat at 40o C in a water bath. Brick red precipitate indicates positive result.

Test for tannins: 2.00 gm of aqueous extract was taken in test tube added 2 drops of 5% ferric chloride if gives green colour then test will be positive.

Test for phlobatannins: Take 10.0 ml of aqueous extract and boil with few drop of 1% HCl. Deposition of red precipitation gives positive result.

Test for saponins: Take 1.0 ml aqueous extract in test tube and add 5.0 ml de-ionized distilled water and shake it vigorously allow it for few minutes if froth last for 15 minutes means presence of saponins. Olive oil can also be added for checking oil emulsion.

Test for flavonoids: Add few drop of 1% of NH3 yellow colour observe show presence of flavonoids and can be more confirmed by taking aqueous extract or ethanolic extract and add 10.0 ml DMSO then heat it followed by adding Mg (magnesium) and 6 drop of

conc. HCl gives red colour to confirmed flavonoids.

Test for terpenoids: Take 5.0 ml of aqueous extract add 2.0 ml CHCl3 followed by addition of 3.0 ml conc. H2SO4 observe the reddish brown interface for presence of terpenoids.

Test for alkaloids: Take 1.0 ml of aqueous extract in two separate test tubes. In test tube 1 add 2-3 drop of Dragendoffs reagent it gives orange red precipitate and in test tube 2 add 2-3 drop of Meyer?s reagent white ppt. result will be positive.

Test for steroidal glycosides: Take 1.0 ml of extract add 2.0 ml of acetic anhydride followed by 4 drop of CHCl3 then add few drop of H2SO4 by surface of the beaker. A brown ring at interface and violet colour in the supernatant layer indicates the presence of steroidal of glycosides.

Results

MIC determined by double agar plate method showed a very good response against pathogens.

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 and especially good result against E. coli. 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 S. aureus, E. coli, MRSRA1103 MH, MRSRA1102 MH, MRSRA1101 MH, Microspora and T. rubrum 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. Antimicrobial activity due to reducing sugars, terpenoids and terpenoids serves leaf to be a good traditional diabetic?s treatment. Leaves strengthened the skin and increase the concentration of antioxidant. Terpinoid also known as heart friendly phytochemical. Terpenoids make leaves a rejuvenating agent and useful remedy for ageing and beauty enhancement. By the presence of saponins leaf reduces blood cholesterol and blood pressure. By the combined effect of reducing sugar and terpenoids green fruits are good antimicrobial specially antibacterial and green fruit (unrippened) and is a wound healer and antioxidant because of terpenoids which also serve s it a rejuvenating property. Ripened fruit is a good wound healer than green fruit because of presence of tannins also along with terpenoids. Yellow fruit is also a traditional remedy for urinary tract problem because of tannin.

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Illustrations

Illustration 1

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

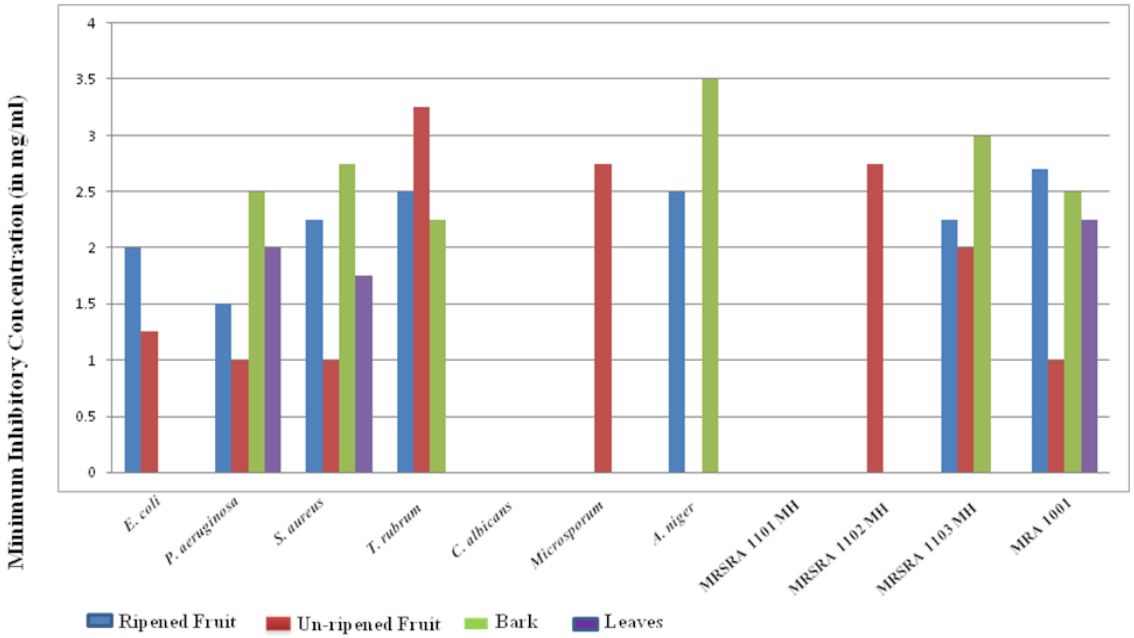

Fig. 1: MIC of methanolic extracts of *A. cadamba* against various bacterial pathogens.

Illustration 2

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

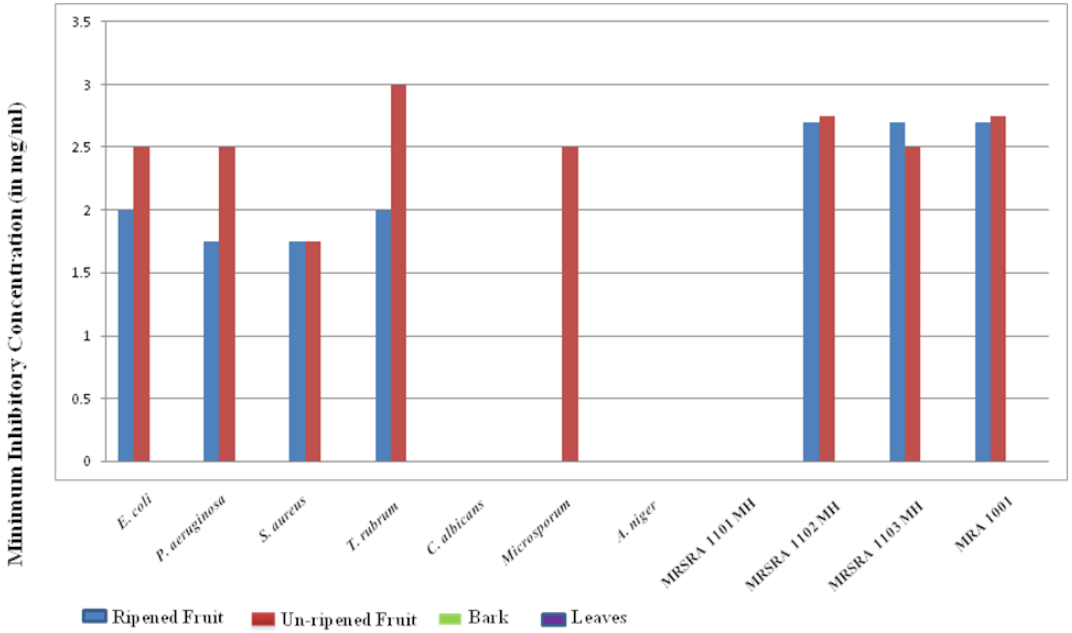

Fig. 2: MIC of ethanolic extracts of *A. cadamba* against various bacterial pathogens.

Illustration 3

MIC of the hot water extracts of plant metabolites against various pathogens was calculated and data shown below (Fig. 3).

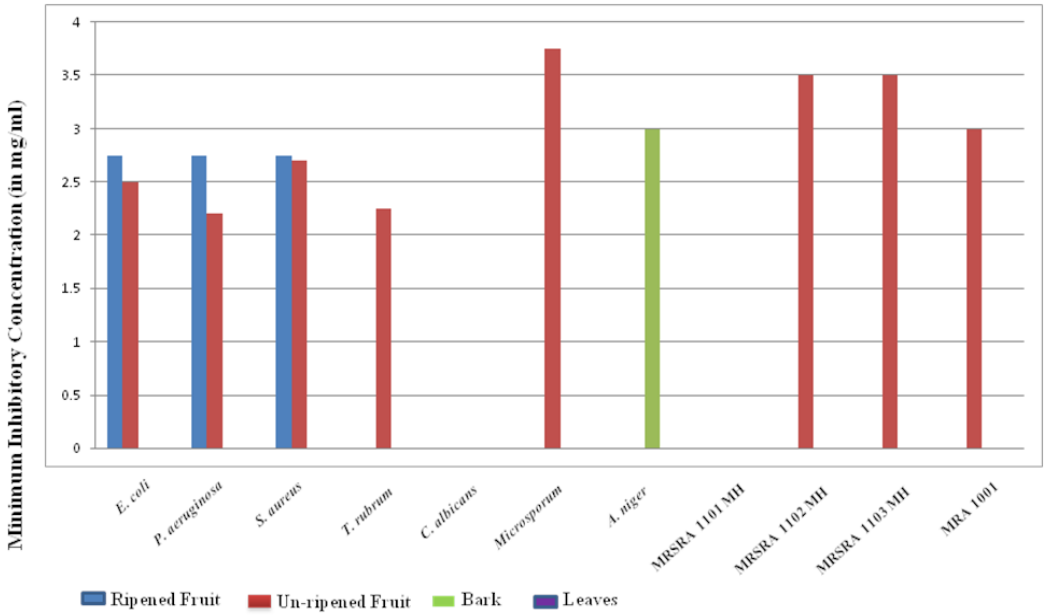

Fig. 3: MIC of hot water extracts of *A. cadamba* against various bacterial pathogens.

Illustration 4

Phytochemical quantification were performed in different part of A. cadamba data shown in table in below:

Phytochemical constituents	Leaf	Bark	Ripened	Unripened
Reducing sugar	+++	--	++++	+++++
Tannins	+++++	++	--	+
Phlobatannins	--	--	--	--
Saponins	+++	+	--	--
Flavonoids	+++++	++	--	--
Cardiac steroids	+	+	--	--
Terpenoids	+++	--	+	++

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