

## **Antibacterial properties of *Anthocephalus cadamba* fruits**

**\*Ram Prakash Mishra<sup>1</sup> and Liza Siddique<sup>2</sup>**

<sup>1</sup>*R&D Division, MRD LifeSciences, Lucknow, U.P. INDIA*

<sup>2</sup>*Department of Biotechnology, Bundelkhand University, Jhansi. U.P.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.

**Keywords:** *Anthocephalus cadamba*, Antibacterial properties, *S. aureus*.

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### **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 [1]. Some scientific studies have been carried out to reveal its antimalarial [2] and antihepatotoxic activities [3]. The major constituents of bark are triterpenes, tripernoid glycosides, saponins, indole alkaloids cadambine, 3 a-dihydrocadambine, cadamine, isocadamine and isodihydrocadambine [4-7]. 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 [8-11].

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 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 - 20°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 4°C until required for use. Single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 °C 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:**

10.0 ml nutrient agar media was poured in a sterile Petri dish, 100  $\mu$ 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 $\mu$ l plant extracts with positive (Tetracycline; 50 $\mu$ g/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 °C 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 (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 (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 °C 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.

**RESULTS**

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

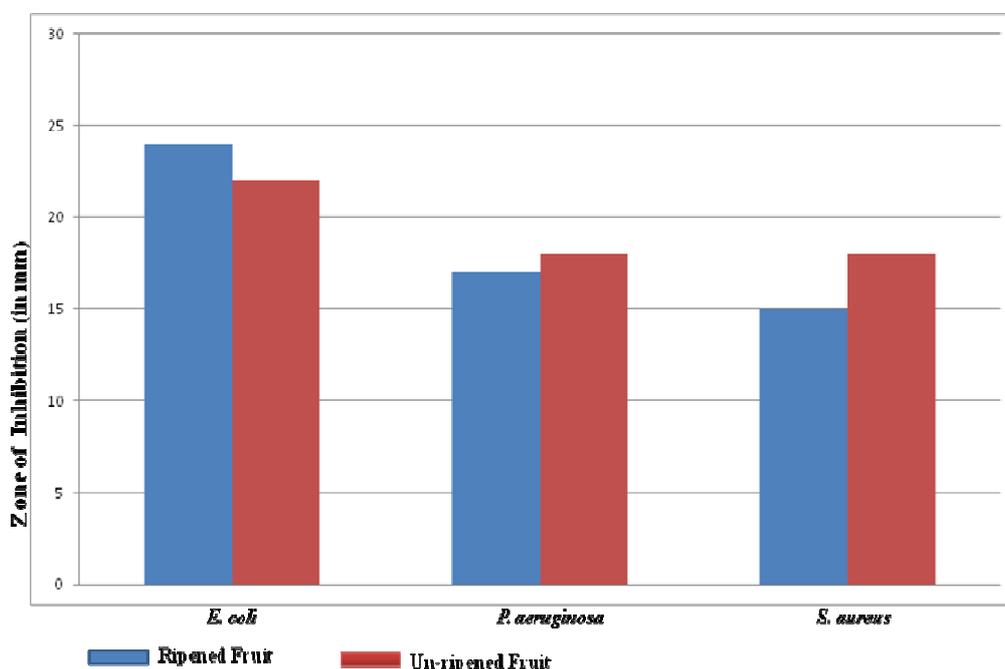


Fig. 1: Zone of inhibition of methanolic extracts of *A. cadamba* Fruits against various bacterial pathogens.

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

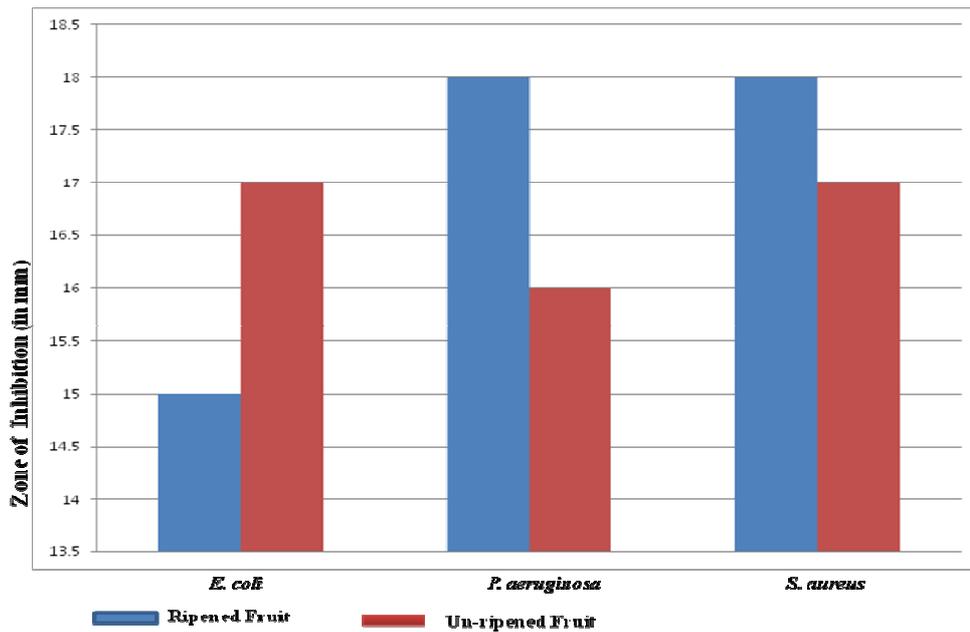


Fig. 2: Zone of inhibition of ethanolic extracts of *A. cadamba* fruits against various bacterial pathogens.

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

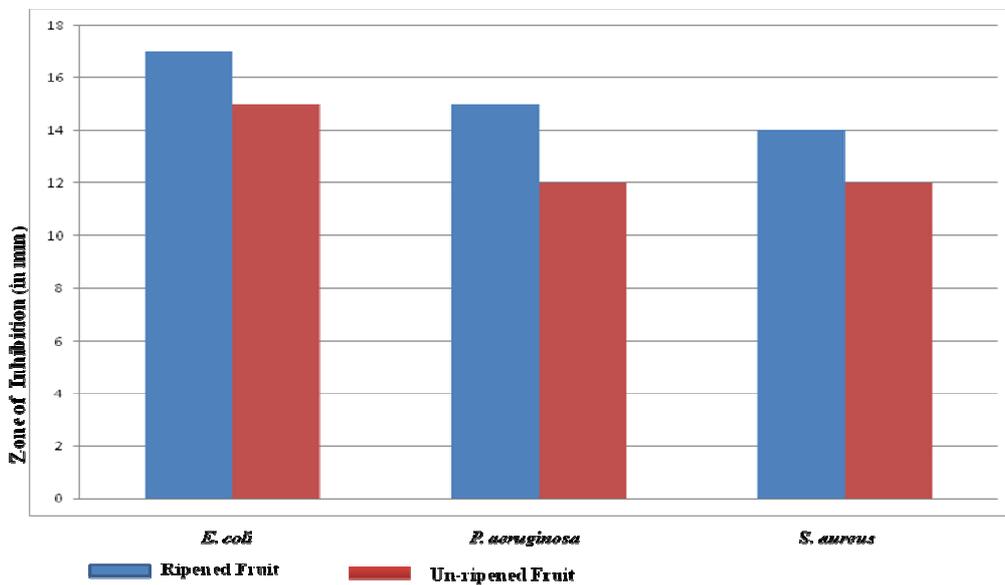


Fig. 3: Zone of inhibition of ethyl acetate extracts of *A. cadamba* fruits against various bacterial pathogens.

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

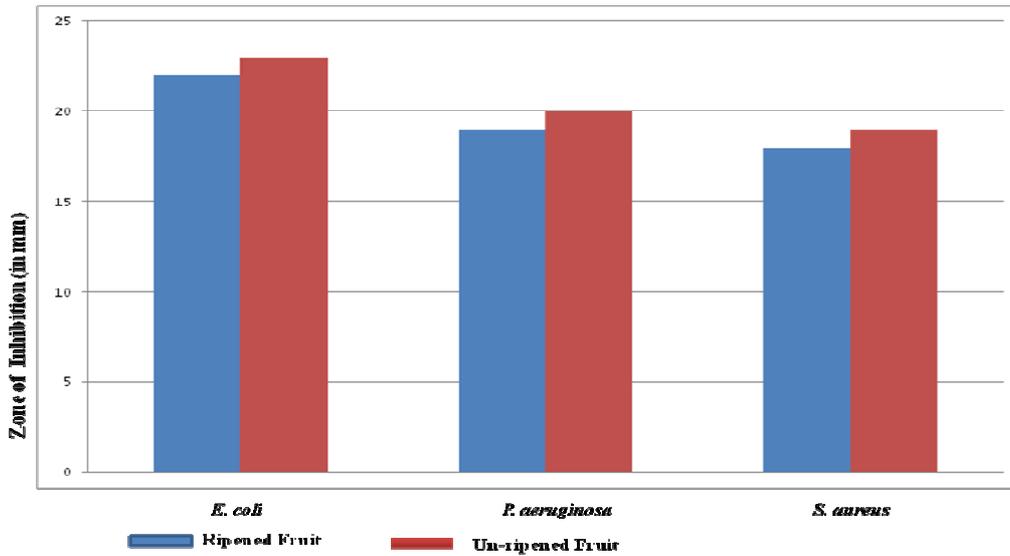


Fig. 4: Zone of inhibition of hot water extracts of *A. cadamba* fruits against various bacterial pathogens.

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).

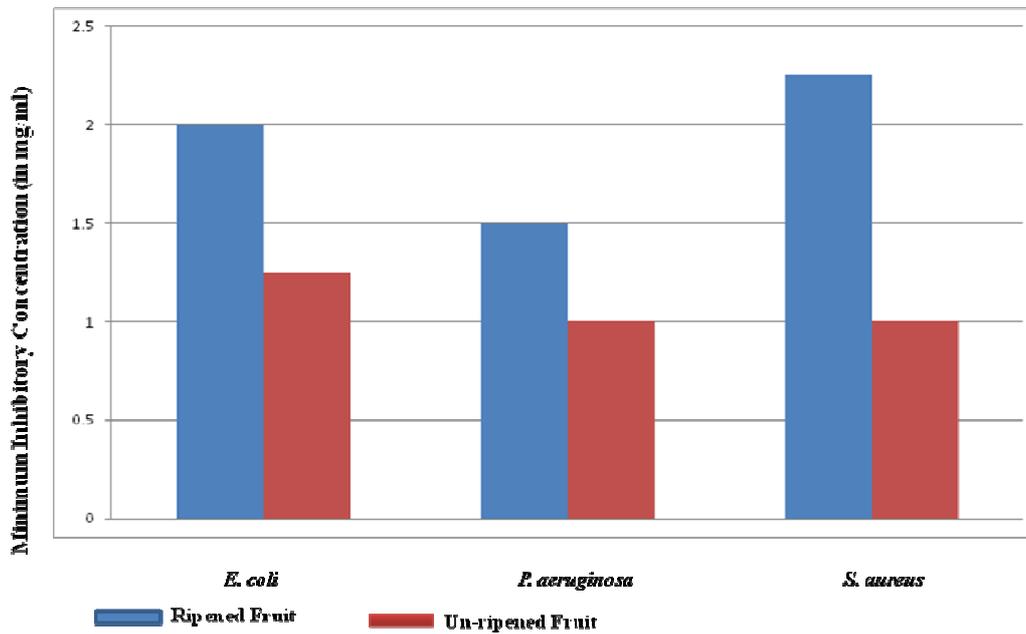


Fig. 5: MIC of methanolic extracts of *A. cadamba* fruits against various bacterial pathogens.

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

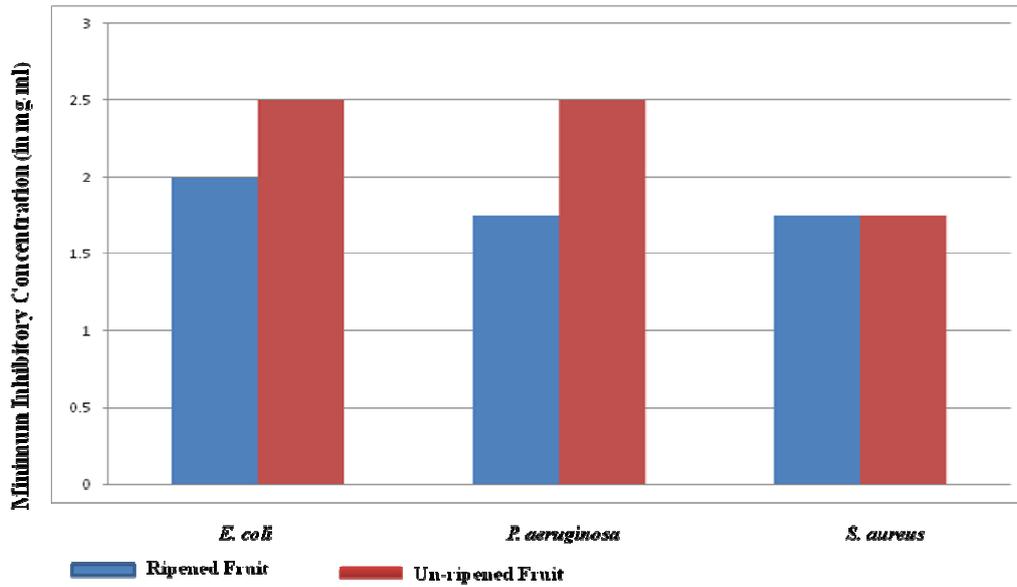


Fig. 6: MIC of ethanolic extracts of *A. cadamba* fruits against various bacterial pathogens.

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

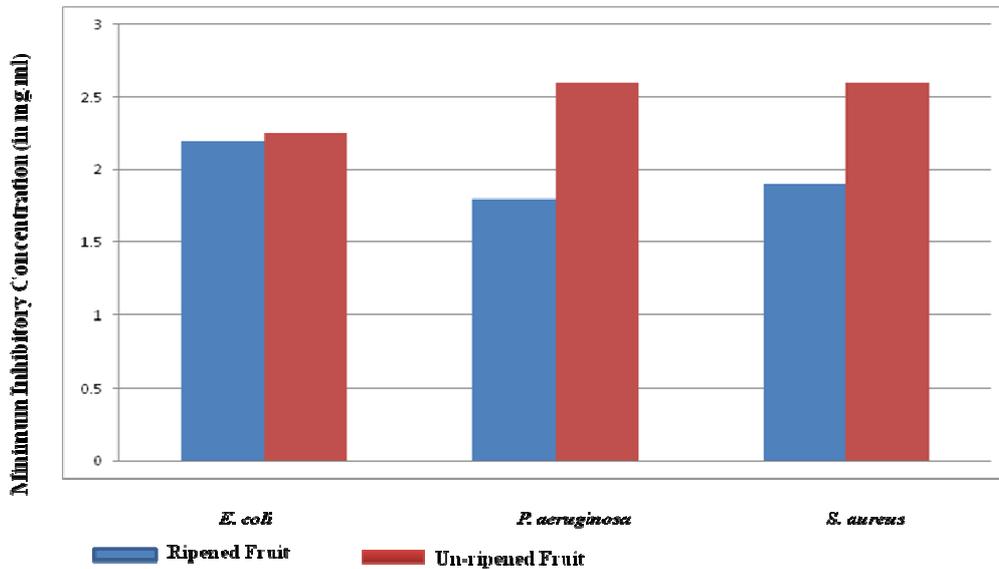


Fig. 7: MIC of ethyl acetate extracts of *A. cadamba* fruits against various bacterial pathogens

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

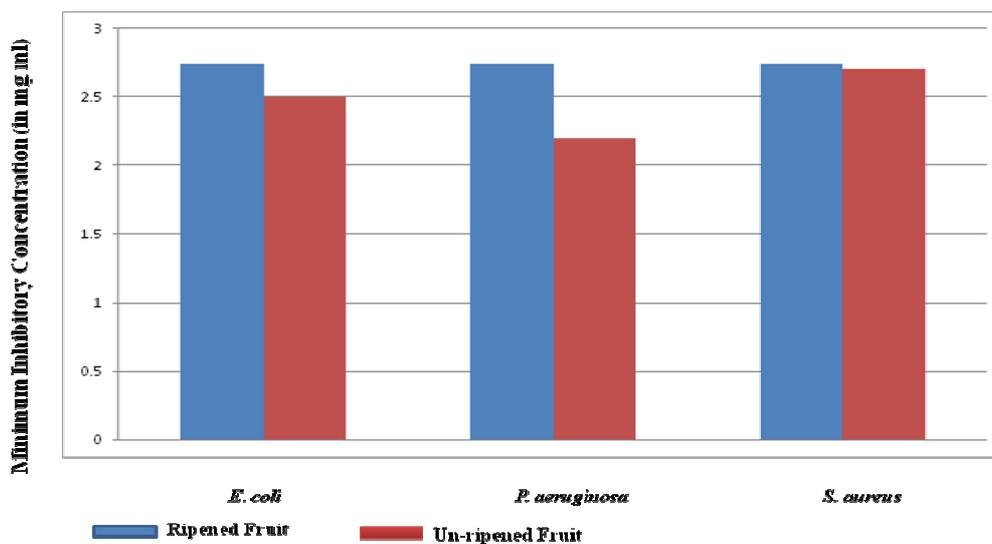


Fig. 8: MIC of hot water extracts of *A. cadamba* fruits against various bacterial pathogens.

## DISCUSSION

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.

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