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Research Article

EVALUATION OF INVITRO ANTHELIMINTHIC ACTIVITY AND ANTI BACTERIAL ACTIVITES OF ETHANOLIC BARK EXTRACT OF ANNONA MURICATA

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ABSTRACT

In the current study, In-vitro experiments were conducted to determine the possibleanthelminthic and anti-bacterial activities of crude ethanolic bark extract of *Annonamuricata*. The anthelminthic activity was tested on earthworms at a concentration of 100 and 150mg/ml and compared with Albendazole(20mg/ml) as the standard. The antibacterial activity was assessed by agar dilution method against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Pseudomonas putida, Mycobacterium luteus*at a concentration of 150 and 200mg/ml respectively. Gentamycin(20µg/mL) was used as a reference standard for antibacterial activity. The extract was found to show significant anthelminthic activity as compared to that of standard drug albendazole and also showed anti-bacterial activity against gram positive and gram negative bacteria in a concentration dependant manner.

Keywords: Anthelminthic , Antibacterial , Albendazole , Gentamycin , Pherithimapostuma.

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial.The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. In India, medicinal plants form the back bone of several indigenous traditional systems of medicine[1].There are several reports on the antibacterial and anthelminthic activity of different herbal extracts in different regions of the world .Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics , recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicines. In the present study medicinal plant *Annonamuricata* belonging to the family *Annonaceae* was selected to asses antibacterial and anthelminthic activty[2]. The medicinal properties of *Annonamuricata* include anti-inflammatory, anti hypertension. Annona muricata have many health benefits as they as good source of proteinsand other vitamins. Annonamuricata bark also helps in maintaining healthy blood vessels, nerves and tissues.

MATERIALS AND METHODS

Plant material

The stem bark of plant *Annonamuricata* was collected from Jalluru local area of East godavari District, Andhra Pradesh . The plant was identified and authenticated by T.Raghuram Taxonomist, Maharani College, Peddapuram.

Extraction

The freshly collected bark of plant were cleaned from dirt, dried under shade and then coarsely powdered manually. The powder was macerated in ethanol for a period of 7 days

and then subjected to hot Percolation for 8hrs.Then the solution was filtered, concentrated and dried.

Antibacterial Activity

Theethanolic bark extract of*Annonamuricata* was studied for antibacterial activity by employing sterile nutrient agar mediumagainst several gram positive and gram negative organisms[5]. The various organisms like *Staphylococcus aureusATCCBAA 1026,Bacillus subtilisATCC 11774 ,Pseudomonas aeruginosaATCC 10662,Escherichia coliATCC 10536, Pseudomonas peptida ATCC 700007Micrococcus luteus ATCC 9341* are procured from Microbes speciality lab,Danaviapeta, Rajahumundry. The reference standard Gentamycin was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad. The antibacterial activity of ethanolic bark extract was performed by using Agar cup-plate method. 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37^0C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile agar broth (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Ethanolicbark extracts of *Annonamuricata* was dissolved in water to obtained different concentration (100,150mg/ml) and sterilized by filtration through a Whattman filter paper no.1, and 0.05 ml of the different concentrations of extract were added to the respective bores.0.05ml of Gentamycin at a concentration of (25µg/ml) was taken as reference standard All the plates were kept in a refrigerator at 2 to 8^0 C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37ºc for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

Anthelmenthic Activity

Anthelminthic activity was performed according to the method of Ghosh et al.[6] with slight modifications. The bark of *Annonamuricata* was studied for anthelminthic activity using earth worms (Pheretimaposthuma) collected from the Aditya gardens in surampalem .Because of easy availability, earthworms have been used widely for the evaluation of Anthelmintic compounds.All the earth worms were of approximately equal size (6cm). 50ml containing 5 concentrations each of crude ethanolic extract (100,150mg/ml) were prepared and six worms were placed in it. The standard drug and extract solutions were prepared freshly before starting the experiment. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed by fading away of their body colours. The anthelminthic activity of ethanolic extract of *Annonamuricata* is compared with standard reference drug Albendazole(20µg/ml).

DISCUSSION

Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of chronic and debilitating in nature .The development of Anthelminthic resistance and the high cost of conventional Anthelminthic drugs led to the evaluation of medicinal plants as an alternative source of Anthelminthic[3].Indian earthworm resembles intestinal round worm parasiteso these worms are used for the study.[4]*Annonamuricata* bark extract has shown significant anthelminthic activity evident from the TABLE 1 at a concentration 100mg/ml and 150mg/ml against*Pheretimaposthuma*. Anthelminthic activity was found to be increased with dose . In light of this, the results of the present study suggest that the extract of *Annonamuricata* could be used in the control of helminthic infections namely ascariasis, hookworm infections etc; as the worms used in the study are in resemblance with the intestinal parasitic worms. Phytochemical analysis revealed the presence of tannins as one of the chemical constituent. Tannins were shown to produce Anthelminthic activity[5]. Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelminthics(Niclosamide, Oxyclozanide and Bithionol) are shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation[6]. It is possible that tannins contained in the extracts of *Annonamuricata* produced similar effects . Another possible anthelminthic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal[7] or glycoprotein on the cuticle of the parasite[8].The presence of tannins in the ethanolic bark extract of *Annonamuricata*may be responsible for

anthelminthic activity. The antimicrobial activity of extract was evaluated by determining the diameter of zone of inhibition against gram negative and gram positive bacteria using the cup plate diffusion method. The diameter of the inhibition zones were measured in millimeters .*Annonamuricata* bark extract has shown excellent antibacterial activity against gram positive organisms compared to that of gram negative organisms which is evident from the Table.2. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms[9]. Several studies indicates the presence of these bioactive compounds in plant materials to antibacterial activity . The presence of alkaloids, flavonoids in the ethanolic bark extract may be responsible for antibacterial activity.

CONCLUSION

This investigation has opened up the possibility of the use of this plant in drug development. However, before coming to the conclusive statement further research is needed investigate the bioactive constituents which are responsible for these biological activities.

RESULTS

ANTHELMINTHIC ACTIVITY

Table 1: Anthelminthic activity of Ethanolic bark extract of *Annonamuricata*

Group	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Ethanolic extract of *Annonamuricata* bark	100 150	30±1.63 26.3±1.24	37.7±2.05 32±2.01
Standard Albendazole	20	20±1.63	23±1.25
Distilled water	-	-	-

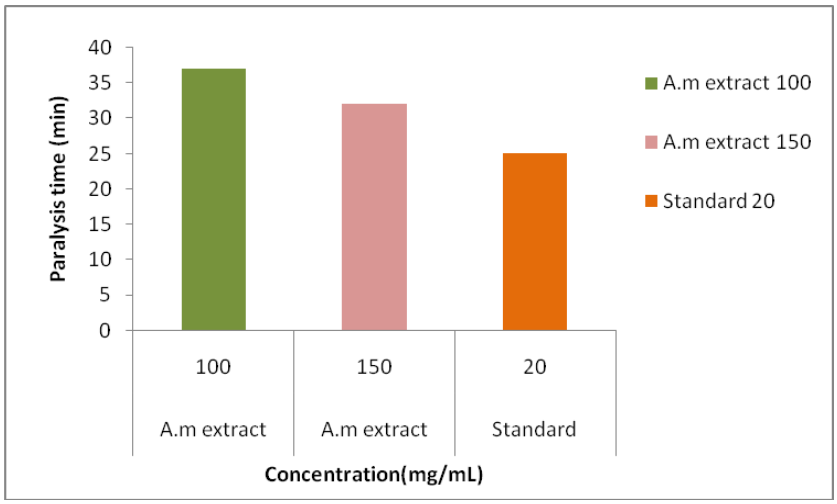

Fig. 1: Plot of time taken for paralysis of *Annonamuricata bark* extract

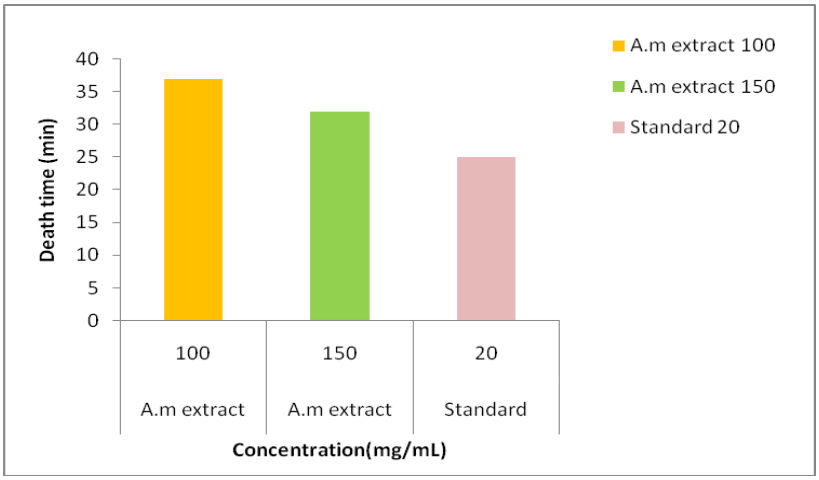

Fig. 2: Plot of time taken for death of *Annonamuricata bark* extract

ANTI BACTERIAL ACTIVITY

Table 2: Anti bacterial activity of ethanolic extract annona muricata bark

ZONE OF INHIBITION			
Micro organisms	**150mg/ml**	**200mg/ml**	**Gentamycin 50µg/ml**
Gram positive			
Staphylococcus aureus	14.6±0.19	18.7±0.17	20.5±0.13
Bacillus subtilis	14.8±0.12	16.7±0.15	25.8±0.4
Mycobacterium luteus	15.8±0.12	16.8±0.12	19.5±0.12
Gram negative			
Pseudomonas aeruginosa	10.8±0.16	14.8±0.14	24.4±0.12
Escherichia coli	11.7±0.16	12.8±0.13	22.5±0.16
Pseudomonas putida	10.7±0.16	10.8±0.14	18.5±0.12

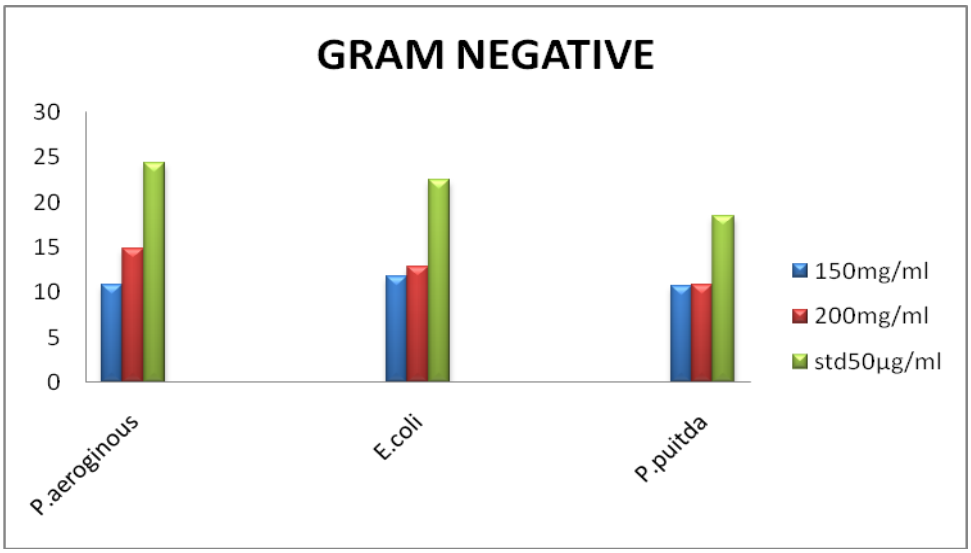

Fig. 3: Plot of zone of inhibition of *Annonamuricata bark* extract against Gram negative organism

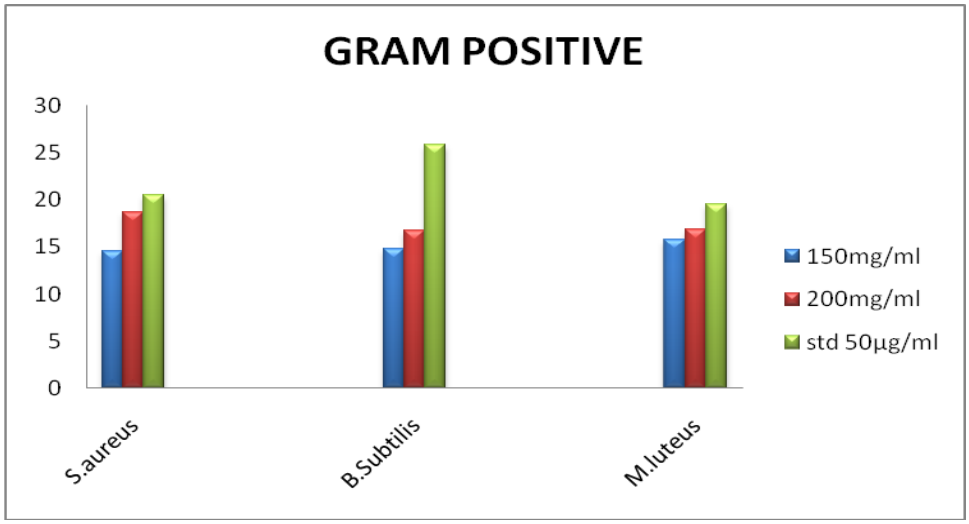

Fig. 4: Plot of zone of inhibition of *Annonamuricata bark* extract against Gram positive organism

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