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Evaluation of Antimicrobial and Antifungal Properties of *Annonamuricata*Leaf Extracts

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ABSTRACT

The worldwide increase of multidrug resistance associated bacterial infections has gained the attention of researchers to warrant an effective antimicrobial therapy. *Annonamuricata* (Graviola) has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use for many ailments. The present study was designed to screen for selected phytochemicals and antibacterial properties (antibacterial and antifungal)using different solvent extracts (Hydroethanolic, Chloroform, Ethylacetate and Petroleum ether)in graviola leaves. The results obtained shows that *Annonamuricata* contains alkaloids, flavanoids, tannins, terpenoids, steroids, glycosides, and reducing sugar. Hydroethanolic extract was noticed to exert significant inhibitory effect against the growth of *P.aeruginosa, Klebsiella, and E.Coli. Stapylococcusaureus*was sensitive to petroleum ether and chloroform extracts. Ethyl acetate extract was found to be effective against the fungal strains (*Candida albicans, A.fumigatus and* and*A.niger*). In addition, minimum inhibitory concentration of the extracts was found to be ranging from 250 to 350µg /ml against all tested bacterial strains. The results could justify the traditional use of *Annonamuricata* in the treatment infectious diseases.

Keywords:*Annonamuricata,*antifungal, antibacterial, hydroethanol.

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INTRODUCTION

Infectious diseases account for 41% of the global diseases and causes public health problem while noninfectious diseases and injuries causes 43% and 16% respectively[1]. The natural development of bacterial resistance to various antibiotics remains responsible for these infectious diseases[2]. These multidrug-resistant (MDR) bacteria evolve because of the accumulation of different antibiotic resistance mechanisms within the same strains[3]. This situation has drawn the attention of researchers to search for development of herbal based better-quality drugs with improved antibacterialand antifungal efficacy[4].World health organization (WHO) has reported that 80%of the global population use indigenously available herbal drugs as per their traditional system of medicine[5]. Herbal plants are considered to bethe effective and safer sources against various infectious diseases since phytotherapypossessesreduced toxicity, uncomplicated availability,and low side effects[6]. Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. Plant based products or extracts are cheaper alternatives to the development of synthetic drugs[7]. Numerous medicinal plants havebeen extensively evaluated for treatment of infectious diseases such as urinary tract infections,gastrointestinal disorders, respiratory ailments and cutaneousdiseases[8]. *Annonamuricata* L. belonging to the family of*Annonaceae* has a widespread tropical distribution.This small tree natively grows in warmest tropical part of Central America, Caribbean, Northeast and Southeast regions of Brazil, and North America[9]. Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties[10]. However, there is no much report available to validate the antimicrobial effect of *Annonamuricata*.With this background information, the present work was carried out to perform studies on antibacterial and antifungal activity in 4 different leaf extracts viz., hydroethanolic, petroleum ether, ethyl acetate and chloroform of *Annonamuricata*.

MATERIALS AND METHOD

Preparation of plant extracts

Fresh leaves of *Annonamuricata* were collected from the well grown trees in the regions of Coimbatore District, Tamilnadu and washed using tap water. The leaves were shade dried for few days and then powdered with blender. Dry leaf powders were subjected to successive extraction with 50% ethanol, chloroform, ethyl acetate and petroleum ether solvents following cold maceration procedure. The extracts were filtered through Buchner funnel

using Whatman filter paper no.1. The filtrate was evaporated to dryness under reduced pressure and crude extracts were obtained.

Microbial strains

The bacterial cultures used in the present study include *Staphylococcus aureus, Pseudomonaaeruginosa, Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia*. The cultures were obtained from the Department of Microbiology, P.S.G College of Arts and Science, Coimbatore. A.niger, Candida albicans, A. fumigatus ,Penicillium and Mucor were also procured from the same laboratory.

Evaluation of antibacterial activity

Dehyrated media and standard antimicrobial drugs were obtained from Hi-Media Laboratories Ltd, India. All the media were prepared in sterile glass petriplates according to manufacturer's instructions.The agar cup method was used to study the antibacterial activity of theextracts[11]. Mueller-Hinton agar (MHA) (Hi-Media, India) was used as bacteriological medium. MHA plates were prepared by pouring molten media into sterile Petri plates. The plates were allowed to solidify for 5 min. Wells were prepared in seeded agar plates. 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 min. The extracts were diluted in 100% Dimethyl sulfoxide (DMSO). A total of 5 mm diameter wells were punched into the agar and filled with the 50 µl (5 mg/ml in DMSO) extracts, 20µl DMSO (negative control) and 5 µl of standard antibiotic (Ciprofloxacin at concentration 10 µg/ml) were used as a positive control. The plates were incubated at 37 ºC for 24 h. After the incubation period formation of zones around the wells were measured to confirm the antibacterial activity of the respective extracts. The same procedure was followed for each strain and extract. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Minimum inhibitory concentration(MIC)

Minimum inhibitory concentration was determined using broth dilution technique. Seven test tubes containing 1 ml of sterile Sabourauds Dextrose broth were prepared. For assaying plant extract, the starting concentration kept at 200µg/ml in the first tube containing 1 ml of sterile Sabourauds dextrose broth. The plant extractswere serially diluted at the concentrations of 400, 350, 300, 250, 200, 50, 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml. To each of this test tube, 0.1 ml of 6 hr culture of bacteria was added. The tubes were incubated at 30°C for about 24-48 hr. The test tubes were examined for visible turbidity. Now, the absorbance of each tube was measured spectrophotometrically at 620nm.The end point of complete inhibition wasdefined as the minimum inhibition concentration of the extract in the original tube which fails to yield visible growth when sub cultured[12].

Antifungal Activity

Aagar-well diffusion method was followed to determine the antifungal activity using spore suspension with agar at 45°C. Wells (4.6mm in diameter) were cut in a similar way as for the antibacterial activity with a sterile borer and 60µl extract solutions were delivered into them. The plates were incubated at 28°C for 3 days after which diameter of zones of inhibition (DIZ) were measured. Amphotericin B and fluconazole were used as positive reference[13].

RESULTS AND DISCUSSION

Many phytochemicals such as alkaloids, flavanoids, tannins, saponins, terpenoids, steroids, glycosides and reducing sugar were found to be present in the *Annonamuricata* leaf extract as summarized in the Table 1. These secondary metabolites have been reported to possess antimicrobial activity[14]. Plants are reservoir of valuable bioactive chemical constituents. In particular, the flavonoids were reported to be responsible for anti-microbial activity associated with some ethanomedicinal plants[15]. The increasing emergence of antibiotic resistance has deviated the attention of researchers towards the medicinal herbs in search of new and non-toxic drugs[16]. Therefore, this study was carried out to evaluate the antibacterial and antifungal activities of the various extracts of *Annonamuricata* leaf. Hydroethanolic, chloroform, ethyl acetate and petroleum ether were tested for antibacterial activity against the 5 bacterial *Staphylococcus aureus, Pseudomonaaeruginosa, Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia* strains and the results are summarized in table 2.Hydroethanolic extract was found to produce significant zone of inhibition against *Pseudomonaeruginosa*(14 mm), *Escherichia coli* (15 mm), and*Klebsiella pneumonia* (14 mm).Minimum inhibitoryzone was found in the case of *Staphylococcus aureus*(4 mm) and *Salmonella typhi*(5 mm). Petroleum ether extract exhibited moderate effect against *Staphylococcus aureus*with zone of inhibition of 6 mm. 8 mm zone of inhibition was observed when ethyl acetate extract was tested against *Escherichia coli* strain. Chloroform extract was found to exert inhibitory effect on the growth of *Staphylococcus aureus*alone with 7 mm zone of inhibition. All test strains of bacteria were found to be sensitive to standard drug (Ciprofloxacin) with the zone of inhibition in the range between 19mm and 27mm[17]. DMSO was used as the negative control which did not exhibit any zone of inhibition against tested bacteria.

Table 1: Phytochemicals detected in different extracts of *Annonamuricata*

Plant part	Solvent	Alkaloids	flavanoids	Tannins	Saponins	Terpenoids	Steroids	Glycosides	Reducing sugar
Leaves	Hydroethanol	+	+	+	+	+	+	+	+
	Chloroform	+	-	+	+	+	+	+	-
	Ethyl acetate	+	+	+	+	-	+	-	-
	Petroleum ether	+	+	+	+	-	+	-	-

Table 2: Effect of *A.muricata* leaf extracts against the growth of bacterial strains

Extract/Drug (50µg)	Diameter of zone of inhibition (mm)				
	E. coli	***P.aeroginosa***	***S. typhi***	***Klebsiella pneumonia***	***Stapylococcusaureus***
Hydro ethanolic	15 ± 0.57	14 ± 0.58	5 ± 1.18	14 ± 1.12	4 ± 0.51
Petroleum ether	0.0	1 ± 0.59	0.0	3 ± 2.19	6 ± 2.66
Ethyl acetate	8 ± 0.35	2 ± 0.66	5 ± 0.68	2 ± 1.01	3 ± 0.77
Chloroform	3 ± 0.69	3 ± 0.76	2 ± 0.59	4 ± 1.00	7 ± 0.87
Ciprofloxacin(5 µg)	25.7 ± 1.73	27 ± 2.08	21 ± 2.08	12 ± 1.98	19.6 ± 1.00

*Values are mean ± SD of triplicates

Table 3: Antifungal activity of *A. muricata*leaf extracts

Extract/Drug(50µg)	Diameter of zone of inhibition (mm)				
	A.niger	***Candida albicans***	***A.fumigatus***	***Penicillium***	***Mucor***
Hydroethanolic	2 ± 0.76	4 ± 0.78	2 ± 0.67	2 ± 0.69	3 ± 0.75
PetroleumEther	2 ± 1.03	2 ± 1.09	3 ± 0.96	4 ± 0.99	2± 1.01
Ethyl acetate	6 ±0.59	6 ± 0.65	5 ± 0.75	6.5 ± 0.78	4.5 ± 0.68
Chloroform	3 ± 2.57	2 ± 2.45	4 ± 2.11	6 ± 0.97	2 ± 1.95
Amphotericin (100 µg)	18 ± 0.79	14 ± 0.84	16 ± 1.01	18 ± 1.19	15 ± 0.98

*Values are mean ± SD of triplicates

The results obtained for screening of antifungal activity have been shown in Table 3. Ethyl acetate extract exhibited effective inhibition against all the fungal strains tested with zone of inhibition ranging from 4.5 mm (*Mucor*), 5 mm (*A.fumigatus*), 6 mm (*A.niger, Candida albicans*), and 6.5 mm (*Penicillium*) respectively. Chloroform extract was found to affect the growth of *Candida albicans*and*Penicillium* with zone of inhibition 4 mm and 6 mm respectively. The moderate or less antifungal affect of petroleum ether was found against all the tested fungi except *Penicilliumnottatum*with 4 mm. All fungal strains were found to be sensitive with standard antibiotic Amphotericin. DMSO was used as the negative control which showsno zone of inhibition against tested fungi.

The minimum inhibitory concentration (MIC) was determined by making the dilutions of different extracts of *Annonamuricata* from 400 to 3.0125µg/ml. The MIC values of hydroethanolic, petroleum ether, ethyl acetate and chloroform extracts are summarized in table 4. The results illustrate that MIC of different extracts of *A.muricat* against bacterial strains ranged from 350 to 250µg/ml. The data reveal that all the strains were susceptible to petroleum ether extract when compared with hydroethanol, chloroform, and ethyl acetate extracts. From all MIC values of different *A.muricata* extracts, lowest MIC values for *E. coli* was found to be 250µg/ml with hydroethanolic and petroleum ether extracts; 300 µg/ml with ethyl acetate and chloroform extracts. 250µg/ml MIC value of *S. aureus*was exerted by petroleum ether and chloroform extracts; 300 and 350µg/ml were found when treated with hydroethanolic and ethylacetate extracts respectively. *Pseudomonaaeruginosa*was sensitive to MIC of 300µg/ml (Petroleum ether) and 350µg/ml (Hydro ethanolic, Chloroform, and ethyl acetate). Lowest MIC value for *Klebsiellapneumonia*(250 µg /ml)was exhibited by ethyl acetate extract. In case *Salmonella typhi* , Petroleum etherextract had lower MIC value of 250µg /ml while hydroethanolic extract had 300µg /ml; both ethyl acetate and chloroform had 350µg /ml.

Table 4: Minimum Inhibitory Concentration of different solvent extracts of *Annonamuricata*against tested bacteria.

S. No	Bacterial strains	Minimum Inhibitory Concentration (µg)			
		Hydro ethanolic	Petroleum ether	Ethyl acetate	Chloroform
1.	*Staphylococcus aureus*	300	250	350	250
2.	*Pseudomonaaeruginosa*	350	300	350	350
3.	*Escherichia coli*	250	250	300	300
4.	*Klebsiella pneumonia*	300	300	250	300
5.	*Salmonella typhi*	300	250	350	350

CONCLUSION

The present study confirms that *Annonamuricata* has significant antibacterial and antifungal activity along with the presence of valuable phytochemicals. Different solvent extracts exhibited wide range of antibacterial activity with MIC values (250 to 350µg/ml)indicating that *Annonamuricata* could be a good sourceto combat multidrug resistant bacterial infections. Antifungal effect of extracts was also found to be moderate against the fungal strains except ethyl acetate extract which was much effective. In conclusion, our findings suggest that further studies are required to isolate the active principles from the plant for the development of antimicrobial drugs.

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