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IN VITRO ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF ANNONA MURICATA

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ABSTRACT

Annona muricata is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. A wide array of ethnomedicinal activities is contributed to different parts of A. muricata, and indigenous communities in Africa and South America extensively use this plant in their folk medicine. Numerous investigations have substantiated these activities, including anticancer, anticonvulsant, anti-arthritic, antiparasitic, antimalarial, hepatoprotective and antidiabetic activities. This research aimed to evaluate the potential antimicrobial effects from extracts of leaves of soursop by agar well diffusion method. The100 µl of methanolic extract inhibited Streptococcus mutans(20mm), Klebsiella pneumonia (22mm), Staphylococcus aureus (20mm), Pseudomonas aeroginosa (21mm). and Escherichia coli (22mm). The100 µl of ethanolic extract inhibited Enterococcus (20mm), Klebsiella pneumonia (18mm), Staphylococcus aureus (20mm), and Escherichia coli (20mm). In klebsiella pneumonia, E. coli and S. Aureus methanolic extracts were found to be having more inhibitory activity than ethanolic extracts. In Candida albicans the100 µl methanolic extract of Annona muricata obtained an inhibition zone of 20mm and in Aspergillus niger it showed an inhibition zone of 15mm. The antimicrobial activity of the tested extracts are comparable with the standard drugs.

KEY WORDS

Annona muricata, *antimicrobial activity, agar well diffusion, Aspergillus niger*

INTRODUCTION

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Ahemedulla and Nayar, 1999). All over the world the herbal medicine acts as the representative of the most important fields of traditional medicine. The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs (Parekh 2007). Medicinal plants have been used for the treatment of illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C gives many references of the utilization of the medicinal plants. "Virikshayurveda" is one of the remotest works in the traditional herbal medicine which was compiled even before the beginning of Christian era. "Rig Veda" is one of the oldest literatures which was written around 2000 B.C. and mentions the use of Cinnamon (Cinnamomum verum), Ginger (Zingiber officinale), Sandalwood (Santalum album) etc was used not only in the religious ceremonies but also in the medical preparations (Prasad Palthur *et al*., 2010). The relationship between food and medicine was quoted as "Let food be thy medicine and medicine be thy food". (Bentley *et al.,* 1880) Plants and plant-based medicaments are used as the basis of many of the modern pharmaceuticals that we use today in order to treat our various ailments (Abraham,1981).

Multidrug resistance has been a biggest threat to the medical world as bacteria are acquiring antibiotic resistance day by day. Interesting conundrums have been encountered in investigations of links between antibiotic use and the development of antibiotic resistance (Julian Davies *et al*., 2010). Nowadays more and more bacteria are becoming resistant which were earlier sensitive to the antibiotics. Newer antibiotics are not invented or slowdown in the process of inventing a newer molecule of antibiotics. The medicinal plants look promising as it has proved in the past like a saviour to the medical world.

Annona muricata is a member of the family of Custard apple trees called Annonaceae and a species of the genus *Annona* known mostly for its edible fruits Anona. *Annona muricata* produces fruits that are usually called Soursop due to its slightly acidic taste when ripe. *A. muricata* trees grew natively in the Caribbean and Central America but are now widely cultivated and in some areas, escaping and living on their own in tropical climates throughout the world. A wide array of ethnomedicinal activities is contributed to different parts of *A. muricata,* and indigenous communities in Africa and South America extensively use this plant in their folk medicine. Numerous investigations have substantiated these activities, including anticancer, anticonvulsant, anti-arthritic, antiparasitic, antimalarial, hepatoprotective and antidiabetic activities

The aim of this research work is to check the antimicrobial activity of *Annona muricata* against pathogenic organisms like *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* and *Klepsiella pneumonia.* Antifungal activity was checked against *Candida albicans* and *Aspergillus niger.*

MATERIALS AND METHODS

Collection of Plant sample:

The mature leaves of *Annona muricata* were collected during july 2014 from Arayoor, Trivandrum District, kerala, India. The plant material was shade-dried with occasional shifting and then powdered with mixer, and stored in an airtight container.

Extraction:

50 g of fresh plant material of plant was grounded with different solvents and soaked in 200 ml of different solvents for 3-7 days filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to attain a clear filtrate. The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum and stored in a small bottle in fridge at 5 °C.

The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. 50 g of the fine powder was soaked in 200 ml of different solvents for 3-7 days filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to attain a clear filtrate. The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum and stored in a small bottle in fridge at 5 °C.

ANTIMICROBIAL ACTIVITY

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

REAGENTS

1**. Muller Hinton Agar Medium (**1 L**)**

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2**. Nutrient broth** (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Antibacterial Activity of Ethanolic Extracts Of Annona Muricata

PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains such as*, Staphylococcus aureus, E coli, Pseudomonas, Klebsiella and Enterococcus.* Wells of approximately 10mm was bored using a well cutter and methanolic sample of 25, 50, and100 µl conc: were added. The plates were then incubated at 37°C for 24 hours. The

antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Gentamycin (standard antibacterial agent, concentration: 20mg / ml) was used as a positive control.

Antibacterial Activity of Methanolic Extracts of Annona Muricata

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Klebsiella pneumonia, E.coli, Pseudomonas aeroginosa, Staphylococcus aureus and Streptococcus mutans* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and sample of 25, 50, and100 µl conc: were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin (standard antibacterial agent, concentration: 10mg / ml) was used as a positive control.

ANTIFUNGAL ACTIVITY

In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown species of fungus such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and methanolic samples of 50 µl and 100 µl concentration were added, the zone of inhibition was measured after overnight incubation and compared with that of standard antibiotics.

RESULTS AND DISCUSSION

Identification of The Plant

Based on the taxonomical characters the plant was identified as Annona muricata of family Annonaceae

ANTIMICROBIAL ACTIVITY

In the present study, the antibacterial and antifungal activities of *Annona muricata* was recorded against different bacterial strains including *E. coli, P. aeruginosa, S. Aureus, Streptococcus mutans*, *klebsiella pneumonia,and Enterococcus* and fungal strains like *Aspergillus niger* and *Candida albicans.*(Fig1-11,Table1-3) The different extracts of this plant showed variable activities.

ANTIMICROBIAL ACTIVITY

Fig 1Antibacterial Activity of Ethanolic Extracts Of *Annona muricata* against *Enterococcus*

Fig 2Antibacterial Activity Of Ethanolic Extracts Of *Annona muricata* against *Staphylococcus aureus*

Fig 3Antibacterial Activity Of Ethanolic Extracts Of *Annona muricata* against *Klebsiella pneumonia*

Fig 4Antibacterial Activity of Ethanolic Extracts Of *Annona muricata* against *Escherichia coli*

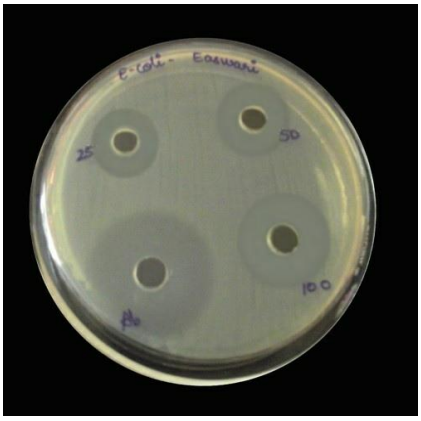

Table 1 Antibacterial Activity Of Ethanolic Extracts Of Annona Muricata

Test organisms	Concentration of extracts(µl)			Positive control Gentamycin 20mg/ml
	25	50	100	
	Zone of inhibition(mm)			
Enterococcus	10	15	20	40
Klebsiella pneumonia	8	14	18	33
Staphylococcus aureus	9	14	20	34
Escherichia coli	10	17	20	36

Fig 5 Antibacterial Activity of Methanolic Extracts Of *Annona muricata* against *Staphylococcus aureus*

Fig 6Antibacterial Activity of Methanolic Extracts of *Annona muricata* against *Escherichia coli*

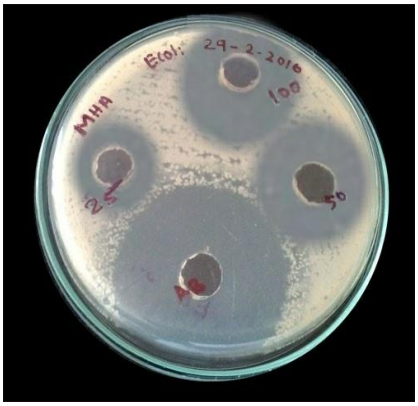

Fig 7Antibacterial Activity of Methanolic Extracts of *Annona muricata* against *Klebsiella pneumonia*

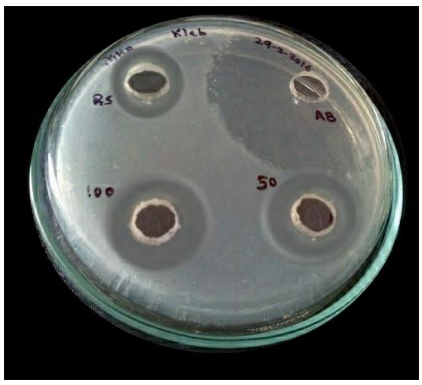

Fig 8Antibacterial Activity of Methanolic Extracts of *Annona muricata* against *Streptococcus mutans*

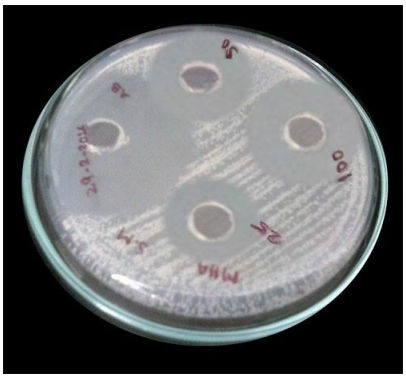

Fig 9Antibacterial Activity of Methanolic Extracts of *Annona muricata* against *Pseudomonas aeroginosa*

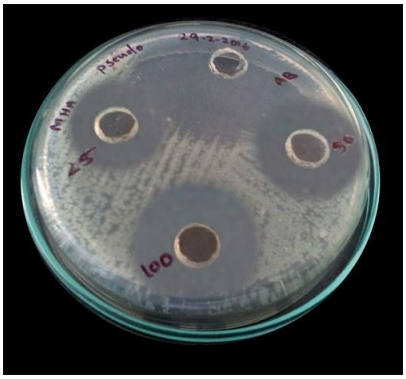

Table 2Antibacterial Activity of Methanolic Extracts of *Annona muricata*

Test organisms	Concentration of extracts(µl)			Positive control Streptomycin 10mg/ml
	25	50	100	
	Zone of inhibition(mm)			
Streptococcus mutans	14	18	20	41
Klebsiella pneumonia	10	14	22	39
Staphylococcus aureus	8	14	20	40
Escherichia coli	15	20	22	40
Pseudomonas aeroginosa	10	15	21	41

ANTIFUNGAL ACTIVITY

Fig 10 Antifungal Activity of Methanolic Extracts of *Annona muricata* against *Aspergillus niger*

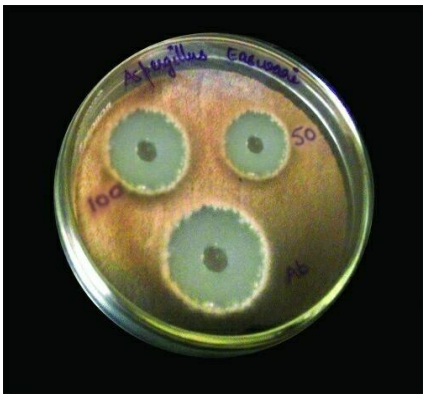

Fig 11Antifungal Activity of Methanolic Extracts of *Annona muricata* against *Candida albicans*

Table 3Antifungal Activity of Methanolic Extracts of *Annona muricata*

Test organisms	Concentration of extracts(µl)		Positive control clotrimazole
	50	100	
	Zone of inhibition(mm)		
Candida albicans	**18**	**20**	**19**
Aspergillus niger	**9**	**15**	**22**

Antibacterial Activity of Different Solvent Extracts of *Annona muricata*

The methanolic and ethanolic extracts of *A.muricata* were evaluated for *in vitro* antibacterial activity against, *E. coli, P. aeruginosa , S. Aureus, Streptococcus mutans , klebsiella pneumonia,and Enterococcus* indicating different zones of inhibition (Fig 1-9,Table 1,2) .In *klebsiella pneumonia, E. coli and S. Aureus* methanolic extracts were found to be having more inhibitory activity than ethanolic extracts. The inhibitory activity

increased with increase in concentration of the sample. It was found that 100 µl of extract of *Annona muricata showed* significantly higher zone of inhibition (18-22mm) against *E. coli, P. aeruginosa, S. Aureus, Streptococcus mutans*, *klebsiella pneumonia,and Enterococcus*. While 50 µl of extract of *Annona muricata* showed moderate activity (14-18mm) against the tried bacterial strains. (Fig1-9, Table 1,2)

The methanolic extracts of *A.muricata* also showed significant antibacterial activity against *Streptococcus mutans, Klebsiella pneumonia,Staphylococcus aureus, Pseudomonas aeroginosa* .

and *Escherichia coli. (*Fig (5-9), Table 2*).* In *Streptococcus mutans* and *Staphylococcus aureus* the100 µl of methanolic extract showed an inhibition zone of 20mm. The100 µl of methanolic extract inhibited *Klebsiella pneumonia* and *Escherichia coli* with a zone diameter of 22mm. An inhibition zone of 21mm was obtained in the100 µl of methanolic extract of Annona muricata for *Pseudomonas aeroginosa* .

Our results are supported by the study of R. Vinothini and Lali Growther (2016) in which they reported that methanol and water extracts of *Annona muricata* can be effective against tested bacteria *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis*. The inhibition zones obtained were similar to our results.

The ethanolic extracts of *A.muricata* also showed significant antibacterial activity against *Enterococcus, Klebsiella pneumonia,Staphylococcus aureus* and *Escherichia coli.(*Fig(1-4)Table 1). In *Enterococcus* the100 µl of ethanolic extract showed an inhibition zone of 20mm. the100 µl of ethanolic extract inhibited *Klebsiella pneumonia* with a zone diameter of 18mm. an inhibition zone of 20mm was obtained in the100 µl of ethanolic extract of Annona muricata for *Staphylococcus aureus* and *Escherichia coli.*The investigation of Kamath etal (2017) also showed similar activity against *Klebsiella pneumonia* and *Escherichia coli* .

The Comparative antibacterial activity between ethanolic extract of *Annona muricata* and standard antibiotic gentamycin and methanolic extract of *Annona muricata* and standard antibiotic streptomycin were studied. The antimicrobial activity of the tested extracts are comparable with the standard drugs. These activities may be due to strong occurrence of different chemical compounds such as flavonoids, tannins, alkaloids, steroids, phenols and saponins.

ANTIFUNGAL ACTIVITY

Antifungal activity for methanolic extract of *Annona muricata* was seen against *Candida albicans* and *Aspergillus niger.*(Fig 10,11 ,Table-3) The methanolic extract showed significant activity against both the tested organisms. In *Candida albicans* the100 µl methanolic extract of *Annona muricata* obtained an inhibition zone of 20mm and in *Aspergillus niger* it showed an inhibition zone of 15mm. The antifungal activity of the tested extracts are comparable with the standard drug clotrimazole. *Annona muricata* was also reported to have significant antifungal activity (B.H Midhun Pai *et., al* 2016 and R. Vinothini and Lali Growther(2016).

CONCLUSION

This study revealed that *Annona muricata* extract possess activity against bacteria responsible for some common microbial dieseases. These promissory extracts open the possibility of finding new clinically effective antimicrobial compounds. Further research is necessary to determine the identity of the antimicrobial compounds from the leaves of Annona muricata and also to determine their full spectrum of efficacy. This could also play an important role in the establishing of new pharmaceutical agents.we hope that this study would be a source of enlightenment and motivation for researchers to further perform *in vitro*,*in vivo* and clinical investigations on the biological activities of *A. muricata* to gain insight into developing new agricultural and pharmaceutical agents.

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