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ANTIBACTERIAL AND ANTIOXIDANT ASSAY OF *ANNONA MURICATA* LEAF EXTRACT

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ABSTRACT
Annona muricata also referred to as Soursop is a, flowering evergreen tree native to Mexico, Cuba, Central America and parts of India. It is a natural cancer killer and found to be 10,000 times stronger than chemotherapy. The aim of the present study was to extract phytochemicals from *Annona muricata* leaves, and to determine the percentage yield of crude Annona muricata leaf extract. Also, an attempt was made to observe the Antimicrobial activity of the leaf extract. The antioxidant properties of the leaf extract was calculated using Anti-oxidant (DPPH) assay.

INTRODUCTION
Annona muricata belongs to the family of custard apple tree called Annonaceae. Its fruit is called as sour-sop due to its slightly acidic taste when ripe.[1] The fruit is juicy, acidic, whitish and aromatic with abundant seeds. The flavour of the fruit is described as a combination of strawberry and pineapple with a hint of citrus flavour in a creamy texture. 80% of the fruit is water and the remaining is composed of 1% protein, 18% carbohydrates with micro-nutrients such as Vitamins B, B2 and C, Potassium and fibers.[2] The average weight of 1000 fresh seeds is 470g and has an average oil content of 24%.[3] When dried for three days in 60ª the average seed weight was 322g and were tolerant of the moisture extraction showing no problems for long-term storage under reasonable conditions. It is reported that the natural compounds are predominantly found in stem, bark and fruit seeds. In a 1997 clinical study, novel alkaloids found in *Annona muricata* fruits exhibited anti-depressive effects in animals.[4,6]

All the components of *Annona muricata* are useful. It has been used as a folkloric herbal medicine in many regions. It is considered to be anti-spasmodic and anti-emetic. A decoction of *Annona muricata* leaves is used to kill bed bug, head lice and to reduce fever. This can be taken orally or added to bathing water. The leaves are also used for faster healing of skin eruptions. A poultice of these leaves is also used to alleviate rheumatism, skin infections and inflammations. Pulverized *Annona muricata* seeds is also used as a caterpillar repellent spray.[4] The fruit's juice is also taken to eliminate worms, parasites, reduce fever, increase mother's milk production after child birth and is also used as an astringent for diarrhoea and dysentery.[1] The dark leaves are considered as a sedative and antispasmodic.[1]

Annona muricata also played an important medicinal role in indigenous communities of Peruvian Andes, Guyana, Brazilian Amazon, Jamaica, Haiti and the West Indies. The crushed seeds were used to kill parasites. The bark, leaves and roots were used to treat diabetes.

In the Brazilian Amazon communities, the tea of its leaves were used for liver problems and the oil of the leaves and unripe fruits are mixed with olive oil and used externally for neuralgia, rheumatism and arthritic pain.[5]

Phytochemicals are biologically chemical agents produced in plants as secondary metabolites. They are found in fruits, vegetables, grains legumes and green tea. Many approaches have been found to extract phytochemicals from plants using suitable solvents. As reported, *Annona muricata* has been found to possess many phytochemicals and exhibit anti-0xidant properties.

In this paper, antimicrobial activity and anti-oxidant properties of Annona muricata leaf extract was performed and the results are tabulated. Further research on its antimicrobial properties as well as the determination of its biochemical components must be done at different concentrations to determine its phytochemical activity.

MATERIALS AND METHOD

1. Extraction from *Annona muricata* leaves

Annona muricata leaves were collected and sun-dried for three days. The leaves were powdered in a pulveriser. 20g of the crude leaf powder was weighed and taken in a muslin cloth and tied. The cloth was placed in a Rotary Evaporator containing 300mL of Methanol which is the solvent used. The Rotary Evaporator was allowed to run for 48 hours until the disappearance of green colour in the tube containing the powdered leaves. The amount of the crude extract was weighed.

Figure 1: Rotary Evaporator.

Figure 2: Extraction process.

2. Anti-bacterial activity of methanol extract of *Annona Muricata*

The four Microbial culture collections were purchased from NCCS, Pune. Sub-cultures of the 4 species of the bacteria were made on nutrient agar plates and were incubated at 37ª for 24 hours. The growth of pure cultures of the 4 species were made ready for the experiments.

For antibacterial studies, 38g of Mueller Hinton Agar (Hi Media) medium powder was weighed and placed in 1000mL sterile conical flask. 1L of deionised water was added to the flask. The flask contents were autoclaved at 15 PSI (121 C) for 15 mins. The autoclaved medium was poured in 25mL amounts into sterile 90mm Polystyrene Petri dishes (Tarsons) in laminar air flow hood. After allowing 1 hour for perfect cooling and solidification of the medium, the culture media were used for experimental purpose.

1000mg of the crude leaf extract of *Annona muricata* in Methanol was dissolved in 10mL of Dimethyl sulphoxide (DMSO) to get a stock suspension of 100mg/mL (100000ug/mL). From this stock suspension of leaf extract in Methanol, 7 different working suspensions of the concentrations of 1000, 2000, 3000, 4000, 5000, 6000 and 7000 which were duly labelled. Using separate sterile cotton swabs, the subcultures of the 4 pathogenic species of bacteria viz. *S. aureus, E. faecalis, S. epidermidis and K. pneumoniae* were made as suspensions in sterile normal saline. The turbidity of the resultant bacterial suspensions with sterile cotton swabs the test bacteria were inoculated on duly labelled sterile Mueller Hinton agar medium.Using sterile micro tips 8 wells were punched in the culture media with a gap of 1 cm between each well. The wells were marked at the base of the plate as 1, 2, 3, 4, 5, 6, 7 and control. With sterile micro tip 100uL of each one of the 7 working plant extract suspensions was added to the duly marked wells. The bacteria were also exposed to an antibiotic disc containing 5ug of Ciprofloxacin as an antibiotic control. The culture plates were incubated in the bacteriological incubator (Equitron) at 37 ª for 24 hours. After incubation period, the plates were examined for any zone of inhibition. The zones of inhibition are tabulated.

3. DPPH (1, 1, diphenyl 2, 2 picryl hydrazyl) Assay

DPPH (1, 1, diphenyl 2, 2 picryl hydrazyl) is a stable, free radical with an unpaired valence electron at one atom of nitrogen bridge. It uses the property of radical scavenging for determining antioxidant properties of phytochemicals. It is dark violet coloured powder which is crystalline in nature. The colour changes to colourless or pale yellow when neutralized. The In this experiment, Methanol is used as a solvent. DPPH is dissolved in methanol and its final concentration is 2mg/Ml. Ascorbic acid dissolved in Methanol is taken as control with concentration of 1mg/mL. The Annona muricata leaf extract is dissolved in Methanol (or DMSO) and its final concentration is of 10mg/Ml. The working concentrations were 10µg, 20µg, 30µg, 40µg, 50µg, 60µg, 70µg, 80µg, 90µg and 100µg. All the 10 samples, control and standard tubes are placed in dark at room temperature for 20 minutes. The Optical density (O.D.) value of the 12 samples are recorded at 517nm using Methanol as blank in a Spectrophotometer (Labman).

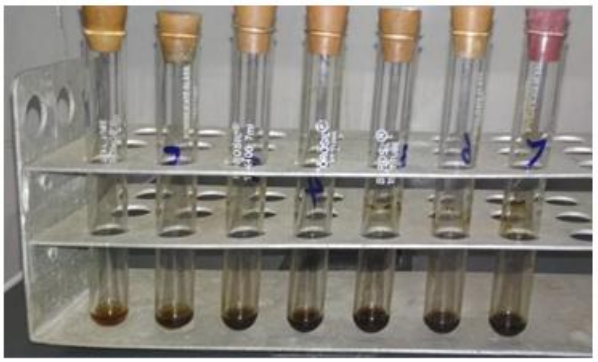

Figure 7: Setup for DPPH Assay.

The Antioxidant activity (% Scavenging) of each concentration calculated using the formula:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS

1. Percentage yield of crude *annona muricata* extract

Amount of plant material taken = 20g

Amount of the extract = 1.596g

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of crude leaf powder}} \times 100$$

$$= \frac{1.596}{20} \times 100$$

$$= 7.98\%$$

Therefore, the % yield was calculated to be 7.98%.

2. Antibacterial study of methanol extract of *annona muricata* leaf

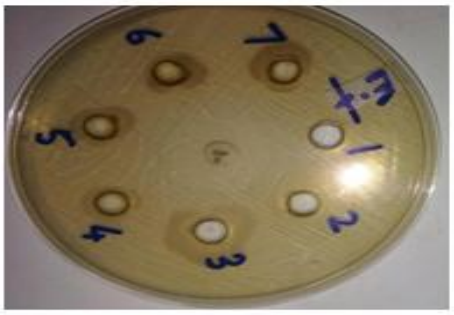

Figure 3

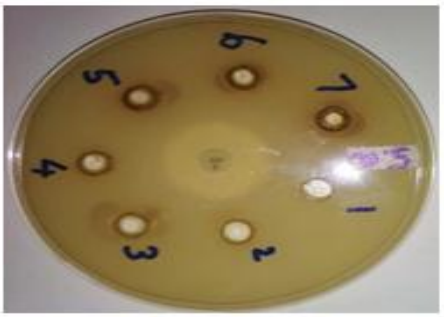

Figure 4

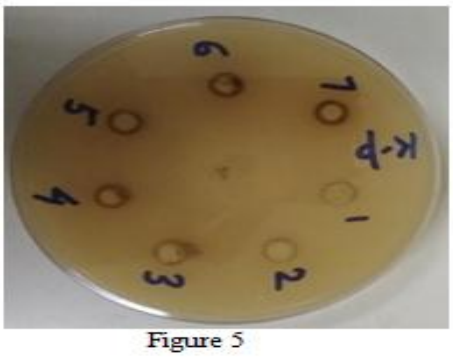

Figure 5

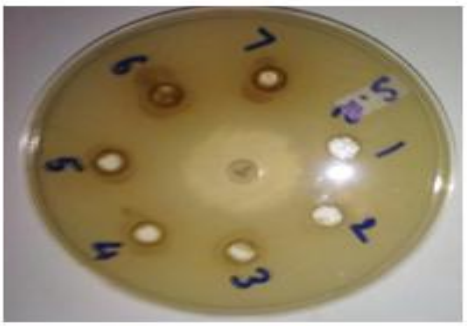

Figure 6

Table 1: Zones of inhibitions.

Concentration of leaf extract	*Staphylococcus aureus*	*Enterococcus faecalis*	*Staphylococcus epidermidis*	*Klebsiella pneumonia*	Result
1000µg	0mm	0mm	0mm	0mm	Resistant
2000µg	0mm	0mm	0mm	0mm	Resistant
3000µg	0mm	0mm	0mm	0mm	Resistant
4000µg	0mm	0mm	0mm	0mm	Resistant
5000µg	0mm	0mm	0mm	0mm	Resistant
6000µg	0mm	0mm	0mm	0mm	Resistant
7000µg	0mm	0mm	0mm	0mm	Resistant
DMSO(control)	0mm	0mm	0mm	0mm	Resistant
Ciprofloxacin(5µg) (control)	30mm	30mm	30mm	30mm	Highly sensitive

Table 2: DPPH Assay.

S. No.	Stock plant extract (10mg/mL)	Methanol	Concentration of extract	Absorbance	%Scavenging
1.	10µL	90µL	10µg	0.286	70.99
2.	20µL	80µL	20µg	0.271	72.51
3.	30µL	70µL	30µg	0.263	73.32
4.	40µL	60µL	40µg	0.251	74.54
5.	50µL	50µL	50µg	0.240	75.65
6.	60µL	40µL	60µg	0.231	76.57
7.	70µL	30µL	70µg	0.222	77.48
8.	80µL	20µL	80µg	0.212	78.49
9.	90µL	10µL	90µg	0.200	79.71
10.	100µL	-	100µg	0.183	81.44
11.	Standard 100µL	-	-	0.294	70.18
12.	Control	-	-	0.986	-

CONCLUSION

The antimicrobial property of *Annona muricata* leaves were not exhibited at the concentration used in this experiment. No zones of inhibitions were observed in the wells filled with the plant extract (Figures 3-6, Table 1). Zone of inhibition was visible only around the antibiotic disc. There have been reports that say that antimicrobial properties were exhibited at these concentrations. It could be checked when the experiment is performed again.

The percentage of scavenging is tabulated (Table 2). The leaf extract showed a moderate anti- oxidant activity compared to the control (Ascorbic acid) which showed a reading of 0.986. At different concentrations, the range of the anti-oxidant activity was between 70.99% to 81.44%. The lowest anti-oxidant activity was observed at 10µg and the highest activity was observed at 100µg.

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