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RESEARCH ARTICLE

Antioxidant and antibacterial activity of (*Annona muricata* L.) leaf aqueous extract

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SUMMARY

Annona muricata L. (Soursop) of Annonaceae is a medicinal plant whose aqueous leaf extract was tested and the same possessed antioxidant properties including radical scavenging activity. The antibacterial activity was tested by agar diffusion method against *Escherichia coli*, *Enterobacter aerogens, Klebsiella pneumoniae* and *Streptococcus pneumoniae*. The minimum inhibitory (MIC) and bacterial concentration (MBC) values varied for the four bacterial genus tested.

Key Words : Antibacterial activity, Antioxidant activity, *Annona muricata,* DDPH, Minimum Inhibitory concentration, Minimum bacterial concentration

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Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited (Adewole and Caxton-Martins, 2006). There are several reasons why the use of medicinal plants should be studied: herbal remedies may have recognizable therapeutic effects (Bailey and Day, 1989); they may also have toxic side-effects (Keen *et al*., 1994). The use of medicinal plants provides an indication of beliefs about illness and its treatment that may conflict with beliefs of workers in the formal healthcare system (Morgan and Watkins, 1988).

Several studies have described the medicinal purposes of *Annona muricata* and outlined the social history of the plant's use (Ayensu, 1981). *A. muricata* L. family Annonaceae, commonly called 'Soursop' is a small, upright evergreen tree growing 5 to 6 meters in height. Young branchlets are rusty-hairy, the malodorous leaves, normally evergreen, are alternate, smooth, glossy, dark green on the upper surface, lighter beneath, oblong, elliptic or narrow-obovate, pointed at both ends, 6-20 cm long and 2-6 cm wide. The flowers are borne simply, and may emerge anywhere on the trunk, branches or twigs. They are short stalked, 4-5 cm long, plump, and triangular or conical; the 3 fleshy, slightly spreading, outer petals yellow-green, with 3 closet inner pale-yellow petals (Vasquez, 1990; de Feo, 1992). The fruit is more or less oval or heart-shaped, sometimes irregular, lopsided or curved, due to improper carpel development or insect injury. The size ranges from 10-30 cm long and upto 15 cm in width. The fruit is compound and covered with reticulated leathery - appearing but tender, inedible bitter skin from which protrude a few or many stubby or more elongated and curved soft, pliable 'spines'. The tips break off easily when the fruit is fully ripe. The skin is dark-green in the immature fruit, becoming slightly yellowish-green at maturity and the fruit is soft to touch. Its inner surface is cream-coloured and granular and separates easily from the mass of snow-white, fibrous, juicy segments - much like flakes of raw fish - surrounding the central, soft pithy core. In aroma, the pulp is somewhat pineapple-like, but its musky, subacid to acid flavour is unique (Schultes and Raffauf, 1990). Most of the closely-packed segments are seedless. In each fertile segment, there is a single oval, smooth, hard, black seed, 1.25-2.0 cm long and a large fruit may contain from a few dozen to 200 or more seeds (Morton, 1980). The plant is indigenous to

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most of the warmest tropical areas in South and North America including Amazon. *A. muricata* has become naturalized in many countries, and now has a wide distribution throughout tropical and sub-tropical parts of the world, including tropical India.

All parts of *A. muricata* tree are used in natural medicine in the tropics including the bark, leaves, root and fruit-seeds (Holdsworth, 1990). Many bioactive compounds and phytochemicals have been found in *A. muricata* and its uses in natural medicine have been validated (Weniger *et al*., 1986). Several studies by different researchers demonstrated that the leaf, bark, root, stem and seed extracts are antibacterial *in vitro* against numerous pathogens (Misas, 1979; Heinrich, 1992; Sundarrao, 1993) and that the bark has antifungal properties (Lopez Abraham, 1979).

Annona muricata has a long history of use in herbal medicine in the tropical areas in South and North America including the Amazon and West Africa. In view of this, the present study was designed to evaluate the antioxidant and antibacterial activity of *A. muricata* leaf aqueous extract on four bacterial genus.

MATERIALS AND METHODS

***Collection and identification of plant material* :**

Fresh leaves of *Annona muricata* L. were collected from a private garden in Tiruchirappalli, Tamil Nadu (Fig. A). The taxonomic identities of these plants were confirmed by Flora of the Presidency of Madras (Gamble, 1925). Fresh leaf material was washed under running tap water, air dried in shade and then homogenized to fine powder and stored in sterile air-tight bottles for the experimental work.

Bacterial cultures :

The bacterial cultures tested in this work *Escherichia coli* NCBT 001, *Enterobacter aerogens* NCBT 012, *Klebsiella pneumoniae* NCBT 018, *Streptococcus pneumoniae* NCBT 062 were maintained in immobilized condition in Nutrient Gelatin medium containing Gelatin 15 g, Nutrient broth 100 ml with pH 7.2 (Harrigan and McCance, 1969) in Microbiology Lab, Department of Biotechnology, National College, Tiruchirappalli.

Preparation of leaf extract :

100 g of dried leaf powder was extracted using 300 ml of sterile distilled hot water and 90 per cent methanol (v/v). The immersed leaf powder was kept in a shaker (60 rpm) for a week and filtered through Whatman No.1 filter paper. The extract was concentrated using simple distillation and lyophilisation method and stored in sterile vials at 4° C for further work.

Total phenolic content assay :

Total phenolic content assay was conducted using Folin-Ciocalteu reagent method (Sato *et al*., 1996). An aliquot of 0.5 ml (100 mg/ml in 80% methanol) of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 0.05 ml of 10% Na_2CO_3

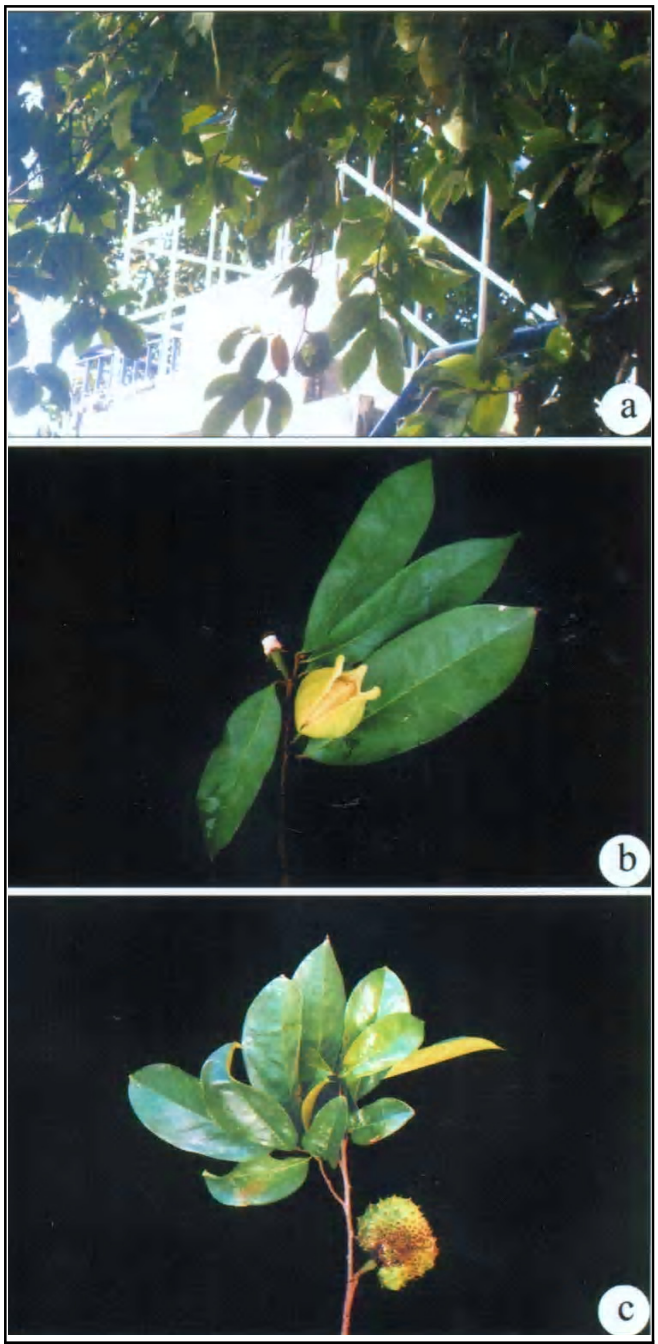


Fig. A: ***Annono muricata*** **L. (*Annonaceae*) (a) Habit, (b) Twig with flower, (c) Twig with young fruit**

and absorbance was measured at 735 nm after 1 hr of incubation at room-temperature. Gallic acid was used as the standard for the calibration curve and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extract.

Measurement of α, α-Diphenyl-β-picrylhydrazyl radical scavenging activity :

Quantitative measurement of radical scavenging

properties was carried out in a universal bottle. The reaction mixture contained 50 µl of test samples (0.1 mg/ml) or 80% MeOH as a blank and 5 ml of 0.04% (w/v) solution of α,α-Diphenyl-β-picryl- hydrazyl in methanol (Oktay *et al*., 2003). Two different known antioxidants such as Vitamin E (0.1 mg/ml) and butylated hydroxytolune (BHT, Sigma) (0.1 mg/ml) were used for comparison as positive control. The colorimetric test for free radicals relies on the reaction of a specific antioxidant (AH) with α,α-Diphenyl-β-picrylhydrazyl (α,α-Diphenyl-b-picrylhydrazyl-H+A) and the method was adopted from Ohnishi *et al*. (1994). In the radical form α,α-Diphenyl-β-picrylhydrazyl resulted in a maximum absorption at 517 nm but upon reduction by an antioxidant, the pale-yellow non-radical form was produced and hence the absorption at 517 nm disappeared.

Decolourization was measured at 517 nm after incubation for 30 min. Measurements were taken in triplicate. Scavenging effect of α,α-Diphenyl-β-picrylhydrazyl was calculated using the equation.

$$\text{Scavenging effect of } \alpha,\alpha-\text{diphenyl}-\beta-\text{picrylhydrazyl (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample (Oktay *et al*., 2003). The actual decrease in absorption induced by the test compounds was compared with the positive controls. Extract concentration providing 50 per cent inhibition (IC_{50}) was calculated from the graph of percentage of inhibition against extract concentration. Tests were carried out in triplicate.

Measurement of reducing power :

The reducing power of the leaf extracts of *Annona muricata* and butylated hydroxytolune were determined according to the method of Yen and Chen (1995). The extracts (1-10 mg/ml) and butylated hydroxytolune (1-6 mg/ml) were mixed with an equal volume of 0.2 m phosphate buffer (pH 6.6) and 1 per cent potassium ferricyanide and then incubated at 50° C for 20 min. An equal volume of 1 per cent trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged at 4500 rpm for 10 min. The supernatant was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2 (v/v/v) and then absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Antioxidant studies - DPPH and reducing power :

The scavenging effect of the leaf methanol extracts on DPPH radical exhibited strong radical scavenging activity. It might be due to their hydrogen-donating ability (Oyaizu, 1986; Hyang-Sook *et al*., 2000) and is generally associated with the presence of reductants (Pin-Der and Duth, 1998). The total phenolic content of the crude extract of the leaf (100 mg/ml in 80% methanol) of *A. muricata* was 14.5 mg (mg gallic acid equivalent/g).

Scavenging effect on α, α - Diphenyl -β -picrylhydrazyl radical :

The leaf extract was screened for DPPH radical scavenging activity according to the method Ohnishi *et al.* (1994). *A. muricata* leaf extract exhibited stronger radical scavenging activity than butylated hydroxytoluene and vitamin E (Table 1).

Table 1: Scavenging effect percentage of crude leaf extract of *Annona muricata* and known antioxidant BHT and Vitamin-E at 0.1 mg/ml respectively

Sample	Scavenging effect %
BHT	8.5
Vitamin-E	4.0
A. muricata leaf extract	9.5

The concentration of the crude extract decreased α,α -Diphenyl-β-picrylhydrazyl to 50 per cent of the initial radical concentration (IC_{50}) in 30 min. This parameter is widely used to measure antioxidant power. A low IC_{50} is indicative of strong antioxidant compounds such as flavones, catechins and xanthan (Shimada *et al*., 1992; Schinella *et al*., 2000). Strong radical scavenging activity of leaf extract of *A. muricata* might be due to their hydrogen donating ability as stated by Oyaizu (1986), Hyang-Sook *et al*. (2000) and is generally associated with the presence of reductants (Pin-Der and Duth, 1998). At proper concentration, the crude extracts may act as electron donors and react with free radicals to convert them to more stable products and terminate radical chain reaction (Duh and Yen, 1997). The results demonstrated that the leaf crude extracts of *A. muricata* had potent free radical scavenging activity.

Reducing power of *A. muricata* leaf extract :

The presence of reductants (antioxidants) in the leaf extract would result in the reduction of the Fe^{3+} ferricyanide complex to its ferrous form. The formation of Perl's Prussian blue measured at 700 nm can be used to monitor the amount of Fe^{2+} complex. The dose-response curve for the reducing power of the leaf extract is shown in Fig. 1. Butylated hydroxytolune (BHT) was used as positive control and the reducing powers of leaf extract also increased with the increase in the concentration. At a dose of 10.0 mg/ml, the leaf extract showed reducing power value 2.0 when compared with the BHT control 2.5, respectively. These results revealed that the leaf extract acts as electron donor and could react with free radicals, converting them to more stable products

and terminating the radical chain reaction (Yen and Chen, 1995; Yamaguchi *et al.,* 1998).

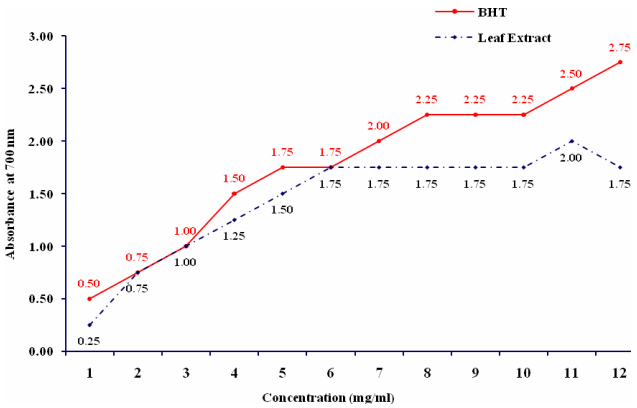


Fig. 1 : Reducing power of the crude leaf extract of *A. muricata* and known antioxidants butylated hydroxytolune (BHT). Increase in the absorbance at 700 nm indicates the reducing power

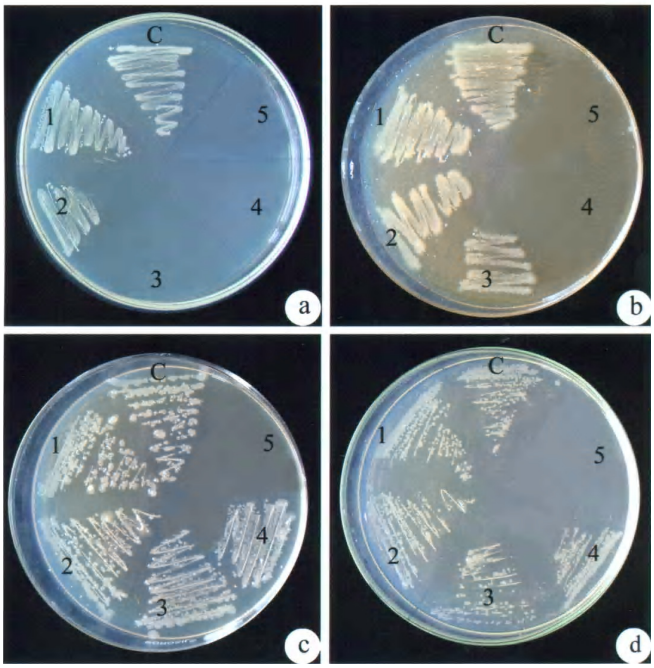
The above results suggest that the *A. muricata* leaf extract can eliminate human health problems in which free radicals play a major role (Karbownik and Lewinski, 2003; Lugasi *et al*., 1999). There is a continuous effort in the food industry to seek additional plant sources that are safer and better antioxidants *A. muricata* leaf extract serves as a suitable natural source of antioxidant.

Antibacterial activity :

The minimal inhibitory concentration (MIC) value and the minimum bacterial concentration (MBC) value varied for the four bacteria tested for this study. *Escherichia coli* showed minimum value. MIC 60 mg/5 ml and MBC 70 mg/5 ml, *Enterobacter aerogens* showed MIC 70 mg/5 ml and MBC 80 mg/5 ml, *Klebsiella pneumoniae* showed MIC 80 mg/5 ml and MBC 90 mg/5 ml whereas *Streptococcus pneumoniae* showed maximum value, MIC 80 mg/5 ml and MBC 90 mg/5 ml (Fig.1).

Traditionally plant leaf extracts are used to treat microbial infections. Annonaceous plants and *Annona muricata* plant extract are found in antibacterial, antitumor, anti-cancer and cytotoxic studies (Haddock, 1994; Oberlies *et al*., 1995; Mor, 2000; Chang, 2001; Padma *et* al., 2001; Liaw, 2002; Chang *et al*., 2003; Pathak *et al*., 2003; Yuan *et al.*, 2003; Kojima, 2004).

A. muricata tree is rich in many phytochemical compounds like ellagic acid, saponins, cardiac glycosides, lactones, tannins, isoquinoline alkaloids, steroids, flavonoids. Many annonaceous acetogenins including annocatalin, annohexocin, annomonicin, annomontacin, annomuricin, annomutacin and annonacin are active against multi-drug resistant (MDR) cancer cells by ATP-blocking properties (Oberlies *et al*., 1995; Pathak *et al.,* 2003; Tormo *et al*., 2003; Kojima, 2004). Such bioactive compounds of *A. muricata* leaf extract possess antimicrobial properties and active against the tested bacteria.



C- Control
1- 50 mg/5 ml
2- 60 mg/5ml
3- 70mg/5ml
4- 80mg/ml
5- 90mg/5ml

Fig. 2 : Antibacterial activity MIC/MBC of *Annona muricata*L. leaf aqueous extract (a) *Escherichia coli* (b) *Enterobacter aerogens* (c) *Klebsiella pneumoniae* (d) *Streptococcus pneumoniae*

In conclusion, *Annona muricata* leaf extract serves as a safe natural plant antioxidant and can provide the raw material to the food industry. In addition it can also be a potential antibacterial source and the bioactive compounds will find a place in the formulation of herbal medicine to treat bacterial mediated infections like gasteroenteritis and respiratory infections.

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