

EVALUATION OF IN VITRO ANTI OXIDANT ACTIVITY OF *ANNONA MURICATA* BARK

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ABSTRACT

The present study was an endeavor to evaluate the anti oxidant activity of Ethanolic bark extract of *Annonamuricata*. In vitro study of anti oxidant activity was carried out by DPPH (1, 1-Diphenyl 2 picrylhydrazyl), Hydroxyl radical scavenging assay and reducing power method with Gallic acid as the standard in all the three methods. The results revealed that the extract of *Annonamuricata* possess significant antioxidant activity.

Keywords: *Annonamuricata*, DPPH, Hydroxyl radical scavenging assay, reducing power method.

INTRODUCTION

Anti oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease¹.

Annonamuricata belongs to family Annonaceae commonly called Soursop, guayabano, lakshamana phalam². The bark leaves and roots are considered sedative, anti spasmodic, hypoglycemic, hypotensive, smooth muscle relaxant and nervine³. Bark has anti fungal properties⁴. Stem bark yielded one acetogenin, solamin, and two triterpenoids, stigmasterol&sitosterol⁵.

MATERIALS AND METHODS

DPPH was obtained from research lab fine chemical industries, Mumbai. Gallic acid was a gifted sample. Potassium ferricyanide, Trichloroacetic acid, Ferroussulphate, Hydrogen peroxide, sodium salicylate were used for this study are of analytical grade.

Plant Material

The stem bark of *Annonamuricata* was collected during December –February 2013 from Jalluru, East Godavari Dist, Andhra Pradesh. The stem bark of *Annonamuricata* was identified and authenticated by the Taxonomist Dr. T.V.Raghavarao, Maharani College, Peddapuram.

Preparation of Extract

The freshly collected barks were cleaned from dirt and they were 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 then dried.

DPPH METHOD⁶

The free radical scavenging activity was followed by preparing 0.002% DPPH solution in methanol. Gallic acid was taken as the reference standard.

Different concentrations of the extract (50, 100, 300 µg/ml) and standard drug (1, 2.5 & 5 µg/ml) were prepared using methanol. 1ml of 0.002% DPPH solution is mixed with 3ml of all the concentrations of both extract and standard separately. These mixtures are kept in dark for about 30 min and the optical density was measured at 517 nm. 0.002% DPPH and the methanol mixture is the blank. Finally the % inhibition of the DPPH activity is calculated using the formula.

$$\text{DPPH Scavenged (\%)} = \frac{[A_{\text{control}} - A_{\text{test}}]}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control reaction (containing all reagents except sample extract) and A_{test} is the absorbance in the presence of the sample of the extracts. The anti oxidant activity of the ethanolic bark extract was

expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration (in $\mu\text{g/ml}$) of extract that scavenges the DPPH radicals by 50%.

REDUCING POWER ASSAY⁷

Different concentrations of the extract (50, 100, 300, 500 $\mu\text{g/ml}$) and standard drug (1, 2.5, 5 $\mu\text{g/ml}$) were prepared using distilled water. 1% Potassium Ferricyanide, 10% Trichloro acetic acid, 0.1% ferric chloride and 0.2M Phosphate buffer were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1ml of each concentration of both extract and standard were taken separately and mixed with 1ml of 0.2M phosphate buffer (p^H 6.6) and 1ml of Potassium ferricyanide. Incubate all these samples at 50°C for 30 min. Then add 1ml of 10% Trichloroacetic acid and centrifuge at 3000 rpm for 10 min. Now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5 ml of freshly prepared ferric chloride. Finally measure the absorbance at 700nm.

HYDROXYL METHOD⁸

The scavenging ability of the extracts on hydroxyl radicals was determined according to the method described by Smirnoff and Cumbe (1989) with some modifications. Briefly, individual sample extract (1ml) at different concentrations (100, 300, 500 $\mu\text{g/ml}$) was added to the reagent containing 1ml (1.5mM FeSO_4 , 0.7ml 6mM Hydrogen peroxide and 0.3 ml 20mM sodium salicylate). After incubation for 1hr at 37°C, absorbance of the reaction mixture was read at 562nm. The scavenging ability on hydroxyl radicals was calculated using the following equation

$$\text{Scavenging ability on hydroxyl radicals (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control reaction (containing all reagents except sample extract) and A_{test} is the absorbance in the presence of the sample extract. The anti oxidant activity of the ethanolic bark extract was expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration

(in $\mu\text{g/ml}$) of extract that scavenges the Hydroxyl radical by 50%.

RESULTS AND DISCUSSIONS

DPPH METHOD

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which may be induced by antioxidants. **Table 1** shows the % of DPPH radical scavenged by Gallic acid and ethanolic extract of bark at various concentrations ($\mu\text{g/ml}$). **Figure 1, 2** illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in the ethanolic extract of bark of *Annonamuricata*. The IC_{50} values were found to be 109 $\mu\text{g/ml}$ and 3.5 $\mu\text{g/ml}$ for Ethanolic bark extract of *Annonamuricata* and Gallic acid respectively.

REDUCING POWER ASSAY

Reducing power assay is based on the principle that substances which have reduction potential, react with Potassium ferricyanide (Fe^{+3}) to form Potassium ferrocyanide (Fe^{+2}), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. **Table 1** shows the reducing power of ethanolic bark extract of *Annonamuricata* from **Figure 3, 4** it was found that the absorbance of the extract increased with the increase in concentrations. Reducing power capabilities of extract was found to be closer to Gallic acid.

HYDROXYL METHOD

The reduction capability of hydroxyl radical was determined by the decrease in its absorbance at 562nm, which may be induced by antioxidant. **Table 1** shows the % of hydroxyl radical scavenged by Gallic acid and ethanolic extract of bark at various concentrations ($\mu\text{g/ml}$). **Figure 5, 6** illustrates a decrease in the concentration of hydroxyl radical due to the scavenging ability of the soluble constituents in the ethanolic extract of bark of *Annonamuricata* and standard Gallic acid. The IC_{50} values were found to be 120 $\mu\text{g/ml}$, and 0.5 $\mu\text{g/ml}$ for ethanolic bark extract of *Annonamuricata* and Gallic acid respectively.

Table 1: Effect of Ethanolic Extract of *Annonamuricata* bark on different antioxidant models

Concentration($\mu\text{g/ml}$)	%Inhibition		
GALLIC ACID	DPPH	HYDROXYL METHOD	REDUCING METHOD
1	23 \pm 1.24	55.6 \pm 0.72	0.0075 \pm 0.002
2.5	37.6 \pm 1.51	65.3 \pm 1.9	0.0201 \pm 0.004
5	60 \pm 0.47	70 \pm 0.28	0.0412 \pm 0.004
IC ₅₀	3.5 $\mu\text{g/ml}$	0.5 $\mu\text{g/ml}$	
<i>ANNONA MURICATA</i> (TEST DRUG)($\mu\text{g/ml}$)			
50	23.6 \pm 0.27	-	0.0795 \pm 0.008
100	37.6 \pm 1.08	47.3 \pm 0.21	0.1256 \pm 0.001
300	95 \pm 0.47	64.5 \pm 0.70	0.3390 \pm 0.001
500	-	77.3 \pm 1.10	0.5765 \pm 0.001
IC ₅₀	109 $\mu\text{g/ml}$	120 $\mu\text{g/ml}$	

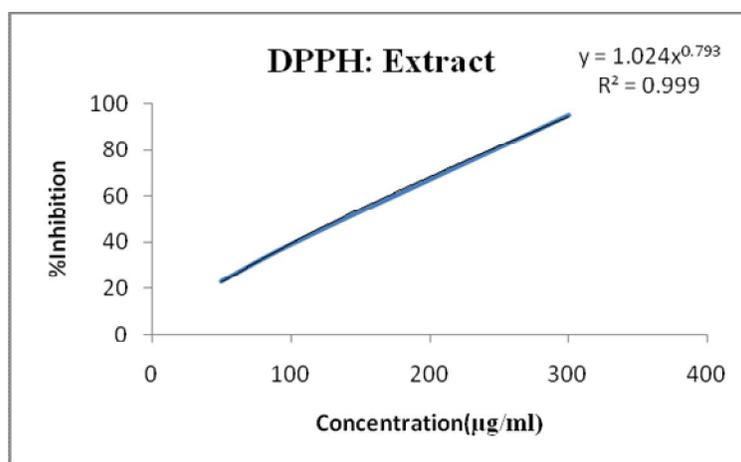


Fig. 1

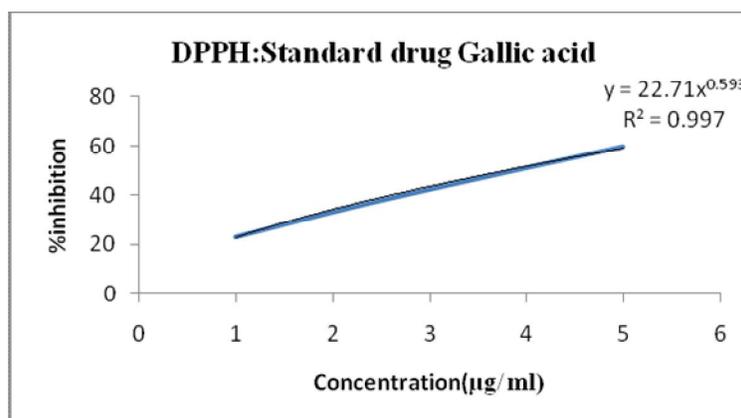


Fig. 2

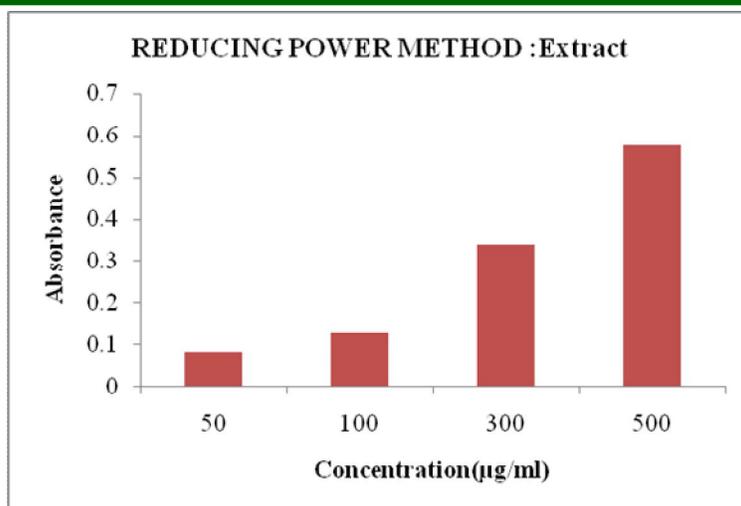


Fig. 3

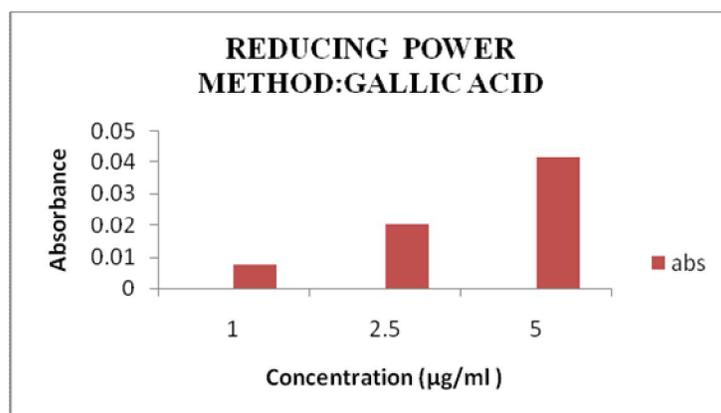


Fig. 4

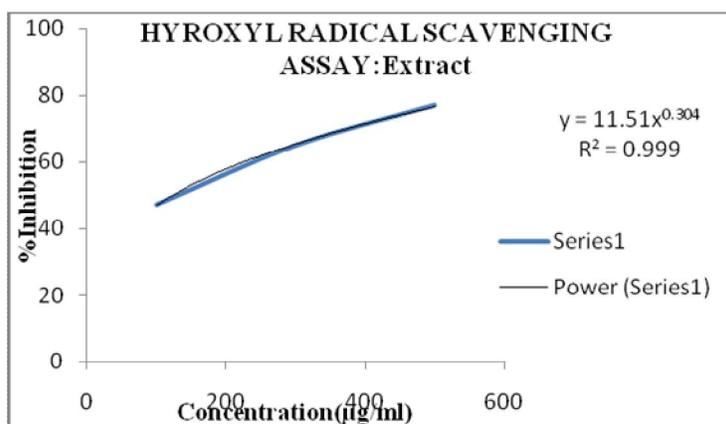


Fig. 5

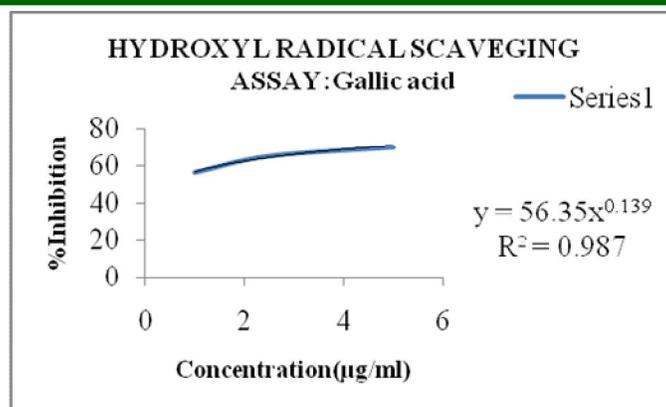


Fig. 6

CONCLUSION

The study was performed to evaluate the invitroanti oxidant activity of Ethanolic bark extract of *Annonamuricata*. The results obtained indicates the significant antioxidant activity in all the three methods, and the results were compared with standard reference Gallic acid. Further research investigations may be carried out to isolate the actual phytoconstituents responsible for antioxidant activity.

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