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RESEARCH ARTICLE

Analysis of Phytochemical and Antioxidant Activity of *Annona muricata* L. Flower in Alcoholic Extracts

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Abstract

Background: The phytochemicals found in plants have served as a fundamental and indispensable to the functioning of a pharmacological development during the past century. *Annona muricata* L. is one such medicinal plant to be explored. **Aim:** To investigate if *A. muricata* L. flower extracts could be used to treat cancer based on its antioxidant properties. **Methods:** In this study, seven solvents like Petroleum ether, Acetone, Hexane, Chloroform, Carbinol, Ethyl alcohol and Aqueous were employed for the phytochemical analysis. Alkaloids, Phenols, Flavonoids, Tannins, Triterpene and Steroids are the most common phytochemical components determined and extracted from the flower. **Results:** The TPC of the solvent-extracted carbinol of *A. muricata* flower was found to range from 33.9±0.001to 97.2±0.001µg/ml and in ethyl alcohol extract, the range was from 35.9±0.002 to 97.5±0.33 µg/ml with GAE. The TFC of carbinol solvent extract of *A. muricata* flower was found to range from 0.15±0.002 to 0.48±0.002µg/ml; and the ethyl alcohol solvent extract was found to range from 0.18±0.03 to 0.50±0.003µg/ml with standard (RE). The result of antioxidant activity using DPPH assay showed the IC_{50} value of 60.94 µg/ml in ethyl alcohol extract. In FRAP assay, the highest values were 1.63± 0.12 µg/ml and 1.88± 0.003 µg/ml in ethyl alcoholic and carbinolic extracts of *A. muricata* flower. **Conclusion**: These results indicate the strong potential antioxidant nature of *A. muricata* flower that could be further utilized for product development to treat cancer.

Major Findings: The findings of the current study support that Carbinol extract of *A. muricata* flower shows better result which could act as a source of antioxidant.

Keywords: *A. muricata* L., Antioxidant Activity, Phytochemical, Total Phenol, Total Flavonoid

1. Introduction

One of the major threatening health issues in the current scenario is cancer. Millions of people worldwide die from cancer, according to a UN report by ICMR. Globally, breast cancer appears to be the most frequent cancer. An essential first step in lowering cancer mortality is raising knowledge of the early warning signs and symptoms of cancer. This will allow for early diagnosis and treatment of the diseases before they progress. Canceris getting more common, which raises questions about how effectively current treatments perform. Soursop, also known as *A. muricata* L., is a widely used anticancer drug and traditional medicinal herb and demands further research[1].

Since ancient period, people have used natural goods, particularly those made from plants, to cure any kind of health issues[2]. All portions of the *A. muricata* tree, like those of other Annona species similar as A. *squamosa* and *A. reticulata*, are extensively employed as traditional drugs to treat a wide range of fatal situations and conditions, including cancer and opportunistic infections. This study suffers from the lack of research on the flowers of *A. muricata*.

Despite these examinations, only a small number of plant species have experienced expansive scientific

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scrutiny, and our understanding of their probable function in nature is still rather limited. Thus, thorough exploration of these plants natural processes and their primary phytochemicals is needed to achieve a meaningful perception of natural products[3]. The following exploration section discusses about *A. muricata* L. the phytochemical analysis and antioxidant activity in flower. For the current study, only fallen flowers were collected and used for further process.

2. Description

A. muricata L., widely known as soursop belongs to Annonaceae family, which has 130 genera and 2300 species[4]. It's inherent to the tropical parts of South and North America[5]. *A. muricata* is a 5-8 metre (Figure 1) tall erect, evergreen, terrestrial tree with massive, glossy, dark green leaves. The tree bears gigantic, heart-shaped, green, edible fruits with spikes on the skin with diameters ranging from 15 to 20 cm[6]. Short, plump stalk flower measuring 4 to 5 cm in length and shaped like a triangle with six petals, three pale yellow close-set petals on the inside and three yellow-green, fleshy petals on the outside. Acetogenin is the main compound that can be found in plants leaves, stems, fruits, and roots. The presence of flavonoids, in addition to acetogenin, as an active ingredient in *A. muricata* added benefits for effective therapeutic results. Flavonoids have anticancer effects as well[1].



Figure 1. *A. muricata* flower.

3. Methodology

3.1 Groundwork of Plant Extract

The flower was washed completely to remove soil. The Fresh flowers used for extraction were shade dried and powdered using a blender. Fine powder was attained by sieving. The powder was stored in clean air tight screw cap bottles. The fine powder was soaked in different solvents like Petroleum Ether, Acetone, Hexane, Chloroform, Carbinol, Ethyl alcohol, Aqueous in 1:10 ratio and kept in orbital shaker for 72 hours. After 3 days, the extract was filtered and allowed to dematerialize to get crude extract.

3.2 Phytochemical Analysis

The qualitative screening was carried out in order to learn more about the primary and secondary metabolites found in the *A. muricata* flower. Alkaloids, flavonoids, tannins, steroids, proteins, amino acids, terpenoids, and phenols in the extracts of petroleum ether, acetone, chloroform, carbinol, ethyl alcohol and aqueous extracts were analysed[7-9].

3.3 Quantitative Analysis

3.3.1 Total Phenolic Content

Estimation of Total Phenolic Content (TPC) was carried out using the Folin- Ciocalteau (FC) method[10]. The absorbance was read using a UV-visible double ray spectrophotometer (LABMAN). FC reagent reacts with phenols in plant extricates and changes into dark blue colour. All the concentrations are carried out in triplicate. The add up to phenolic substance were calculated using the equation:

$$C = c \times \frac{v}{m}$$

3.3.2 Total Flavonoid Content

Estimation of Total Flavonoid Content (TFC) was done using the $AlCl_3$ Method[10]. TFC was quantified as Rutin equivalents applying the linear equation derived from the calibration curve. The TFC was calculated using the formula similar to TPC.

3.3.3 2,2 Diphenyl 1- picrylhydrazyl Assay

The antioxidant activity of the plant samples was evaluated using DPPH (2,2′-diphenyl-1-picrylhydrazyl)[11]. The IC_{50} value represent the radical scavenging potential of

the sample at 50%. The IC_{50} of the extract and measures was calculated graphically. The antioxidant activity % was calculated using the following formula:

$$Radical\ scavenging\ activity\ (\%) = \frac{Abs\ control - Abs\ sample}{A\ control} \times 100$$

where, control was 2 mL carbinol + 1 mL DPPH solution.

3.3.4 Ferric Reducing Antioxidant Power Assay

The determination of FRAP of the extracts was performed as described by the Optical Density readings (OD) were used to calculate the FRAP values[12]. Higher OD indicates the reducing power. The following formula was used to get the FRAP value:

$$FRAP\ Value = \frac{As - Ao}{Ac - Ao} \times 2$$

Were, A_s – Abs of Sample, A_o – Abs of Negative Blank, A_c – Abs of Positive Control.

4. Result and Discussion

The phytochemical screening of *A. muricata* L. flower was done in seven solvents: Petroleum Ether, Acetone, Hexane, Chloroform, Carbinol, ethyl alcohol, and Aqueous.

The phytochemical screening (Table 1) of the *A. muricata* flower extracts exposed the presence of alkaloids, tannins, flavonoids, protein, amino acid, phenol, triterpenoid and steroid in the extracts of carbinol and ethyl alcohol in higher amount compared to other solvents.

In the earlier studies, phytochemical screening showed the occurrence of alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, saponins in water and 96% ethyl alcohol *A. muricata* leaves[13].The same phytoconstituents like alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, and triterpenoids in the ethyl alcohol extract of *A. muricata* rind[14]. Acetogenins are the main constituents, followed by phenols, alkaloids, and other substances. Most

Table 1. Phytochemical screening of *A. muricata* L. flower

Tests	Petroleum Ether	Acetone	Hexane	Chloroform	Carbinol	Ethyl alcohol	Aqueous
Test for Alkaloids: Mayer's test							
Wagner's test							
Dragendroff's test							
Test for Flavonoids: Ferric chloride test							
Alkaline reagent test							
Magnesium ribbon test							
Test for Titerpenoids: Libermann-Burchard test							
Salkowski's test							
Test for Tannins: Lead Acetate test							
Ferric chloride test							
Test for Protein: Biuret test							
Warming test							
Folin's lower method							
Test for Amino Acid: Ninhydrin test							
Million's test							
Test for Phenols: Ferric chloride test							
Libermann's test							
Test for Steroid:							

= highly present; = moderately present; = present; = absent

phytochemicals have been found from organic extracts, although aqueous extracts have also received attention recently[15].The existence of alkaloids, saponins, terpenoids, anthraquinones, tannins, phenols and phytosterols are in higher amount and flavonoids in low amount, coumarins and lactones, cardiac glycosides are present in average amount in the samples of the ethyl alcoholic and water extracts of *A. muricata* leaves[16].

4.1 TPC

TPC of the selected flower extracts was expressed with GAEs, and the contents were obtained using calibration curve Y = 0.0143x + 0.1051; R^2 = 0.9821. The high TPC was observed in carbinol extract of *A. muricata* (97.2±0.001µg/ml), flower. Using the standard calibration curve of gallic acid, the TPC of carbinol solvent extract of *A. muricata* flower is found to range from 33.9±0.001to 97.2±0.001µg/ml; in ethyl alcohol extract, the range was from 35.9±0.002 to 97.5±0.33 µg/ml with GAE (Table 2). This shows that carbinol *A. muricata* flower extract possesses high phenolic content than ethyl alcohol (Figure 2).

The ethyl alcohol extract of *A. muricata*, measured with the FC reagent, had a TPC of 609.08 ± 5.82 mg GAE/g. This value was represented as mg GAE per gram of dried material (mg QE/g)[13]. Result has indicated that the ethyl alcohol extracts of *A. muricata* leaves had a total phenol concentration of 278.60 ± 4.21 mg GAE/g[17]. As per the previous work, the total phenolics in the ethyl alcoholic extract were measured to be 372.92±0.15 g/mL GAE[16], but in the aqueous extract they were found to be 683.69±0.09 g/mL GAE.

Table 2. TPC in ethyl alcohol and carbinol

Concentration (µg/ml)	Gallic Acid	Ethyl alcohol	Carbinol
20	28.8 ± 0.004	35.9 ± 0.002	33.9±0.001
40	46.8 ± 0.003	36.6 ± 0.001	43.1±0.001
60	67.7 ± 0.001	50.3 ±0.006	54.1±0.002
80	79.8 ± 0.003	86.5 ±0.015	83.4±0.001
100	90.6 ± 0.001	97.5 ±0.033	97.2±0.001
SEd CD (p<0.05)	0.0020 0.0044	0.0014 0.0031	0.0013 0.0029

The table represents each value as TPC ± SD (n=3) and is significant** at (p<0.05)

4.2 Total Flavonoid Content

TFC of the selected flower extracts was expressed with RE, and the contents were obtained using calibration curve y = 2.197x + 0.0291; R^2 = 0.9966. Using the standard calibration curve of Rutin, the TFC of carbinol solvent extract of *A. muricata* flower was found to range from 0.15±0.002 to 0.48±0.002µg/ml; and the ethyl alcohol solvent extract was found 0.18±0.03 to 0.50±0.003µg/ml with RE (Table 3). This shows that flower extract using ethyl alcohol has a high flavonoid content (Figure 3).

The carbinol and ethyl alcohol extracts showed the highest TFC and L-MeOH extract showed 81.32 mg/CEg[18]. The total flavonoid content of carbinol extract of *A muricata* L. leaves and roots are 1.12±0.03 and 0.25±0.01 (mg GAE/mL)[19]. The studies showed that the level of flavonoids exhibited higher concentration in hexane compared to other solvents[20].

4.3 DPPH Assay

A UV-Vis double beam spectrophotometer was used to measure the antioxidant activity of the ethyl alcohol

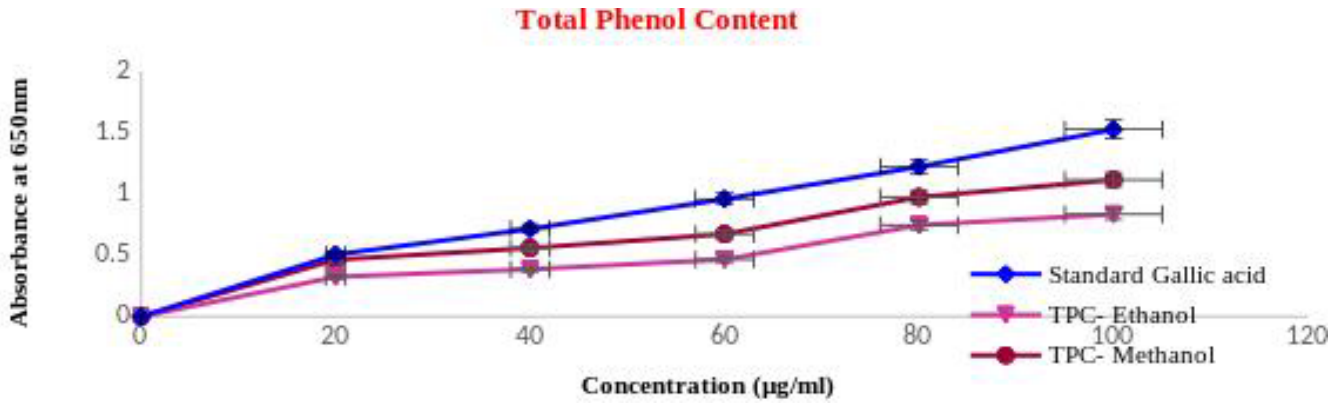


Figure 2. Total phenol content of ethanol and methanol extract of *A. muricata* flower.

and carbinol extracts of *A. muricata* flower. The DPPH method was selected because of its simplicity, convenience of use, and low sample requirement[21,22]. When DPPH, a persistent free radical with a purple hue, combines with antioxidant. According to the results, ethyl alcohol extract has an IC_{50} value of 60.94 µg/ml (Table 4), making it a powerful antioxidant (Figure 4). The IC_{50} value of L-Ascorbic acid as standard is 372.29 µg/ml. molecules, it transforms into a yellow substance. Antioxidants donate electrons to DPPH in this process.

Annona muricata ethyl alcohol extracts demonstrated free radical scavenging activity, with an IC_{50} value of 2.58 µg/mL[17]. With an IC_{50} value of 724.98 ± 3.00 ppm[23], the fruit pulp extract of *A. muricata* demonstrated free radical scavenging action. The *Annona muricata* leaf extract's ethyl acetate and n-butanol fractions revealed extremely low IC_{50} values of roughly 4.3±0.7 and 9.3±0.8µg/mL[13,24]. The ethyl alcohol extract of *A. muricata* exhibited strong antioxidant activity in earlier research (IC_{50} = 20.75±0.28 µg/ml)[13].

Table 3. Total flavonoid content of *A. muricata* L. flower

Concentration (µg/ml)	Rutin	Ethyl alcohol	Carbinol
20	0.12±0.01	0.18±0.003	0.15±0.002
40	0.20±0.02	0.23±0.003	0.22±0.002
60	0.29±0.05	0.30±0.003	0.32±0.002
80	0.41±0.02	0.37±0.003	0.39±0.002
100	0.50±0.01	0.50±0.003	0.48±0.002
SEd CD (p<0.05)	0.0261 0.0582	0.0022 0.0049	0.0014 0.0032

The values in the table are expressed as TFC ± SD (n=3) and is statistically significant** at (p<0.05).

4.4 FRAP Assay

Ethyl alcohol and carbinol extracts showed signs of antioxidant activity as determined by the FRAP technique. It estimates the reductive potential of the antioxidants that is, it will reduce the iron present in the chemical reagent. Greater the absorbance, greater the antioxidant molecules. It will develop a bluish colour compound indicating the presence of reducing power. The highest values were 1.63± 0.12 µg/ml and 1.88± 0.003 µg/ml in ethyl alcoholic and carbinolic extracts of *A. muricata* flower (Table 5). It was observed that the

Table 4. DPPH assay of Soursop flower

Concentration (µg/ml)	Ascorbic Acid	Ethyl alcohol	Carbinol
20	6.3 ± 0.001	15.8 ± 0.001	14.5 ± 0.001
40	13.2 ± 0.001	44.3 ± 0.003	44.8 ± 0.002
60	17.2 ± 0.001	48.6 ± 0.001	53.2 ± 0.001
80	23.9 ± 0.002	70.9 ± 0.001	65.0 ± 0.001
100	35.6 ± 0.001	71.2 ± 0.001	71.1 ± 0.001
IC_{50}	372.29	60.94	61.66
SEd CD (p<0.05)	0.0012 0.0027**	0.0008 0.0017**	0.0008 0.0019 **

All values in the table are significant** at (p<0.05) and are expressed as %RSA ± SD (n=3).

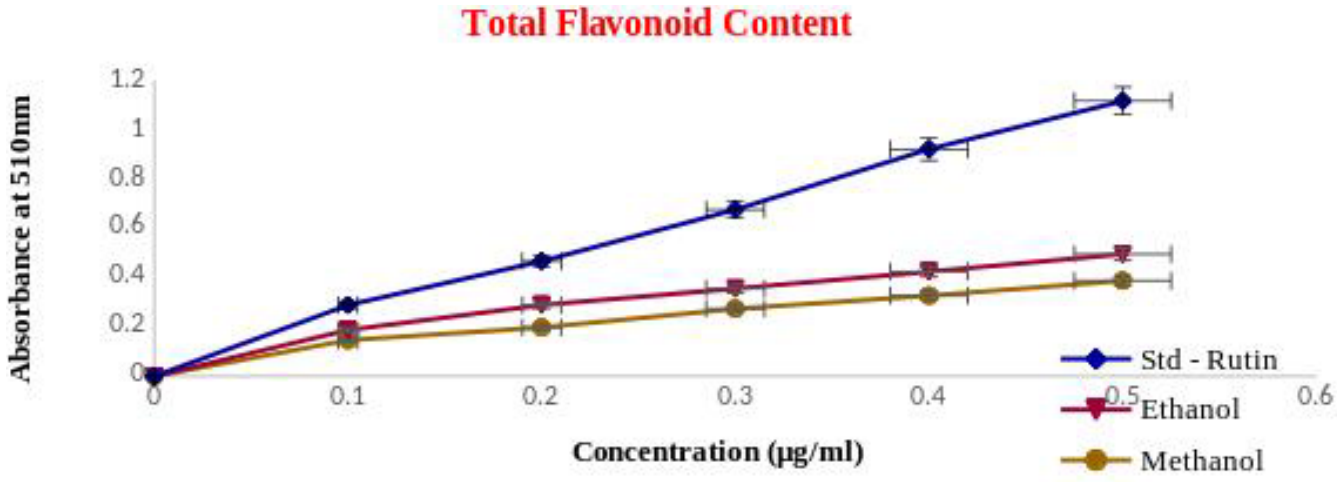


Figure 3. Total flavonoid content of *A. muricata* flower.

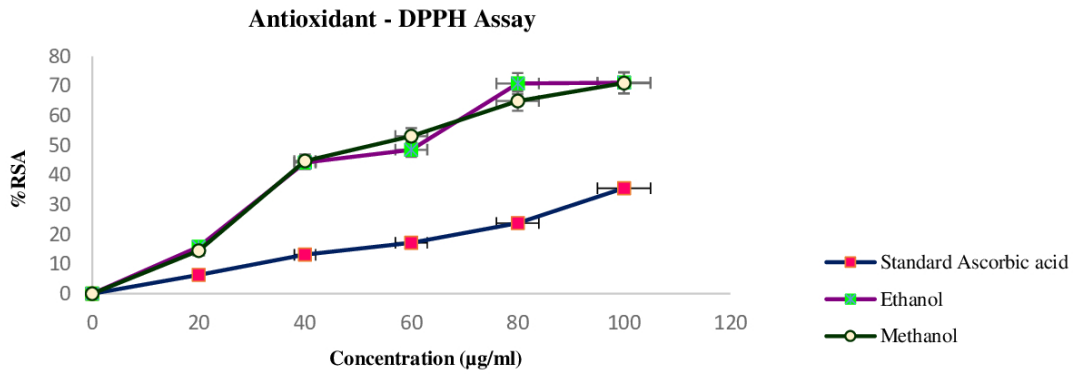


Figure 4. Antioxidant potential of *A. muricata* flower - DPPH assay.

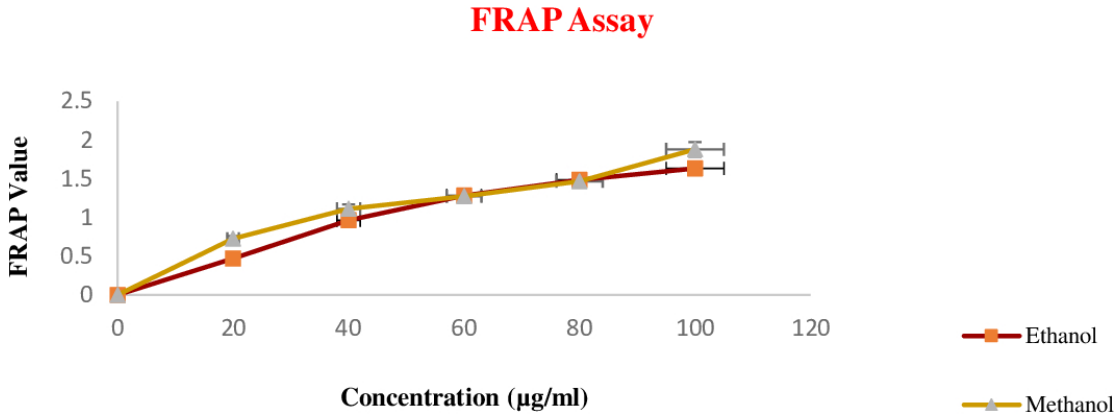


Figure 5. Antioxidant potential of *A. muricata* flower - FRAP assay.

Table 5. Antioxidant activity using FRAP assay

Concentration (µg/ml)	Ethanol	Methanol
20	0.47 ± 0.12	0.73 ± 0.002
40	0.96 ± 0.09	1.11 ± 0.002
60	1.28 ± 0.05	1.27 ± 0.001
80	1.48 ± 0.01	1.47 ± 0.003
100	1.63 ± 0.12	1.88 ± 0.003
SEd CD (p<0.05)	0.0017 0.0038**	0.0008 0.0019 **

The table shows FRAP Value ± SD (n=3) and significant** at (p<0.05).

antioxidant activity measured by FRAP was highest in carbinol extract (Figure 5).

The earlier studies, results revealed that water; carbinol and ethyl alcohol extract of *A. muricata* leaf was able to reduce Fe^{3+} ions, the maximum activity was found to be at a concentration of 500µg[25]. The previous study revealed that fruits injected with *C. siamense* exhibited a late response on storage, with an increase in their antioxidant potential (16.81 mg EAA/100 g FW)[26]. FRAP activity of *A. muricata* fruit extract was found to be equivalent to vitamin C. This implies that the *A. muricata* fruit pulp extract contains bioactive substances, underscore the potential health benefits due to their antioxidant properties[23].

5. Conclusion

This investigation was conducted to assess *A. muricata* L. Flower's phytochemical composition and antioxidant activity. For the phytochemical analysis, seven solvents were used and the better result shown in the ethyl alcohol and carbinol extract and it showed the highest antioxidant activities compared to that of other solvents. Research into natural remedies for cancer is always intriguing, and exploring the potential of the soursop flower sounds promising. It's essential to thoroughly investigate different solvents like ethyl alcohol, carbinol, and ethyl acetate to understand

their efficacy in extracting beneficial compounds from *A. muricata*. This could open the door to more environmentally friendly treatments. However, before any conclusions can be drawn, rigorous animal studies are necessary to confirm the antioxidant properties and assess its potential as an anticancer agent. Such studies are crucial steps in the process of developing new treatments derived from natural sources.

6. Author Contribution

Allen Princy AP: Collection of samples, designing the protocol for the study, collecting the data, performing the analysis, interpretation, and manuscript writing. Gayathri. V: Conceptualising the study, designing the methodology, and critically revising the content. All authors have read and agreed to the published version of the manuscript.

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