

# ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF EXTRACTS OF SOURSOP (*ANNONA MURICATA* L.) LEAVES AND FRUIT PULPS

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## ABSTRACT

*Annona muricata*'s role in healthcare has been better understood thanks to recent scientific and technological developments. Acetogenins, coumarins, phenolic acids, alkaloids, and flavonoids are among the phytochemical components. Antioxidants, antimicrobials, anti-diabetes, and a host of other health advantages are provided by the phytochemical composition. The purpose of this research was to detect phenolic and flavonoid phytochemicals in extracts from the leaves and pulp of the *Annona muricata* plant, as well as to compare and contrast the two components' antioxidant and antimicrobial capabilities. A *Annona muricata* tree was used to harvest the pulp and remove the leaves. The disc diffusion technique was used for antimicrobial testing against *S. aureus*, and antioxidant assays such as FRAP, DPPH, and TPC were performed. The investigation included samples with concentrations of 25, 50, 100, 200, and 400 µg/ml. The research found that different concentrations of 25µg, 50µg, 100µg, 200µg, and 400µg of extracts from the leaves and pulp of *Annona muricata* had a satisfactory level of antioxidants. The antibacterial activity of both extracts is effective at such concentrations, however. When looking at the antioxidant content and sensitivity to microbes in the pulp and leaves extracts, the leaves extract comes out on top.

## INTRODUCTION

Scientists have taken a keen interest in exotic and tropical fruits in the last few years. Additionally, there is a rise in consumer interest in them. The possible medicinal properties of several exotic and tropical plants are the reason for this. [1,2] The Annonaceae family includes soursop, graviola, and other members like it (*Annona muricata* L.). Originally from the hottest parts of North and South America, sour oranges are now a common sight in many tropical and subtropical climates throughout the Americas, Africa, and Asia. The soursop fruits are around 15 to 20 centimeters in diameter. [3,4]

There are 55-170 black seeds encased in green peel in the pulp. Soursop fruit has several by-products, including the inedible peels and seeds, which have not been investigated for their potential medicinal components. Nevertheless, there has been a recent uptick in curiosity in the possible high nutritional and bioactive compound content of fruit and vegetable by-products, including vitamins, dietary fiber, and phenolics. [5,6] There was a time when people thought the exotic fruit waste may be a good source of natural, high-quality food additives. [7]

Aromatic sour sop fruits are versatile in the kitchen. You can make juice, ice cream, or jelly out of pulp, and it's also delicious eaten raw. [8,9] In addition, traditional medicine makes use of other sour-sop elements, such as the leaves, bark, roots, fruit, and seeds, to treat a wide range of illnesses, including malignancies, gastrointestinal issues, inflammation, hypertension,

inflammation, diabetes, and inflammatory disorders. Acetogenins, alkaloids, megastigmanes, phenolics, cyclopeptides, and essential oils are some of the phytochemicals that have been linked to the therapeutic and physiological advantages of *A. muricata* L. [10,11]

Both the leaves and the fruits of soursop contain phytochemicals with antioxidant potential, the most important of which are phenolic compounds. The tannins, phenolic acids (mostly hydroxycinnamic acids), and flavonoids found in *A. muricata* L. pulp, seeds, and leaves were among these. [12] To get plant extracts containing phenolic chemicals, solvent extractions are often used. The same standard procedures were used on the soursop components as well. When it comes to extraction, the polarity of the solvent is a key aspect. [13,14] Some studies found that the bioactivity of *A. muricata* extracts varied depending on the solvent used. The majority of phenolic terpenes may be extracted using hexane or petroleum ether. The low-molecular-weight phenolics, such as phenolic acids and flavonoid aglycons, are extracted using ethyl acetate. Hydrogen peroxide, methanol, and ethanol combined with water were able to extract flavonoid glycosides and high-molecular-weight phenolics. [15]

### 1. RESEARCH METHODOLOGY

#### • Chemicals

Sodium carbonate, glycerol, triethanolamine, paraffin oil, methyl paraban, EDTA, butylated hydroxytoluene (BHT), propyl paraben,

ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gramme stain safranin, ethanol, Muller Hilton agar, and phthalic acid.

- **The Annona Muricata extract's source**

The powder of Annona Muricata was extracted by using standard method.

- **Discovering flavonoids and phenolics**

A neutral 5% ferric chloride solution was added to the 50 mg extract after diluting it with 5 mL of distilled water. A shade of dark green or blue-green was used to indicate the existence of phenolic chemicals. In a test tube with some magnesium turnings and some test samples, a few drops of hydrochloric acid were introduced. A change from green to red was used to identify flavonoids.

- **The amount of phenolic compounds (TPC)**

A reagent approach developed by Rajkumar et al. Folin-Ciocalteu was used to quantify the total phenolic content (TPC) of the extracts. The following were added to 50 liters of each extract concentration: 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml. The mixture was then incubated at 45 °C for 30 minutes with 2.5 ml of Folin-Ciocalteu reagent (1/10 dilution) and 2 ml of 7.5 percent Na<sub>2</sub>CO<sub>3</sub> (w/v) solution. Varian, Inc. of California, USA, made the Cary 50 UV-Vis spectrophotometer, which was used to measure the absorbance at 765 nm. Gallic acid equivalent (GAE) in g/ml was the unit of measurement, with gallic acid serving as the reference. Three separate runs of the extraction were carried out. Gallic Acid Equivalent (GAE) was used to determine the phenol concentration.

- **Ferric Ion Reducing Antioxidant Power (FRAP)**

A FRAP reagent was made by combining 200 mL of 300 mM acetate buffer with 20 mL of a solution of 20 mM ferric chloride hexahydrate (FeCl<sub>3</sub>.6H<sub>2</sub>O) in a volumetric ratio of 10:1:1, followed by 20 mL of a solution of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM hydrochloric acid (HCL). The FRAP enzyme was produced just before use, heated in a water bath to 37C, and then wrapped in aluminum foil to protect it from light deterioration in the beaker. To make the reaction mixture, combine 0.1 milliliters of pulp and leaves extract (25µ-400µg /ml)

**Table1:** The Annona muricata leaf and pulp total phenolic content (TPC) at various concentrations (µg/ml).

Part	Concentrations(µg/ml)	N	TPC value	P value
Pulp	25	3	0.073 ± 0.015 <sup>cdegf hij</sup>	(P<0.05)
	50	3	0.082 ± 0.002 <sup>efghij</sup>	
	100	3	0.088 ± 0.002 <sup>aefghij</sup>	
	200	3	0.095 ± 0.002 <sup>aefghij</sup>	
	400	3	0.112 ± 0.003 <sup>abcd fghij</sup>	
Leaf	25	3	0.153 ± 0.003 <sup>abcdeghij</sup>	(P<0.05)
	50	3	0.173 ± 0.006 <sup>abcdefij</sup>	
	100	3	0.178 ± 0.006 <sup>abcdefij</sup>	
	200	3	0.235 ± 0.004 <sup>abcdefghj</sup>	
	400	3	0.248 ± 0.009 <sup>abcdefghi</sup>	

We used the One-Way ANOVA test. The results are shown as the average amount of gallic acid equivalent (GAE) per gram of dry weight sample (DW), with the standard deviation (SD) of three separate samples. Post hoc Tukey's test is being used to compare

with 300µ milliliters of 2% Tween 20 and 3 milliliters of FRAP reagent. Use a sonicator to mix the ingredients while the mixture was in the dark. After 30 minutes of incubation, the reaction mixture was measured in triplicate using a spectrophotometer at 593 nm. The reaction mixtures were compared using ascorbic acid, which ranged from 25µg/ml to 400µg/ml. The standard curve was then produced using ferrous sulphate heptahydrate (FSO4.7H<sub>2</sub>O) by serial dilution within the range of 0.1 to 2.0 mm. This was done

- **The Method of Disk Diffusion**

The disk diffusion technique, which was slightly modified from the Kirby Bauer method provided by Balouiri et al. (2016), was used to perform the antimicrobial test. The antibacterial properties of several extracts from Annona muricata, including the pulp and leaves, are evaluated using this approach. The antimicrobial investigation was done using concentrations of 100µg and 200µg. The medium utilized for S. aureus is Mueller Hinton agar. The microbial suspensions were made using 0.5 McFarland standard and had a concentration of 1.5 X 10<sup>8</sup> CFU/ml. Spreading the microbes to be tested across the agar plate surfaces was the process of inoculation. There was an extract-soaked 6-millimeter filter paper disk on top of the agar. After that, the plates were placed in an incubator set at 37 °C for a whole day to enable the bacteria to develop. The negative control group consisted of a 6-mm filter paper disk that included 100µg and 200µg of each extract, along with distilled water. The positive control group employed a conventional antibiotic disc containing Gentamicin. Each step was performed three times.

- **Statistical Analysis**

Triplicates were used for all tests. An analysis of variance (ANOVA) was performed on the collected data. Excel and the Statistical Package for the Social Sciences (SPSS) 20.0 were used. Statistical significance was determined at a p-value less than 0.05, and the data were presented as the Mean ± SEM (Standard error of mean).

## 2. RESULTS

- **Total Phenolic Content (TPC)**

means among concentration groups. P < 0.05 was used to establish statistical significance:

a: Divergence with pulp part concentrations of 25 µg/ml that is statistically significant b: Divergence with pulp part

concentrations of 50 µg/ml that is also statistically significant section c: Distinct dissimilarity at 100 µg/ml pulp concentrations Section d: Distinct variation at pulp values of 200 µg/ml Part e: Distinct observed variation at pulp values of 400 µg/ml Section f: At pulp concentrations of 25 µg/ml, g: there is a statistically significant difference. part There is a noticeable disparity when using 50 µg/ml of the leaf extract. Section h: Distinct variation at 100 µg/ml of the leaf with respect to statistical significance Part i: A statistically significant variation was seen with 200 µg/ml of the leaf There is a notable disparity at doses of 400 µg/ml of leave with respect to art j. Part The relationship between sample concentration and total phenolic content shows a rising trend. A higher TPC value is indicative of a more concentrated sample. This is backed by many studies that used different plant extracts and found similar results.” The results shown in Table indicate that the TPC value is 0.248µg/ml at a concentration of 400µg/ml of leaf extract and 0.113µg/ml of pulp extract, respectively. That leaves extract has a greater TPC value than pulp extract at a given concentration is shown here. According to a research that was carried out by, the TPC value of methanolic pulp extraction was 10.92 µg/ml, lending credence to this discovery. On the other hand, a TPC value of **Table 2:** displays the findings of the DPPH scavenging activity of the various *Annona muricata* extracts and the ascorbic acid positive control.

19.84µg/ml is produced by the methanolic leaves extract. In each given research, TPC values could vary due to a wide variety of causes. Plant, method, and standard variations in TPC expression; non-phenol specificity of the Folin-Ciocalteu reagent color assay; and the possibility that additional components, including ascorbic acid, react with the reagent all have a role in the observed variation in TPC levels. The extraction solvent also has a significant role in determining the TPC value in a given investigation. Extraction yields of total soluble solids and total extractable polyphenolics from plant extracts were shown to rise with increasing solvent polarity, according to several research. Methanol is supposedly the most effective solvent for plant extraction, according to a recent research. “Since methanol is more easily evaporated than water and may inhibit the polyphenol oxidase process, which oxidizes phenolics, it is believed to be the best solvent for extracting phenolic compounds.

**A. 2,2-diphenyl -1-picrylhydrazyl (DPPH)**

The results of the one way ANOVA test were entered as a (P <0.005). Based on this number, it may be inferred that pulp extract, leaf extract, and ascorbic acid have significantly distinct scavenging activities.

Parts	Concentration (µg/ml)	n	%DPPH scavenging	P value
Leaf	25	3	16.09 ± 4.72 <sup>bcdeghijklmno</sup>	(P<0.005 )
	50		27.27 ± 1.43 <sup>defhijlmno</sup>	
	100		38.72 ± 3.48 <sup>abdefgijlmno</sup>	
	200		50.04 ± 1.35 <sup>abcfgijkno</sup>	
	400		60.37 ± 4.87 <sup>abcdfghiklno</sup>	
Pulp	25	3	15.13 ± 2.07 <sup>bcdeghijklmno</sup>	
	50		25.09 ± 1.76 <sup>defhijlmno</sup>	
	100		32.79 ± 1.44 <sup>defijlmno</sup>	
	200		48.66 ± 3.32 <sup>abcfghjkno</sup>	
	400		55.95 ± 4.22 <sup>abcfghkno</sup>	
Ascorbic Acid	25	3	30.73 ± 2.06 <sup>defijlmno</sup>	
	50		49.75 ± 1.21 <sup>abcfghjkno</sup>	
	100		55.43 ± 0.74 <sup>abcfghkno</sup>	
	200		75.88 ± 1.77 <sup>abcdefgijklmo</sup>	
	400		84.94 ± 1.39 <sup>abcdefgijklmn</sup>	

a: Divergence with pulp part concentrations of 25 µg/ml that is statistically significant b: Divergence with pulp part concentrations of 50 µg/ml that is also statistically significant section c: Distinct dissimilarity at 100 µg/ml pulp concentrations Section d: Distinct variation at pulp values of 200 µg/ml Part e: Distinct observed variation at pulp values of 400 µg/ml The data

shows a statistically significant variation when the concentration of leaves is 25 µg/ml. section g: A statistically significant variation was observed when using 50 µg/ml of residual Section h: Distinct variation at 100 µg/ml of the leaf with respect to statistical significance Part i: A statistically significant variation was seen with 200 µg/ml of the leaf Statistically significant variation was

seen with 400 µg/ml of the leaf extract in part j. Section k: Variation with 25 µg/ml of Ascorbic Acid shows a statistically significant difference. Part l: Variation with 50 µg/ml of Ascorbic Acid shows a statistically significant difference. Part m: Variation with 100 µg/ml of Ascorbic Acid shows a statistically significant difference. Part n: Variation with 200 µg/ml of Ascorbic Acid shows a statistically significant difference. Part o: Variation with 400 µg/ml of Ascorbic Acid is not significantly different from Part k.

The percentage of free radical scavenging activity was estimated for *Annona muricata* leaf, pulp, and ascorbic acid, the reference standard. The graph was constructed using the non-linear equations  $y=10.861x+6.855$  ( $R^2 =0.9957$ ),  $y = 10.521x+3.961$  ( $R^2 =0.9028$ ), and  $y = 13.399x+19.023$  ( $R^2 =0.9778$ ). Next, we calculated the effective concentration of each sample required to scavenge the DPPH radical by 50% by determining the IC50 values." The IC50 values are 2.31µg/ml, 3.97µg/ml, and 4.38µg/ml, respectively, as determined by the linear equation of the graphs of ascorbic acid, leaves extract, and pulp extract. Compared to pulp extract, which had an IC50 value of 4.38µg/ml, leaf extract demonstrated superior antioxidant activity with an IC50 value of 3.97µg/ml. Several research have found the same thing.

Acetogenins, a crucial secondary metabolite and the principal bioactive component of the Annonaceae family, are more abundant in the leaf section. An increase in antioxidant activity is Table 3: Findings of FRAP assay

likely due to the increased concentration of acetogenins in leaf extracts compared to pulp extracts. Factors such as the method of cultivation used to extract the fruit or plant components also affect the antioxidant content of the final product. The greatest concentration of phenolic chemicals in plant aerial parts is a hallmark of ontogenesis, according to a research. Newly developed plants and their components, as well as those that are developing quickly, have the highest levels of antioxidants. When compared to leaves that were one or two months old, those that were six months old had significantly lower antioxidant levels. The DPPH assay's ability to measure antioxidant activity is sensitive to a number of variables, such as the compounds' chemical structures, the solvent's properties, the temperature, the pH, and the reactivity of free radicals. "A further drawback of the DPPH test is that it is susceptible to reacting with other radicals present in the substances. Therefore, the rate of change in the amount of antioxidants needed to achieve stability does not follow a straight line.

• **Ferric Ion Reducing Antioxidant Power (FRAP)**

Upon completion of the one-way ANOVA test, a value of ( $P<0.005$ ) was noted. According to this number, the two kinds of extract have significantly different antioxidant levels. Table shows the results of FRAP assay of the different extracted parts of *Annona muricata* which is the pulp and leave extract

Parts	Concentrations(µg/ml)	N	FRAP value (Mm Fe2+/g)	P value
Leave	25	3	0.56 ± 0.25 <sup>cdefghij</sup>	(P<0.05)
	50	3	0.64 ± 0.73 <sup>defghij</sup>	
	100	3	0.76 ± 0.03 <sup>adefghij</sup>	
	200	3	0.95 ± 0.04 <sup>abcefj</sup>	
	400	3	1.15 ± 0.12 <sup>abcdfgh</sup>	
Pulp	25	3	0.19 ± 0.70 <sup>abdeij</sup>	
	50	3	0.28 ± 0.72 <sup>abceij</sup>	
	100	3	0.43 ± 0.62 <sup>abcej</sup>	
	200	3	0.77 ± 0.98 <sup>abcfjg</sup>	
	400	3	1.04 ± 0.58 <sup>abcdfghi</sup>	

We used the One-Way ANOVA test. The FRAP value (Mm Fe2+/g) and the Mean ± Standard deviation (SD) of three replicate samples are used to display the data.

Post hoc Tukey's test is being used to compare means among concentration groups. P < 0.05 was used to establish statistical significance:

A statistically significant variation was seen with 25 µg/ml of the leaf extract. section b: difference with doses of 50 µg/ml of drop portion c: Distinct variation at doses of 100 µg/ml of the Section d: Distinct statistically different results with 200 µg/ml of the leaf Section e: Distinct variation according to doses of 400 µg/ml of mustard Section f: At pulp concentrations of 25 µg/ml, there is a statistically significant difference. section g: Pulp values of 50 µg/ml showed a statistically significant difference. Part h: A discernible variation at pulp values of 100 µg/ml Section I: A statistically significant variation was seen when pulp contents

were 200 µg/ml. Part j: A statistically significant variation was seen when pulp contents were 400 µg/ml. Part

The Frap value is shown as a function of the concentration of the *Annona muricata* pulp and leaves extract, with values ranging from 25g to 400g. The pattern of a rising FRAP value with increasing sample concentration is clearly seen." Both the pulp and the leaves exhibit this pattern. However, at a certain concentration, the FRAP value of the pulp extract differs from that of the leaf extract. Take the FRAP values of 0.635±0.730 and 0.52±0.414 for the leaf extract and 0.289±0.728 and 0.769±0.975, respectively, for the pulp extract, at concentrations of 50µg and 200µg, as an example.

Leaves have an IC50 of 230µg/ml and pulp has an IC50 of 327µg/ml. As the computed IC50 values supported, this showed that the leaf extract had a greater antioxidant content than the pulp extract. For the leaves extract, the IC50 value was determined to be 230µg/ml, but for the pulp extract, it was

327µg/ml. Previous research, such as that, which found that leaf extracts from different solvents had a greater antioxidant content than pulp, lends credence to the current findings. The leaves of the *Annona muricata* plant are thought to have a greater concentration of antioxidant-rich secondary metabolites such as flavonoids, alkaloids, and phenolics.

The findings of the total phenolics content, which were previously addressed, provide support for this. Another factor contributing to the decreased antioxidant content of pulp extract is the inherent fragility of the pulp itself when it is extracted. Some extraction procedures, such as a longer drying time for pulp than for seed and leaves, may reduce the concentration of secondary metabolites. Among the many benefits of the FRAP test is its suitability for assessing the antioxidant activity of various plant extracts; the approach is both easy to use and affordable, and it does not call for any specific reagents. There are certain downsides to the FRAP test, despite its usefulness. The FRAP assay is a timed-based analytical test that requires additional effort and time to prepare the chemicals for the working solution.

• **Antioxidant activity and total phenolic content correlation**

It has long been known that the chemical components of a medicinal plant's beneficial effects are its secondary metabolites.

**Table 4:** Antioxidant activity and total phenolic content correlation.

Types of extract / Correlation Variable	Pulp extract	Leave extract
TPC value (µg/ml GAE) and DPPH scavenging activity (%)	0.947	0.934
TPC value (µg/ml GAE) and FRAP value Mm (Fe <sup>2+</sup> /g)	0.966	0.949

• **A disk diffusion test for microbiological agents**

Anti-*S. aureus* activity of *Annona muricata* leaf extract

Because of their capacity to contribute hydrogen atoms to free radicals, phenolic compounds are crucial antioxidant components that deactivate free radicals. The structural features of phenols make them excellent free radical scavengers as well. The phenolic content of the extracts and the antioxidant properties assessed by various *Annona muricata* parts were connected via the use of Pearson's correlation coefficient.

Antioxidant capacity is linearly related to total phenolic content, according to several publications in the literature. The findings from this investigation demonstrated a positive association between total phenolics and antioxidant activities, with correlation coefficients ranging from R<sup>2</sup> 0.947 to 0.966, N=15 p<0.001: this correlation is significant at the 0.01 level (2 tailed). All of the data points from the various assays pointed to the same general pattern of activity. "The total phenolic content of the pulp and leaf extract are positively associated according to the FRAP test, with R<sup>2</sup> coefficient values of 0.966 and 0.949, respectively. There was a robust positive correlation (R<sup>2</sup>) of 0.947 and 0.934 between the total phenolic content of pulp and leaf extract and the DPPH test. The scavenging and ferrous reduction of pulp and leaf extracts may be associated with the presence of phenolic hydroxyl groups in phenolic substances.

**Table 5:** Antimicrobial activity of different quantities of pulp extract from *Annona muricata*, using both positive and negative controls, against a set of chosen microorganisms, mean ± standard deviation

Microorganism	Zone of inhibition (mm)		
	Concentration		Negative control
Gram Positive	200µg/ml	400µg/ml	
<i>S.aureus</i>	8.03 ± 0.20 <sup>bcd</sup>	8.73 ± 0.32 <sup>acd</sup>	36.00 ± 0.00 <sup>abc</sup> 6.00 ± 0.00 <sup>abd</sup>

compared to a 200µg/mL leaf extract, there is a statistically significant difference (p<0.05). was shown to be statistically significant (p<0.05) when contrasted with a 400 µg/mL extract of leaves. The difference is statistically significant (p<0.05) when contrasted with the negative control. As compared to the positive control group, it is statistically significant (p<0.05).

significant (p<0.05) when contrasted with the negative control. As compared to the positive control group, it is statistically significant (p<0.05).

**Table 6:** Anti-*S. aureus* activity of *Annona muricata* pulp extract

Microorganism	Zone of inhibition (mm)		
	Concentration		Negative control
Gram Positive	200µg/ml	400µg/ml	
<i>S.aureus</i>	6.00 ± 0.00 <sup>bd</sup>	6.23 ± 0.15 <sup>acd</sup>	36.00 ± 0.00 <sup>abd</sup> 6.00 ± 0.00 <sup>bd</sup>

This is significantly different from the pulp extract at a concentration of 200µg/mL (p<0.05). when contrasted with a pulp extract of 400 µg/mL (p<0.05), was shown to be statistically significant. The difference is statistically significant (p<0.05) when contrasted with the negative control. As compared to the positive control group, it is statistically significant (p<0.05). The antimicrobial test showed an average zone of inhibition of 6.00±0.00 for a concentration of 100µg of pulp extract and

6.23±0.15 for a concentration of 200µg. On the other hand, results of 8.03±0.20 and 8.73±0.32 were observed in the leaves extract when the concentrations were kept constant. The reported zone of inhibition falls within the low inhibition range of 7-10 mm, according to the research. Inhibition ranges of 11 mm and above indicate strong antimicrobial activity. Both components of the study's low zone of inhibition are somewhat distinct from one another." The absence of inhibition at concentrations of 200µg/ml and 400µg/ml in the low-level zone of inhibition suggested that

the pulp and leaf extract of *Annona muricata* could not hinder the development of *S. aureus*. However, there are studies that back up the conclusion, so it's not all bad news.

Even with different doses of 5mg/ml, 10mg/ml, and 50mg/ml of extracts from the same sections, the article demonstrates the low-level inhibitory zone. But according to the same study, the zone of inhibition doesn't begin to grow until concentrations of 150 mg/ml and higher are reached. This demonstrates that antibacterial activity against *S. aureus* bacteria is not shown at low concentrations of *Annona muricata*, but is seen at high concentrations above 150 mg/ml. The solvent used to extract the material is one of the variables that affects the inhibition strength.

Research from also indicates that whilst 300 mg/ml of hot distilled water has a significant zone of inhibition, 300 mg/ml of n-hexane extraction has a low one. From this, we might infer that the antimicrobial capabilities of certain extraction solvents are dependent on the inhibitory consequences they produce. According to this research, the antibacterial activity of the leaf extract is greater than that of the pulp extract. Observing the world via the zone of inhibition verifies this claim. The average zone of inhibition for pulp extract at 400µg/ml is 6.23±0.15, but for leaves extract it is 8.73±0.32.

Perhaps the increased concentration of secondary metabolites in the leaf extract explains why it has different antibacterial action from the pulp extract. It is said that the leaf portion contains more flavonoids than the pulp, a secondary metabolite. According to research, flavonoids are the primary metabolites with antibacterial effects against a wide variety of microbes, including *S. aureus*.

## CONCLUSION

The TPC, FRAP, and DPPH assays demonstrated the antioxidant capabilities of an ethanolic extract of *Annona muricata* pulp and leaves. In addition to exhibiting strong radical scavenging action, the plant extracts also had a high total phenolic content. Hydroxyl groups in the chemical structure of the phenolic molecule may provide the necessary component as a radical scavenger and antioxidant, which may explain the strong scavenging activity of some *Annona muricata* components. But alkaloids, saponins, tannins, and flavonoids aren't the only phytochemicals that may be boosting the plant's antioxidant activity. Using the TPC, FRAP, and DPPH assays, this research also demonstrated that the methanolic pulp extract had lower antioxidant activity than the methanolic leaves extract. Reason being, leaves are more amenable to the intense extraction methods and have a greater concentration of phytochemicals. At lower doses of 200µg/ml and 400µg/ml, the methanolic extract of *Annona muricata* leaves and pulp inhibit the growth of *Stephylococcus aureus*. However, as previously mentioned, inhibition begins to manifest at concentrations greater than 150mg/ml.

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