Antioxidant and Antimicrobial Properties of Annona Muricata Leaf and Pulp Methanolic Extract

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Abstract

Context: Annona muricata is a medicinal plant from the Annonaceae family originally comes from the native America but is now become established in many tropical countries, especially in Malaysia. With the advancement of science and technology further study have been done to establish the function of Annona muricata in the healthcare industries. Some phytochemical constituents are acetogenins, coumarins, phenolic, alkaloids and flavonoids. The phytochemical content is the chemical constituent that gives the antioxidants, anti-microbial, anti-diabetics, and many other health benefits.

Aims: This study aimed to identify the phytochemicals in Annona muricata leaves and pulp extracts specifically phenolic and flavonoids and, to evaluate and compare the antioxidant and anti-microbial activity between the two parts of the plant.

Methods: The pulp and leave extract were obtained from a Negeri Sembilan. Antioxidant tests included FRAP, DPPH, and TPC were conducted, while antimicrobial testing was done against S.aureus using the disc diffusion method. The concentration of the sample used in the study was 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml and 400µg/ml.

Results: The result of the study shows that both parts of leaf and pulp of Annona muricata extracts possess a good antioxidant level with the concentration of 25µg, 50µ100µg, 200µ and 400µg. But in contrast those concentration does not show any significant antimicrobial activity for both extracts. When comparing between the pulp and leaves extracts, the leaves extract elicits higher content of antioxidant level and higher sensitivity towards microorganism compared to pulp extracts.

Keywords: Antioxidant, Antimicrobial, Annona Muricata, Pulp and Leave Methanolic Extract.

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INTRODUCTION

Natural entities are those that are created by nature rather than by humans. Natural products have formed the backbone of traditional healing systems for ages before contemporary technology and revolutionization technologies (Veeresham, 2012). As we know, natural product is still relevant in today's time and is still a vital role in developing newer and more potent drugs for consumers. With advancement of technologies more and more diversity of natural origin drug is being researched to understand the structural component and the advantages that the natural product holds(Harvey, 2008). *Annona muricata* is widely distributed across the globe and different parts of the world have different names for this plant. Soursop, Gaviola, Guanabana, Paw Paw, and Sirsak are some of the other names for it (Haron et al., 2020). From studies conducted there are reported to be found about two hundred and twelve bioactive compound that can be found in *Annona muricata*. Major compounds that can be acquire is phenols and flavonoids followed with predominant compounds of acetogenins. These bioactive compounds are the compounds that are responsible for the antioxidant and antimicrobial properties of *Annona muricata*. Antioxidants are substances that aid in the prevention or reduction of cell damage caused by free radicals, which are unstable molecules produced by the body in reaction to environmental and other stimuli. Many plant-based pharmaceutical products are used as human immunity enhancer since the dawn of time. One of the antioxidants that *Annona muricata* possess is Flavanols. Flavonoids are a class of chemicals that protect biomolecules including carbohydrates, proteins, lipids, and DNA from the harmful effects of oxidative processes.(Anbudhasan et al., 2014). Flavonoids could scavenge free superoxide radicals, which slows the ageing process and reduces the risk of cancer. The most abundant secondary metabolites found in plant diets are phenolic chemicals (also referred to as "flavonoids" or "polyphenols"). Antibiotics, on the other hand, are substances produced by or derived from certain fungi, bacteria, and other organisms that are used to treat bacterial infections by either

preventing the bacteria from developing or inhibiting their growth (bacteriostatic agents) or to kill them outright (bactericidal agents) (Sommer & Dantas, 2011). Flavonoids, steroids, and alkaloids found in *Annona muricata* extracts are thought to be responsible for the plant's antimicrobial bioactivity. (Radji et al., 2015). The synergism of the chemicals is most likely the mechanism of action. (Nugraha et al., 2019), have demonstrated antimicrobial activity by inhibiting glycosidase. Antibacterial activity of *Annona muricata* was comparable to that of the conventional antibiotic streptomycin against both gram-positive and gram-negative bacteria.

METHODOLOGY

A. Chemicals

Cetyl alcohol, Stearic acid, Triethonolamine, Paraffin oil, Metyl Paraban, EDTA, Butylated Hydroxytoluene (BHT), Propyl Paraben, Glycerol, Propylene Glycol, Gallic Acid, Sodium Carbonate, Folin Ciocalteu reagent, Gram Stain Safranine, Ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Muller Hilton agar and distilled water.

B. Source of Annona Muricata extract

Annona Muricata extract was bought from A&T Ingredients Sdn Bhd in Nilai in Negeri Sembilan, Malaysia and it is in powder form.

C. Detection of phenolics and flavonoids

The extract (50 mg) was diluted in 5 mL distilled water, then a few drops of a neutral 5% ferric chloride solution were added to the extract. The presence of phenolic compounds was indicated by a dark green or blue green tint (Xu et al., 2017). A few drops of HCl were added to a test tube containing test samples and a little amount of magnesium turnings. The presence of flavonoids was detected by a shift in hue from green to red (An et al., 2017).

D. Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extracts was determined using Rajkumar et al Folin-Ciocalteau.'s reagent technique. To 50 l of each extract concentration (25µg/ml, 50µg/ml, 100µg/ml,200µg/ml, and 400µg/ml), 2.5 ml Folin-Ciocalteau reagent (1/10 dilution) and 2 ml of 7.5 percent Na2CO3 (w/v) solution were added and incubated at 45 °C for 30 minutes. The absorbance at 765 nm was measured using a Cary 50 UV–Vis spectrophotometer (Varian, Inc., CA, As a standard, gallic acid was used, and the results were expressed as GAE (Gallic acid equivalence) in g/ml. This extraction was conducted in triplicate. The phenol content was calculated as Gallic Acid Equivalent (GAE) (Agu & Okolie, 2017).

E. Ferric Ion Reducing Antioxidant Power (FRAP)

The FRAP reagent was prepared by mixing 200ml of 300mM acetate buffer,20mL of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) dissolved in 40mM hydrochloric acid (HCL) and 20 ml of 20 mM ferric chloride hexahydrate ($FECl3.6H_20$) solution in proportion of 10:1:1 (v/v), respectively. Prior to use, the FRAP enzyme was freshly prepared and warmed to 37C in a water bath, and the beaker was covered in aluminium foil to prevent photodegradation. The reaction mixture was prepared by mixing 0.1ml of both pulp and leaves extract (25µ -400µg /ml) with 300µL of 2% Tween 20 and 3mL of FRAP reagent and vortex mixed using sonicator in dark condition. Then, the reaction mixture was incubated for 30 minutes, and absorbance was recorded at 593nm using spectrophotometer and conducted in triplicate. Ascorbic acid (25µg-400µg/ml) was used as comparative model for the reaction mixtures. The ferrous sulphate heptahydrate ($FSO4.7H_2O$) was then used as the standard curve by using serial dilution at range of 0.1 to 2.0 mm (George et al., 2015).

F. Disk Diffusion Method

The antimicrobial test was conducted by disk diffusion method or also known as Kirby Bauer proposed by (Balouiri et al., 2016) with slight modification. This method is used for assessment of the antimicrobial activities of different parts of *Annona muricata* extracts which is the leaves and pulp. The concentration chosen to be conducted for this antimicrobial study were 100µg and 200µg. Mueller Hinton agar is used as the media for *S.aureus.* Suspensions (1.5×10^8 CFU/ml) of microbial were prepared by using 0.5 McFarland standard. The agar plate surfaces were inoculated by spreading the microbial to be tested over the agar surfaces. 6-mm filter paper disk soaked with extracts were located on the agar. Then, the plates were incubated at 37 °C for 24 hours to allow the growth of the bacteria. 6-mm filter paper disk soaked with 100µg and 200µg of each extract, and distilled water served as negative controls while standard antibiotic of Gentamicin disc was used as the positive controls. The steps were done in triplicate manner.

G. Statistical Analysis

All experiments were carried out in triplicates. Data collection was analyzed using One-way ANOVA. Statistical package for social science (SPSS) version 20.0 and Microsoft excel was used. The results were expressed as the Mean ± SEM (Standard error of mean) and a value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A. Total Phenolic Content (TPC)

Table (1). Total Phenolic Content of (TPC) of Annona muricata Leaf and Pulp in different concentration (µg/ml)

Part	Concentrations (µg/ml)	N	TPC value	P value
Pulp	25	3	$0.073 \pm 0.015^{cdegfhij}$	(P<0.05)
	50	3	0.082 ± 0.002^{efghij}	
	100	3	$0.088 \pm 0.002^{aeffghij}$	
	200	3	$0.095 \pm 0.002^{aefghij}$	
	400	3	$0.112 \pm 0.003^{abcdfghij}$	
Leaf	25	3	$0.153 \pm 0.003^{abcdeghij}$	
	50	3	$0.173 \pm 0.006^{abcdefij}$	
	100	3	$0.178 \pm 0.006^{abcdefij}$	
	200	3	$0.235 \pm 0.004^{abcdefghj}$	
	400	3	$0.248 \pm 0.009^{abcdefghi}$	

One-Way ANOVA test. The values are presented as mean ± standard deviation (SD) of triplicate samples and expressed as mg gallic acid equivalent (GAE)/g of dry weight sample (DW).

Note: Means are being compared between concentration groups using post hoc Tukey's test. Statistical significance was set at $P < 0.05$ where:

[a]: Statistically significant difference with concentrations of 25 µg/ml of pulp part

[b]: Statistically significant difference with concentrations of 50 µg/ml of pulp part

[c]: Statistically significant difference with concentrations of 100 µg/ml of pulp Part

[d]: Statistically significant difference with concentrations of 200 µg/ml of pulp Part

[e]: Statistically significant difference with concentrations of 400 µg/ml of pulp Part

[f]: Statistically significant difference with concentrations of 25 µg/ml of pulp part

[g]: Statistically significant difference with concentrations of 50 µg/ml of leave Part

[h]: Statistically significant difference with concentrations of 100 µg/ml of leave Part

[i]: Statistically significant difference with concentrations of 200 µg/ml of leave art

[j]: Statistically significant difference with concentrations of 400 µg/ml of leave Part

There is an increasing pattern that can be seen between the concentration of sample and level of total phenolic content. As the concentration of sample increase the TPC value increase too. This is supported by various study that conducted on various plant extracts that projected the same findings like the study from (SiqueiRA et al., 2015)[13] and (Nguyen et al., 2020). The finding from Table 3.2 shows that a concentration of 400µg/ml of leave extract gives a TPC value of 0.248µg/ml while a concentration of 400µg/ml of pulp extract gives a TPC value of 0.113µg/ml. This shows that at a given concentration, the TPC value of leaves extract is higher than pulp extract. This finding is supported by a study conducted by (Orak et al., 2019) that states based on their study that the TPC value of methanolic pulp extraction with a TPC value of 10.92 µg/ml. In contrast, methanolic leaves extract gives a TPC value of 19.84µg/ml. There are many factors that can cause variables in TPC values in a particular study. Different levels of TPC may be attributed to the different plants, procedures, and standards used to express the TPCs; the color measurement of Folin-Ciocalteu reagents which was non-specific on phenol, and perhaps other components can react with Folin-Ciocalteu reagent such as ascorbic acid (Agu & Okolie, 2017). Another major determinant in the TPC value in a particular study is the type of solvent used during the extraction. Many studies found that as the polarity of the solvent increased, higher extraction yields of total soluble solids and total extractable polyphenolics were obtained from the plant extract. Based on a study (Haron et al., 2020), the best extraction for plant extraction is said to be Methanol. Methanol is claimed to be the most suited solvent for phenolic compound extraction due to its capacity to prevent the polyphenol oxidase process that results in the oxidation of phenolics and its ease of evaporation in comparison to water (Zulkefli et al., 2013).

B. 2,2-diphenyl -1-picrylhydrazyl (DPPH)

Table (2) shows the results of DPPH scavenging activity of the different extracted parts of *Annona muricata* and the positive control which is the ascorbic acid. After running one way ANOVA test, a ($P < 0.005$) value was recorded. This value indicates that there are significant different regarding the scavenging activity of pulp extract, leaf extract and ascorbic acid.

Parts	Concentration (µg/ml)	n	%DPPH scavenging	P value
Leaf	25	3	$16.09 \pm 4.72^{bcdeghijklmno}$	(P<0.005)
	50		$27.27 \pm 1.43^{adefhijlmno}$	
	100		$38.72 \pm 3.48^{abdefgijlmno}$	
	200		$50.04 \pm 1.35^{abcfgijkno}$	
	400		$60.37 \pm 4.87^{abcdfghiklno}$	
Pulp	25	3	$15.13 \pm$	

			$2.07^{bcdeghijklmno}$
	50		$25.09 \pm 1.76^{adefhijlmno}$
	100		$32.79 \pm 1.44^{adefijlmno}$
	200		$48.66 \pm 3.32^{abcfghjkno}$
	400		$55.95 \pm 4.22^{abcfghkno}$
Ascorbic Acid	25	3	$30.73 \pm 2.06^{adefijlmno}$
	50		$49.75 \pm 1.21^{abcfghjkno}$
	100		$55.43 \pm 0.74^{abcfghkno}$
	200		$75.88 \pm 1.77^{abcdefghijklmo}$
	400		$84.94 \pm 1.39^{abcdefghijklmn}$

[a]: Statistically significant difference with concentrations of 25 µg/ml of pulp part

[b]: Statistically significant difference with concentrations of 50 µg/ml of pulp part

[c]: Statistically significant difference with concentrations of 100 µg/ml of pulp Part

[d]: Statistically significant difference with concentrations of 200 µg/ml of pulp Part

[e]: Statistically significant difference with concentrations of 400 µg/ml of pulp Part

[f]: Statistically significant difference with concentrations of 25 µg/ml of leave part

[g]: Statistically significant difference with concentrations of 50 µg/ml of leave Part

[h]: Statistically significant difference with concentrations of 100 µg/ml of leave Part

[i]: Statistically significant difference with concentrations of 200 µg/ml of leave Part

[j]: Statistically significant difference with concentrations of 400 µg/ml of leave Part

[k]: Statistically significant difference with concentrations of 25 µg/ml of Ascorbic Acid

[l]: Statistically significant difference with concentrations of 50 µg/ml of Ascorbic Acid

[m]: Statistically significant difference with concentrations of 100 µg/ml of Ascorbic Acid

[n]: Statistically significant difference with concentrations of 200 µg/ml of Ascorbic Acid

[0]: Statistically significant difference with concentrations of 400 µg/ml of Ascorbic Acid

The free radical scavenging activity of *Annona muricata* leaf, pulp and reference standard which is ascorbic acid were calculated in percentage form as shown in Figure 3.1. Non-linear equation of 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) were used to construct the graph.IC50 values were then determined to calculate the effective concentration of respective samples needed to scavenge DPPH radical by 50%.From the linear equation of the graphs of ascorbic acid, leaves extract and pulp extract the IC50 is 2.31µg/ml,3.97µg/ml and 4.38µg/ml respectively. The IC50 value of 3.97µg/ml for leave extract shows better antioxidant activity than the IC50 of 4.38µg/ml for pulp extract. This result is supported by multiple studies(Orak et al., 2019) and (Coria-Téllez et al., 2018)[20]. The leaf portion includes a higher concentration of acetogenins, a critical secondary metabolite that is regarded the Annonaceae family's primary bioactive ingredient. Leaves extracts contain more acetogenins than pulp extracts, implying a higher level of antioxidant action. Other factors that play a role in the antioxidant level of a plant extract are when the fruit or parts of the plant are cultivated to undergo extraction. A study conducted by(Coria-Téllez et al., 2018) stated that ontogenesis is characterized by the maximum content of phenolic compounds in the aerial part of plants. The most antioxidant level can be acquired in rapidly growing or newly matured plants and their parts. Leaves that reached six months were characterized by low antioxidant values, compared with leaves of one and two months of age. Several factors can influence the reaction rate of antioxidant activity of the DPPH assay, including the chemical structure of compounds, nature of solvent, temperature, pH, and reactivity of free radicals. DPPH assay also has some disadvantages where DPPH can also react with other radicals in the compounds. Consequently, the time to reach the stable state is not linear to the concentration ration of antioxidants (Shahidi & Zhong, 2015).

C. Ferric Ion Reducing Antioxidant Power (FRAP)

Table (3) shows the results of FRAP assay of the different extracted parts of *Annona muricata* which is the pulp and leave extract. After running a one-way ANOVA test, a (P <0.005) value was recorded. This value indicates that there are significant different on the level of antioxidant between the two types of extract.

Parts	Concentrations (µg/ml)	N	FRAP value (Mm Fe2+/g)	P value
Leave	25	3	0.56 ± 0.25^{cdefghij}	(P<0.05)
	50	3	0.64 ± 0.73^{defghij}	
	100	3	0.76 ± 0.03^{adeghij}	
	200	3	0.95 ± 0.04^{abcefj}	
	400	3	1.15 ± 0.12^{abcdfgh}	

Pulp	25	3	0.19 ± 0.70^{abdeij}	
	50	3	0.28 ± 0.72^{abceij}	
	100	3	0.43 ± 0.62^{abcej}	
	200	3	0.77 ± 0.98^{abcfgj}	
	400	3	1.04 ± 0.58^{abcdfghi}	

One-Way ANOVA test. The values are presented as Mean ± Standard deviation (SD) of triplicate samples and expressed as FRAP value (Mm Fe2+/g).

Note: Means are being compared between concentration groups using post hoc Tukey's test. Statistical significance was set at $P < 0.05$ where:

[a]: Statistically significant difference with concentrations of 25 µg/ml of leave part

[b]: Statistically significant difference with concentrations of 50 µg/ml of leave part

[c]: Statistically significant difference with concentrations of 100 µg/ml of leave Part

[d]: Statistically significant difference with concentrations of 200 µg/ml of leave Part

[e]: Statistically significant difference with concentrations of 400 µg/ml of leave Part

[f]: Statistically significant difference with concentrations of 25 µg/ml of pulp part

[g]: Statistically significant difference with concentrations of 50 µg/ml of pulp Part

[h]: Statistically significant difference with concentrations of 100 µg/ml of pulp Part

[i]: Statistically significant difference with concentrations of 200 µg/ml of pulp Part

[j]: Statistically significant difference with concentrations of 400 µg/ml of pulp Part

The Frap value is plotted against different concentrations of *Annona muricata* pulp and leaves extract in Figure3.2, with concentrations of 25g, 50g, 100g, 200g, and 400g for both extracts. There can be seen the trend of increasing FRAP value as the concentration of sample increases. This trend can be seen in both of pulp and leaves extract. But there can be seen difference of FRAP value between the pulp and leaves extract at a given concentration. For example, at concentration of 50µg and 200µg the FRAP value for leave extract was 0.635±0.730 and 0.52±0.414 while the FRAP value of pulp extract was 0.289±0.728 and 0.769±0.975, respectively. The IC_{50} for leave and pulp is 230µg/ml and 327µg/ml, respectively This indicated that the leaves extract possesses a higher antioxidant level when compared to pulp extract and it was justified by the calculated IC_{50} values. The IC_{50} values for leaves extract was 230µg/ml while for pulp extract was calculated at 327µg/ml. The result is supported by previous studies like (Orak et al., 2019)[22] that stated that leaves extract of various solvent showed higher antioxidant level than pulp. Leaves part of *Annona muricata* is said to have more content of secondary metabolites such as flavonoids, alkaloids and phenolics thus making it having a higher antioxidant level (Gavamukulya et al., 2017). This can be supported by the results of total phenolics content as discussed previously. The other reason why pulp extract has lower antioxidant level is because the vulnerability of the pulp extract itself during the extraction process. Based on the study conducted by (Cerretani & Bendini, 2010)[24] and (Kamiloglu et al., 2016)[25] stated that certain types of extraction steps like long drying process of pulp compared to seed and leaves may decrease the content of the secondary metabolites itself. The FRAP assay has a number of advantages, including the fact that it is a simple and inexpensive method that does not require the use of any special reagents, making it an ideal method for determining the antioxidant activity of diverse plant extracts. (Shah & Modi, 2015)[26]. Although it has benefits, the FRAP test also has drawbacks. In terms of preparing the chemicals for the working solution, the FRAP assay is more time consuming and difficult and it is a timed-based analytical test.

D. Correlation between total phenolic content and antioxidant activities

As established, the secondary metabolites are the chemical compounds responsible for the medicinal benefits that a medicinal plant possesses. Vis- a -vis, Phenolic molecules are the essential antioxidant components responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. Phenolics also have ideal structural characteristics for free radical scavenging (Aryal et al., 2019)[27]. The antioxidant capabilities evaluated by different portions of the *Annona muricata*, as well as the phenolic contents of the extracts, were linked using Pearson's correlation coefficient. Different literature reports indicate a linear correlation of total phenolic content with antioxidant capacity (Asem et al., 2020)[28].In this study, the statistical analysis of the data gathered showed a positive correlation of the Total phenolics on the antioxidant activities ranging from R^2 0.947 to 0.966, N=15 $p<0.001$: correlation is significant at the 0.01 level (2 tailed). The results measured within the individual assay showed the same trend of activity in the whole data set. With coefficient values of R2 of 0.966 and 0.949 respectively, the FRAP assay indicates a good association to the total phenolic content of the pulp and leave extract. The correlation between the total phenolic content of pulp and leave extract and DPPH assay also showed strong positive correlation R^2 of 0.947 and 0.934. As a result, the presence of phenolic hydroxyl group in phenolic compounds can be linked to scavenging and ferrous reduction of pulp and leaf extracts.

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^{2+}/g)	0.966	0.949

E. Antimicrobial test using disk diffusion method

Leave extract of *Annona muricata* against *S.aureus*

Table 3.5 Mean ± SD, antimicrobial activity of various concentrations of *Annona muricata* pulp extract, positive and negative control against selected microorganism.

Microorganism	Zone of inhibition (mm)			
	Concentration		Positive control	Negative control
Gram Positive	200µg/ml	400µg/ml		
S.aureus	8.03 $\pm$ 0.20^{bcd}	8.73 $\pm$ 0.32^{acd}	36.00^{abc} $\pm$0.00	6.00 $\pm$ 0.00^{abd}

[a] statistically significant when compared to leave extract of 200µg/mL ($p<0.05$)

[b] statistically significant when compared to leave extract of 400 µg/mL($p<0.05$)

[c] statistically significant when compared to negative control ($p<0.05$)

[d] statistically significant when compared to positive control ($p<0.05$)

Pulp extract of *Annona muricata* against *S.aureus*

Microorganism	Zone of inhibition (mm)			
	Concentration		Positive control	Negative control
Gram Positive	200µg/ml	400µg/ml		
S.aureus	6.00 $\pm$ 0.00^{bd}	6.23 $\pm$ 0.15^{acd}	36.00^{abd} $\pm$0.00	6.00 $\pm$ 0.00^{bd}

[a] statistically significant when compared to pulp extract of 200µg/mL ($p<0.05$)

[b] statistically significant when compared to pulp extract of 400 µg/mL($p<0.05$)

[c] statistically significant when compared to negative control ($p<0.05$)

[d] statistically significant when compared to positive control ($p<0.05$)

The results from the antimicrobial test showed the average zone of inhibition for concentration 100µg and 200µg of pulp extract were 6.00±0.00 and 6.23±0.15, respectively. In contrast, the leaves extract with the same concentrations showed the result of 8.03±0.20 and 8.73±0.32. Based on the study from (Hudzicki & Kirby-Bauer, 2016), the recorded zone of inhibition is considered low inhibition in the range 7-10 mm. A high inhibition towards the microorganism will show a range of inhibition between 11mm and above. This study elicits low zone of inhibition of both parts with slightly different between them. The low-level zone of inhibition indicated that the concentration of 200µg/ml and 400µg/ml of *Annona muricata* pulp and leave extract was not susceptible to inhibit the growth of *S.aureus*. Despite these results, there are also studies conducted by (Orjiakor, 2019)[30] that support the finding. The paper shows the low-level inhibition zone even with other concentrations of 5mg/ml, 10mg/ml, and 50mg/ml of extracts of the same parts. Nevertheless, from the same paper (Orjiakor, 2019), the zone of inhibition only starts to increase when the concentration is more than 150mg/ml and above. This shows that the low level of concentration of *Annona muricata* does not pose antibacterial properties towards *S.aureus*, but a high concentration of more than 150mg/ml will possess antimicrobial activity. One of the factors that also contribute to the strength of inhibition is the type of solvent used during the sample extraction. Based on the study from (Orjiakor, 2019),also shows that a concentration of 300mg/ml of n-hexane extraction has a low zone of inhibition while 300mg/ml of hot distilled water has a high zone of inhibition. This concludes that different extraction solvents have different inhibition outcomes, thus different antimicrobial properties (Agunloye & Onifade, 2020). This study shows that leave extract elicits higher antimicrobial activity compared to pulp extract. This statement can be confirmed by looking through the zone of inhibition. 400µg/ml of pulp extract gives an average of 6.23±0.15 zone of inhibition while 400µg/ml leaves extract gives an average 8.73±0.32 zone of inhibition. The differences between the antimicrobial activity of pulp and leaves are maybe due to the higher content of secondary metabolites in the leaves extract compared to the pulp extract. The secondary metabolite that are in contention that the leave part contain more than pulp is the flavonoids. Based on the study from (Compean & Ynalvez, 2014), flavonoids are the main metabolites that possess antimicrobial properties towards many microorganism especially *S.aureus*.

CONCLUSION

Methanolic extract of *Annona muricata* leaves, and pulp showed antioxidant properties detected using TPC, FRAP, and DPPH assay. A high level of total phenolic content has also been observed in the plant extracts and both extracts shown a significant radical scavenging activity. The high scavenging ability of distinct *Annona muricata* parts could be attributable to hydroxyl groups in the phenolic compound's chemical structure, which can offer the required component as a radical scavenger and antioxidant. However, other phytochemical compounds may contribute to the antioxidant activity of the plant, such as alkaloids, saponins, tannins, and flavonoids. This study also showed that the methanolic leaves

extract has higher antioxidant activity than methanolic pulp extract when being investigated with TPC, FRAP, and DPPH assay. This is because leaves have higher phytochemical content and more susceptible to undergo the vigorous processes of extraction. Next, methanolic extract of *Annona muricata* leaves and pulp extract do not inhibit the growth of *Steph aureus* at lower concentrations of 200µg/ml and 400µg/ml but will start to show inhibition with a concentration of higher than 150mg/ml as discussed earlier.

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