



## Antioxidant Properties and Antibacterial Activity of Breadfruit (*Artocarpus altilis*) Bark and Leaf Extract against *Staphylococcus Aureus* and *Escherichia Coli*

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**ABSTRACT:** This study comprehensively investigated the antioxidant properties and antibacterial activity of breadfruit (*Artocarpus altilis*) bark and leaf extract against two common bacterial strains, *Staphylococcus aureus*, and *Escherichia coli*. The objective was to assess the potential therapeutic applications of breadfruit extract in combating bacterial infections caused by these organisms. The researchers employed an experimental research design, utilizing controlled experiments to evaluate the effects of breadfruit extract on the target bacteria. Both the bark and leaf parts of the breadfruit plant were examined in the study. The extracts were subjected to analysis to identify and quantify the presence of beneficial compounds. The analysis revealed the presence of various compounds, including but not limited to Phenolic Acid and Flavonoids, which are known for their potential health benefits and antioxidant properties. These compounds were found to be present in relatively high concentrations, suggesting the potential of breadfruit extract as a source of natural antioxidants. In terms of antibacterial activity, the breadfruit leaf extract exhibited promising results by demonstrating significant inhibitory effects against both *Staphylococcus aureus* and *Escherichia coli*. In contrast, the bark extract showed limited effectiveness against *Escherichia coli* but displayed some inhibitory activity against *Staphylococcus aureus*. These findings suggest that breadfruit extract may have potential as a natural antimicrobial agent, particularly in combating *Staphylococcus aureus* infections.

**KEYWORDS:** Experimental, antioxidant properties, beneficial compound, Phenolic Acid and Flavonoids, inhibitory effects, natural antimicrobial agent, Surigao, Philippines.

### INTRODUCTION

The leading cause of global morbidity and mortality is an infectious disease, especially bacterial infection (Bachiretal, 2015). As stated by the World Health Organization (2021), the number of deaths reached about 60 million every year, wherein one-third of these were caused by bacteria and viruses. Around 1 in 10 people who got bacterial meningitis died from the infection, even with treatment. All three infectious diseases were caused by pathogens *Staphylococcus aureus* and *Escherichia coli*.

*Staphylococcus aureus* was a highly notorious and prevalent bacterial pathogen that was responsible for a significant number of uncomplicated skin infections and potentially hundreds of thousands to millions of more severe, invasive infections worldwide each year (Rasigade, et al., 2015). In the Philippines, a case of *Staphylococcus aureus* contamination in candy was reported by Philstar Global in July 2015, affecting almost 2,000 people in CARAGA, mostly 10-14 years of age. *Escherichia coli* (*E. coli*) was a type of gram-negative rod-shaped bacterium that was typically present in the normal intestinal flora of humans. However, it could also lead to intestinal and extraintestinal illnesses. Additionally, *Escherichia coli* had been detected on the surfaces of hospital floors and long-term care facilities. However, when found outside of the intestinal tract, *Escherichia coli* could cause urinary tract infections (UTI), pneumonia, bacteremia, and peritonitis, among others (Jain S, et al., 2015). According to a report by Mindanao News on July 22, 2021, health officials in the municipality of Santo Tomas, province of Davao del Norte confirmed that the water system and water supplies of refilling stations in Barangay Tulalian, Santo Tomas were contaminated with *Escherichia coli* (*E. coli*) bacteria, leading to an outbreak of diarrhea. Mart Sambalud, the information officer of the municipality of Davao, stated that a total of 471 residents were affected by diarrhea after consuming tap water. Of this total, one male patient, aged 58, and



two female patients, aged 81 and 69, all residents of Barangay Tullian, died. Antibiotic resistance had been recognized early by Fleming in the 1940s. In recent years, Pitout (2013) noted the emergence of antibiotic-resistant bacteria such as *Escherichia coli* to fluoroquinolone. *Staphylococcus aureus* could also become drug-resistant by genetic mutations that alter the target DNA gyrase or reduce outer membrane proteins, thereby reducing drug accumulation (Kime, et al., 2019). The Centers for Disease Control and Prevention noticed Staphylococcal resistance to penicillin, vancomycin, and methicillin, also known as MRSA, which all became serious problems. Hence, investigations for new classes of antibiotics became increasingly essential. Throughout history, numerous plants have been recognized for their medicinal qualities and have been utilized for the treatment of various human ailments. *Artocarpus altilis*, a member of the Moraceae family, was among these plants. Commonly known as breadfruit, it acquired its name due to its resemblance to freshly baked bread. *Artocarpus* extracts and metabolites from leaves, stems, fruit, and bark contained numerous beneficial biologically active compounds, and these compounds were used in various biological activities including antibacterial, antitubercular, antioxidant, antiviral, antifungal, and cytotoxicity (Jagtap and Bapat, 2018).

Recently, many researchers investigated the possible utilization of some plant extracts as effective natural preservatives (Clarke et al., 2017). Traditionally, the crude extracts of different parts of medicinal plants, including root, stem, flower, fruit, and twigs, were widely used for the treatment of some human diseases (Khan et al., 2015). Medicinal plants were rich in phytochemicals, including flavonoids, alkaloids, tannins, and terpenoids, which possessed antimicrobial and antioxidant properties (Talib and Mahasneh, 2010). Understanding the mechanism of action of those plant extracts against microorganisms was crucial for their effective use as natural antimicrobial agents. Throughout history, plants have been extensively used for medicinal purposes. In local medical practices, the tea made from Guava leaves (*Psidium guajava Linnaeus*) was commonly employed to treat gastroenteritis and child diarrhea, particularly among those who lacked access to or could not afford antibiotics. The Philippines as a country with rich biodiversity would be a great source for detecting new antibiotic agents.

According to the data that was gathered by the researchers from the City Health Office Surigao, the top common diseases consulted at the health centers and the different hospitals of Surigao City in the year 2022 were upper respiratory tract infections caused by *Escherichia coli* (13.86%), acute respiratory infection for 5 and above caused by *Staphylococcus aureus* (7.74%), acute respiratory tract infection in children below 5 (6.43%), and urinary tract infection caused by *Escherichia coli* (4.23%).

Considering all these facts and data, this prompted the researchers to investigate the antioxidant properties and antibacterial activity of *Artocarpus altilis* bark and leaf extract against *Staphylococcus* and *Escherichia coli* to discover a safe and effective antibacterial agent comparable to the existing plants present in the Philippines that were locally used as an antibacterial agent, to minimize the impacts of these disease-causing microorganisms that were prevalent in Surigao City based on the data gathered from City Health.

## Framework

This study investigated the antioxidant properties and antibacterial activity of breadfruit (*Artocarpus altilis*) bark and leaf extract against *staphylococcus* and *Escherichia coli*. The input of microorganisms and materials needed such as the collection of plants especially the bark and leaf of the breadfruit tree, and the microorganisms such as *staphylococcus* and *Escherichia coli*. The process of breadfruit leaf collection and preparation of plant organisms. Drying and extraction are the methods to help remove excess water and enzyme action present in the cell. Another process to be utilized is a rotary evaporator to separate the concentration of plant, plate diffusion to test the standardized concentration of the organisms, and a zone of inhibition test that will identify the resulting zone of inhibition of *Artocarpus altilis* to the test organisms. The output which identify result of the Antioxidant properties and Antibacterial Activity of Breadfruit (*Artocarpus altilis*) Bark and Leaf Extract Against *Staphylococcus aureus* and *Escherichia coli*.

## Research Objectives

This study determined the antioxidant properties and antimicrobial activity of breadfruit (*Artocarpus altilis*) bark and leaf extract against *Staphylococcus* and *Escherichia coli*.

Specifically, this study determined:

1. The antioxidant properties present in the crude extracts of breadfruit (*Artocarpus altilis*), specifically the bark and leaves.



2. The antibacterial activity of the bark and leaf extracts of breadfruit (*Artocarpus altilis*) against *Staphylococcus aureus*. This will be assessed through the following trials:

- 2.1 Trial 1: Bark Extract
- 2.2 Trial 2: Bark Extract
- 2.3 Trial 3: Bark Extract
- 2.4 Trial 1: Leaf Extract
- 2.5 Trial 2: Leaf Extract
- 2.6 Trial 3: Leaf Extract

3. The significant difference in the inhibition of *Staphylococcus aureus* growth when treated with the bark and leaf extract of breadfruit (*Artocarpus altilis*).

4. The significant difference in the inhibition of *Escherichia coli* growth when treated with the bark and leaf extract of breadfruit (*Artocarpus altilis*).

### METHODS

This study shows the method of how this research will be conducted through the process of research design, materials and equipment, data gathering procedure, and experimentation data analysis.

In this study, the researcher employed a quantitative research approach following the classical experimental design. This approach offers a meticulously controlled environment, where researchers can systematically manipulate independent variables, such as the breadfruit extracts, to observe their impact on dependent variables like antioxidant properties and antibacterial activity. It is particularly apt for establishing causality, enabling the research to draw precise cause-and-effect relationships. By focusing on quantitative data, this design ensures the collection of numerical, precise measurements, which are integral to objective analysis and scientific rigor. Ultimately, the choice of a classical experimental design reflects a commitment to a systematic, controlled, and data-driven investigation, enhancing the reliability and credibility of the research outcomes. The following materials were used throughout the research study: axe, *Artocarpus altilis* bark and leaf extract, test organisms (*Escherichia coli* and *Staphylococcus aureus*), nutrient broth/liquid medium, mechanical vortex mixer, a screw-capped tube of test tube organism nutrient agar, sterilized petri dish, sterilized cotton swab, 13 mm paper disc, Saline solution, forceps, plates/agar plates, and scissors. The research data is subjected to statistical analysis, with a primary focus on T-test analysis. The T-test analysis is a fundamental statistical technique used to compare the means of two independent groups. In cases where the assumptions of the standard T-test (Student's T-test) are violated, the study employs Welch's T-test. This statistical hypothesis test is instrumental when comparing the means of two independent groups with unequal variances and unequal sample sizes. Before applying certain parametric statistical tests, such as analysis of variance (ANOVA) or T-tests, to the research data, Levene's Test is utilized as a preliminary step. This test assesses the equality of variances among different groups, ensuring that the assumption of equal variances (homoscedasticity) is met.

#### FLOW-CHART OF THE STUDY

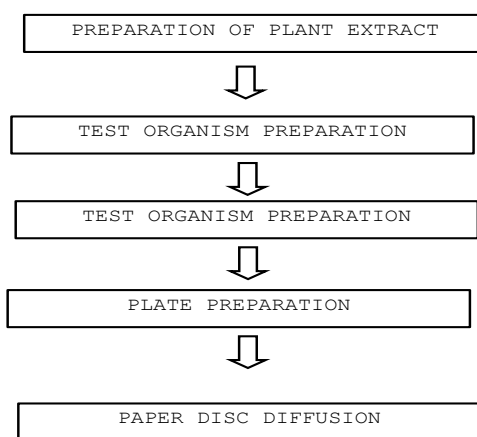


Figure 2: Flow chart of the study entitled Antioxidant Properties and Antibacterial Activity of Breadfruit (*Artocarpus altilis*) Bark and Leaf Extract Against *Staphylococcus aureus* and *Escherichia coli*



Ethics in the conduct of this research were strongly considered for the academic integrity of this study. Ethical research practices in educational institutions are strongly followed since it is always the goal of educational research to contribute to the general welfare of the academic community and to generally create measurable information or data that will eventually add to the increase of human knowledge (Ederio, 2023) such as the essence depicted by this study.

**RESULTS AND DISCUSSION**

**I. Antioxidant Properties of *Artocarpus altilis* Leaf extract using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Assay**

Table 1 presents the antioxidant properties of *Artocarpus altilis* leaf extract using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay

Parameters	Results	Interpretation
Vitamin C	+	Partially Present
Vitamin E	+	Partially Present
Vitamin A	+	Partially Present
Carotenoids	+	Partially Present
Saponins	+	Partially Present
Phenolic Acid	+++	Highly Present
Flavonoids	+++	Highly Present
<b>Parameters</b>		<b>Interpretation</b>
+		Partially present
++		Present
+++		Highly Present

Vitamin C, Vitamin E, Vitamin A, Carotenoids, and Saponins were detected in the leaf crude extract of breadfruit, although their concentrations were categorized as partially present, suggesting they are present but in relatively low amounts. On the other hand, high levels of Phenolic Acid and Flavonoids were observed in the sample, with both receiving a rating of highly present (+++). This suggests that the sample contains significant concentrations of these compounds, indicating potential health benefits and antioxidant properties. These findings provide a basis for further investigation into the health-promoting properties of breadfruit and its potential applications in the development of functional foods or natural remedies.

A study by R. Rahmawati et al. (2023) reported that some studies have higher antioxidant activity in leaf extracts compared to bark extracts, such as in the case of *Dillenia serrata*. The antioxidant activity showed that leaf extract had the highest antioxidant activity, followed by root and bark extract (Z. Zulkifli, 2023).

**II. Antioxidant Properties of *Artocarpus altilis* Bark extract using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Assay**

Table 2 presents the antioxidant properties of *Artocarpus altilis* bark extract using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay

Parameters	Results	Interpretation
Vitamin C	+	Partially Present
Vitamin E	+	Partially Present
Vitamin A	+	Partially Present
Carotenoids	+	Partially Present
Saponins	+	Partially Present
Phenolic Acid	++	Present
Flavonoids	++	Present
<b>Parameters</b>		<b>Interpretation</b>
+		Partially present
++		Present
+++		Highly Present

The results indicate that Vitamin C, Vitamin E, Vitamin A, Carotenoids, and Saponins are partially present in the *Artocarpus altilis* bark extract. This suggests that these compounds are detectable in the extract, although their concentrations may be relatively low. In contrast, Phenolic Acid and Flavonoids are indicated to be present in the extract with a double plus sign (++)



This suggests a potentially higher concentration of these compounds compared to the partially present ones. These findings highlight the presence of various compounds in the *Artocarpus atilis* bark extract, with Phenolic Acid and Flavonoids standing out for their potentially higher concentration and indicating significant antioxidant properties. Further investigation is warranted to explore the specific implications and potential benefits associated with these compounds in the bark extract.

In the case of *Zanthoxylum leprieurii*, the root bark extract exhibited very low antioxidant activity, while leaf and trunk bark extracts showed antioxidant activities (Y. Tine, 2020). The antioxidant properties of different extracts were tested regarding their scavenging activities on ABTS•+ radical. Fruit and stem extracts had low antioxidant potential and root bark extracts exhibited very low antioxidant activity (A. Diallo, 2020).

**III. Antibacterial Activity of *Artocarpus atilis* Bark and Leaf Extract Against *Escherichia coli***

Table 3 presents the antibacterial activity of *Artocarpus atilis* Bark and Leaf Extract Against *Escherichia coli*

Extract	Zone of Inhibition (mm) per Trial (T)			Ave. Zone of Inhibition (mm)	Interpretation
	T1	T2	T3		
Leaf	10	10	10	10	Partially Active
Bark	0.09	0.07	0.07	0.08	Inactive

Parameters	Antibacterial Activity
<10mm	Inactive
10-13 mm	Partially Active
14-19 mm	Active
>19 mm	Very Active

The leaf extract of *Artocarpus atilis* exhibited partial antibacterial activity against *Escherichia coli* (*E. coli*). Zone of inhibition measurements consistently indicated a zone size of 10 mm, categorizing its activity as 'Partially Active.' In contrast, the bark extract of *Artocarpus atilis* demonstrated 'Inactive' antibacterial activity against *Escherichia coli*. The zone of inhibition measurements for *Escherichia coli* were consistently small (0.09 mm, 0.07 mm, and 0.07 mm), with an average of 0.08 mm.

According to the study of Gooma. A. (2018), abutilon leaf extract had maximum activity against *Staphylococcus aureus* (16.8 mm) compared to *Escherichia coli* (7.2 mm). Biogenic AgNPs synthesized using medicinal plants from southern Africa have desirable antibacterial effects against *Staphylococcus aureus* compared to *Escherichia coli* (Aboyewa. J, 2021).

**IV. Antibacterial Activity of *Artocarpus atilis* Bark and Leaf Extract Against *Staphylococcus aureus*.**

Table 4 presents the antibacterial activity of *Artocarpus atilis* bark and leaf extract against *Staphylococcus aureus*.

Strains	Zone of Inhibition (mm) per Trial (T)			Ave. Zone of Inhibition (mm)	Interpretation
	T1	T2	T3		
Leaf	15	14	13	14	Active
Bark	12	12	11	12	Partially Active

Parameters	Antibacterial Activity
<10mm	Inactive
10-13 mm	Partially Active
14-19 mm	Active
>19 mm	Very Active

The leaf extract demonstrated active antibacterial activity against *Staphylococcus aureus* (*S. aureus*), with zone sizes of 15 mm, 14 mm, and 13 mm, and an average zone of inhibition of 14 mm, categorizing its activity as 'Active.' In contrast, the bark extract exhibited 'Partially Active' antibacterial activity against *Staphylococcus aureus* (*S. aureus*), with zone of inhibition



measurements ranging from 11 mm to 12 mm and an average of 12 mm. The findings indicate that the bark extract of *Artocarpus altilis* is not effective against *Escherichia coli* but does show some inhibitory activity against *Staphylococcus aureus*.

According to Senadeera, S. (2021), the aqueous bark extract showed the highest efficacy and potency against *Escherichia coli*, while the methanol bark extract showed the highest efficacy and potency against *Staphylococcus aureus*. Therefore, the plant bark extract has greater antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*.

#### V. Significant Difference in the inhibition of growth of *Staphylococcus aureus* when treated with the leaf and bark extracts of breadfruit (*Artocarpus altilis*).

Table 5 presents the significant difference in the inhibition of growth of *Staphylococcus aureus* when treated with the leaf and bark extracts of breadfruit

Strains	Ave. Zone of Inhibition	p-value (Student's t)	Decision to Ho	Interpretation
Leaf	14	0.004**	Reject Ho	Significant
Bark	12			

\*\*Reject Ho if p-value is  $\leq 0.05$

The average zone of inhibition for *Escherichia coli* (*E. coli*) using the leaf extract of *Artocarpus altilis* is 10 units. The p-value obtained from the Student's t-test comparing the zone of inhibition using leaf and bark extract is 0.004. The null hypothesis (HO) assumes no significant difference in the zone of inhibition between leaf and bark extract. The p-value (0.004) is less than the significance level ( $\alpha = 0.05$ ), leading to the rejection of the null hypothesis. There is a significant difference in the zone of inhibition of *Staphylococcus aureus* when using the leaf and bark extract of *Artocarpus altilis*. The interpretation suggests that the antibacterial activity of leaf extract is significantly different from bark extract.

Based on the results of a study conducted by Kumar et al. (2019), there was a significant difference in the zone of inhibition of plant leaf extract against *Staphylococcus aureus* and *Escherichia coli*. The study evaluated the antibacterial activity of leaf extracts of three plants, namely *Azadirachta indica*, *Ocimum sanctum*, and *Eucalyptus globulus*, against *Staphylococcus aureus* and *Escherichia coli*.

#### VI. Significant Difference in the inhibition of growth of *Escherichia Coli* when treated with the leaf and bark extracts of breadfruit (*Artocarpus altilis*).

Table 6 presents the significant difference in the inhibition of growth of *Escherichia Coli* when treated with the leaf and bark extracts of breadfruit.

Strains	Ave. Zone of Inhibition	p-value (Welch's t)	Decision to Ho	Interpretation
Bark	0.08	0.000**	Reject Ho	Significant
Leaf	10			

\*\*Reject Ho if p-value is  $\leq 0.05$

The zone of inhibition of *Escherichia coli* (*E. coli*) when using the leaf and bark extract of *Artocarpus altilis*. The average zone of inhibition for bark extract is 0.08 units, and the obtained p-value from Welch's t-test is 0.000. By comparing the p-value to the significance level ( $\alpha = 0.05$ ), the null hypothesis is rejected for bark extract, indicating a significant disparity in the zone of inhibition *Escherichia coli*. The interpretation suggests that the antibacterial activity using leaf extract is markedly different from that against bark extract.

A study conducted by Singh et al. (2017) found that the bark extract of *Acacia nilotica* had a higher antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*. Similarly, a study by Kaur et al. (2018) found that the bark extract of *Terminalia arjuna* had a higher antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*.



## CONCLUSIONS AND RECOMMENDATIONS

The following conclusions have been drawn based on the study's findings:

1. The analysis reveals the presence of a range of beneficial compounds in the examined sample. While some compounds are present moderately, Phenolic Acid and Flavonoids stand out for their high concentrations, warranting further investigation into their specific implications and potential benefits for health promotion.
2. The presence of various compounds in the *Artocarpus altilis* bark extract, particularly the higher concentrations of Phenolic Acid and Flavonoids, suggests its potential as a source of natural antioxidants. These findings provide a foundation for further exploration into the antioxidant properties and potential applications of *Artocarpus altilis* bark extract, with emphasis on Phenolic Acid and Flavonoids, as potential contributors to its beneficial effects.
3. The *Artocarpus altilis* leaf extract demonstrated promising antibacterial properties, particularly against *Staphylococcus aureus*. Further research can delve into the specific compounds and mechanisms underlying its antibacterial activity and explore potential applications in natural antimicrobial treatments.
4. The antibacterial activity of *Artocarpus altilis* bark extract was investigated against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The findings suggest that the extract does not possess significant antibacterial activity against *Escherichia coli*, as indicated by the small zone of inhibition measurements and categorization as "Inactive." However, it does exhibit some level of inhibitory activity against *Staphylococcus aureus*, as indicated by the larger zone of inhibition measurements and categorization as "Partially Active."
5. The antibacterial activity against *Escherichia coli* showed a significant difference when treated with bark and leaf extracts, as indicated by the rejection of the null hypothesis. These results suggest that the leaf extract of *Artocarpus altilis* may possess selective antibacterial properties, showing varying effectiveness against different bacterial strains.
6. There was a significant difference in the zone of inhibition when using bark and leaf extract against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The obtained p-value of 0.004 (*Staphylococcus aureus*) and 0.000 (*Escherichia coli*) and rejection of the null hypothesis for both bark and leaf extract indicate a substantial contrast in the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. These findings highlight the differential effectiveness of the bark and leaf extract against these bacterial strains, suggesting its potential for targeted antimicrobial applications.

Based on the research findings of "Antioxidant Properties and Antibacterial Activity of Breadfruit (*Artocarpus altilis*) Bark and Leaf Extract Against *Staphylococcus aureus* and *Escherichia coli*," the following recommendations can be made:

1. **Further Investigation:** Conduct additional research to explore the specific mechanisms of action and potential health benefits associated with the identified compounds, such as Phenolic Acid and Flavonoids, present in the breadfruit bark and leaf extract. Understanding the underlying processes can provide insights into their optimal utilization and potential applications.
2. **Application Development:** Explore the development of products or formulations utilizing breadfruit bark and leaf extract, considering their observed antioxidant properties and antibacterial activity. This can involve formulation optimization, stability testing, and efficacy evaluations to harness the extract's potential for therapeutic or functional applications.
3. **Targeted Antimicrobial Applications:** Given the differential antibacterial activity observed against *Staphylococcus aureus* and *Escherichia coli*, further investigate the extract's potential for targeted antimicrobial applications. Assess its efficacy against a broader range of bacterial strains to determine its spectrum of activity and identify potential areas of application, such as in the development of antibacterial agents or topical treatments.
4. **Safety and Toxicity Assessment:** Conduct comprehensive safety and toxicity evaluations of the breadfruit bark and leaf extract to ensure its suitability for human or animal use. This includes assessing its cytotoxicity, genotoxicity, and potential adverse effects to establish its safety profile and determine appropriate dosage guidelines.
5. **Industrial Scale Production:** Investigate the feasibility of large-scale production of breadfruit bark and leaf extract, considering its potential therapeutic properties and market demand. This can involve optimizing extraction processes, evaluating scalability, and assessing economic viability to support commercial production.

Overall, the researchers recommend that instead of air drying the leaves and bark of the tree, it is recommended to microwave them in the chosen laboratory site for better drying results and to prevent spoilage, which can lead to improved



antioxidant testing properties and antibacterial activity. In addition, since this study is focused on leaf and bark extract, it is also possible to shift the focus toward the roots of a potential tree that has antibacterial and antioxidant properties.

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