



Phytochemical Screening of *Artocarpus odoratissimus* (Marang) Seed Extract and Its Antimicrobial Potential against *Staphylococcus aureus* & *Escherichia coli*

Genaro F. Alderite Jr ^{a,b,++*}

^a Department of Biology, Graduate School, Davao Oriental State University, Philippines.

^b School of Business Management, Education, Arts & Science, San Pedro College, Davao City, Philippines.

Author contribution

The sole author of this paper is responsible for conceptualizing the study, developing the methodology, conducting the investigation, analyzing the data, and writing the manuscript.

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ABSTRACT

Aims: *Artocarpus odoratissimus*, often called locally as "Marang," is well-known in the Philippines for its economic and nutritional value. No phytochemical studies have been conducted on *Artocarpus odoratissimus* cultivated locally in Davao City. The purpose of this study is to establish an initial chemical profile of the species by examining the secondary metabolites found in *Artocarpus odoratissimus* seed ethanol and methanol extract and determining their antimicrobial

⁺⁺ Student and Faculty Member;

*Corresponding author: E-mail: genarojr_alderite@spcdavao.edu.ph;

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activity against selected soil microorganisms, particularly *Staphylococcus aureus* and *Escherichia coli*.

Study Design: Laboratory experiments were conducted at San Pedro College, Davao City, to assess the qualitative phytochemical components and antibacterial activity of crude ethanolic and methanolic extracts of *A. odoratissimus* against selected soil bacteria.

Place and Duration of Study: At San Pedro College, the study was conducted in the science laboratories between December 2022.

Methodology: Seed extracts were prepared using ethanol and methanol solvents through maceration techniques. Standard methods were employed to identify the presence of various phytochemicals qualitatively. The disc diffusion method was utilized to assess the effectiveness of the extracts against specific bacterial strains, including *E. coli* and *S. aureus*. The extent of inhibition zone formation was measured to determine the antimicrobial potential.

Results: Qualitative analysis revealed the presence of various secondary metabolites in *Artocarpus odoratissimus* seed extracts, including flavonoids, tannins, phenolics, saponins, steroids, terpenoids, and alkaloids. Furthermore, the seed extracts exhibited limited antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* as determined by the zone of inhibition assay.

Conclusion: Seed extracts from *Artocarpus odoratissimus* showed promise due to the presence of health-promoting compounds. While initial tests against bacteria were limited, further research can improve extraction methods and explore how these compounds work together for broader antibacterial effects. Future studies should also precisely measure the compounds, test against more bacteria, and explore potential uses based on the extract's profile.

Keywords: Phytochemical screening; *Artocarpus odoratissimus*; antimicrobial potential; soil.

1. INTRODUCTION

Soilborne microorganisms are essential in many biological processes, including nutrient cycling, plant nutrition, disease suppression, water purification, and soil structure maintenance [1].

Two of the most widespread bacterial species in many soil ecosystems are *Escherichia coli* and *Staphylococcus aureus*. These two bacterial species cause invasive health-related infections in human populations [2]. *Escherichia coli* has been found in tropical and subtropical soils [3], of which their survival rate is influenced by high and low temperatures [4], limited moisture [5], and variation in soil texture [6]. On the other hand, *Staphylococcus aureus* is found in different land uses, and their concentrations are associated with varying soil properties [7].

Natural products or secondary metabolites have been recognized as critical active ingredients in natural medicines for treating many diseases [8]. These natural products are derived from medicinal plants and are applied to many vector-borne diseases, fungal infections, and all other life-threatening ailments [9].

Artocarpus odoratissimus, a native fruit of Southeast Asia, including the Philippines, is one potential antibacterial agent that may be useful in controlling *Staphylococcus aureus* and

Escherichia coli infections. In some areas, the fruit is known as marang or tarap. Its fruits and leaves are antibacterial. A chemical profiling study on marang conducted in Malaysia identified several phytochemicals that have been shown to support the potency of its antibacterial properties [10]. According to a separate study, the genus *Artocarpus*, which includes marang, contains secondary metabolites such as flavonoids, which have some antibacterial activity [11].

However, no phytochemical investigation of *Artocarpus odoratissimus* locally grown in Davao City has been reported. Therefore, developing an initial chemical profile of the species would be interesting. Moreover, with the pressing health-related problem and the availability and medical potential of secondary metabolites found in this plant, this study aims to fill the research gap by investigating the secondary metabolites found in *Artocarpus odoratissimus* seed extract and to determine their antimicrobial impact against selected soil microorganisms, particularly *Staphylococcus aureus* and *Escherichia coli*.

2. MATERIALS AND METHODS

2.1 Study Design

This laboratory experiment evaluated the qualitative phytochemical components and the

antibacterial activity of the crude ethanolic and methanolic extracts of *Artocarpus odoratissimus* against a selection of soil bacteria. The microbiology laboratory at San Pedro Hospital is where the test bacteria were obtained. At San Pedro College, the study was conducted in the science laboratories.

2.2 Ethanol Extraction of Plant Extract

85 grams of powdered dried seeds were used to make the extracts, which were then macerated with 500 mL of 95% ethanol in an Erlenmeyer container, covered with aluminum foil, and allowed to settle for 4 days while being stirred intermittently. The filtrate (1) and residue were then produced by filtering the mixture through paper. The residue was then macerated once more (remacerated) with 250 mL of 95% ethanol, and the erlenmeyer was then covered with aluminum foil and left for 2 days while being stirred occasionally. The material underwent filtration to create filtrate after two days (2). In order to obtain a thick extract, the filtrates (1) and (2) were combined and evaporated using a rotary evaporator [12].

2.3 Methanol Extraction of Plant Extract

Plant seeds were mashed after being sun-dried. Each seed sample was macerated, and 200 g was extracted with 500 ml of methanol and placed in a mechanical shaker for 48 hours. The extract was filtered with No.1 Wattman filter paper. The resulting extracts were concentrated, dried in a rotary evaporator, and stored in the refrigerator for future use [13].

2.4 Phytochemical Analysis

Using standard methods [14,15,16] to qualitatively determine the contents the presence of flavonoids, tannins, phenolics, saponin, steroids, terpenoids, and alkaloids were analyzed in the seed extracts of *Artocarpus odoratissimus*.

2.5 Test for Flavonoids

Approximately 1 mL of extract and a few drops of 10% NaOH were added. Flavonoids were present because of the orange color.

2.6 Test for Tannins

20 mL of water was used to boil the extract before it was filtered. FeCl₃ was added in a few

drops to the sample. If the color appears greenish-brown or black-blue, tannin was said to react favorably.

2.7 Test for Phenolic

The extract is diluted with distilled water to a volume of 5 ml. Add a few drops of the neutral, 5% FeCl₃ solution afterward. The dark green color indicates the presence of phenolic compounds.

2.8 Test for Saponin

The extract was cooked in a water bath with 20 mL of water. After shaking, the extract was let to stand for 15 minutes. The development of a stable foam revealed positive samples with saponin content.

2.9 Test for Steroids

To 2 ml of extracts, 2 ml of acetic anhydride was added. To create a deeper layer, 2 ml of conc, H₂SO₄ was added. Changing from violet to blue, the color indicated the presence of steroids.

2.10 Test for Terpenoids

A small amount of conc. was added to a mixture of 5 ml of extract, 2 ml of chloroform, 2 ml of acetic anhydride, and a few drops. Added was H₂SO₄. Terpenoids were present, as evidenced by the reddish violet color.

2.11 Test for Alkaloids

After treating the 5 ml of extract with Dragondroff's reagent and 2 ml of diluted hydrochloric acid, an orange-brown precipitate that formed indicated the presence of alkaloids.

2.12 Test Microorganisms

Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) organisms were used in this study. The microorganism stock culture was obtained from San Pedro Hospital.

2.13 Disc Diffusion Method

A stock solution was prepared by dissolving 0.1 g of the extract with 100 mL of their respective solvents (distilled water and absolute ethanol) to produce a final 100 mg/mL concentration. The stock solution was diluted to 50 and 100 mg/mL of extract. 20 μ L of each dilution was impregnated into sterile, 6 mm diameter blank

discs. Distilled water and ethanol-loaded discs were negative controls for aqueous and ethanol extracts. Ampicillin for *E. coli* and oxacillin disc for *S. aureus* are positive controls. All discs were thoroughly dried before the application on the bacterial lawn. The susceptibility of the test organisms to *Artocarpus odoratissimus* seed extract was estimated by measuring the diameter of zone inhibition in millimeters. The values average three replicates (NARMS, C., 2008). Tables of the American Society for Microbiology, 2016 were used to obtain a standard qualitative report of sensitive (S), intermediate (I), or resistant (R).

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Profile of *Artocarpus odoratissimus* (Marang) Seed Extract

By utilizing methanol and ethanol extracts from the *Artocarpus odoratissimus* (Marang) seed, a

qualitative phytochemical screening process was used to detect the presence or absence of several secondary metabolites. As shown in Table 1, the Marang seed extract's rich phytochemical profile is suggested by flavonoids, tannins, polyphenols, saponins, steroids, terpenoids, and alkaloids.

The investigation findings are supported by literature indicating abundant secondary metabolite chemicals in *Artocarpus* plants [17]. Some *Artocarpus* species were reported to possess high concentrations of flavonoids, phenols, steroids, tannins, saponins, and triterpenoids [18]. For instance, the *Adoratissimus lacucha* plant, which is native to North Sumatra, Indonesia, and is a related species of *Artocarpus odoratissimus*, has secondary metabolites and bioactive chemicals that give it the ability to serve as an antidiarrheal, immunostimulant, anticholesterol, and hepatoprotective agent [19].

Table 1. Phytochemical constituents of *Artocarpus odoratissimus* seed extract

Phytochemicals Present	Methanol Extract	Ethanol Etract
Flavonoids	+	+
Tannins	+	+
Phenolic	+	+
Saponin	+	+
Steroids	+	+
Terpenoids	+	+
Alkaloids	+	+

(+) - indicates presence of secondary metabolites

(-) - indicates absence of secondary metabolites

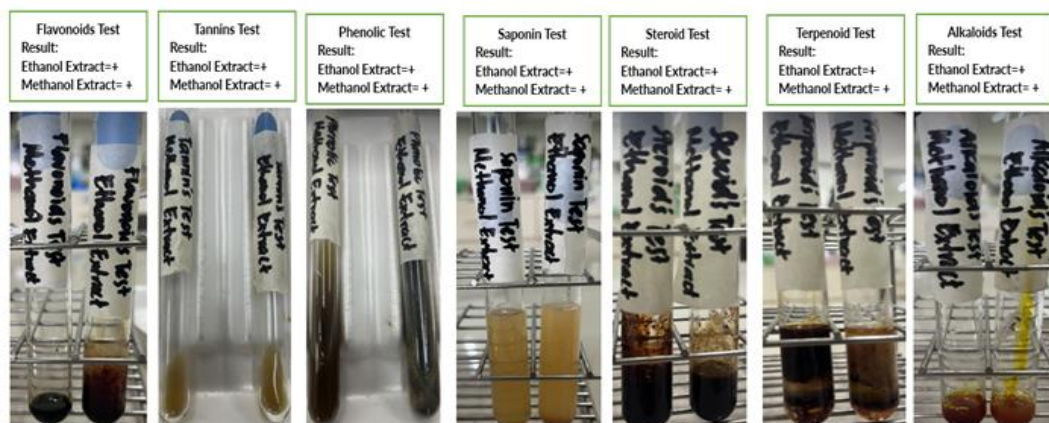


Fig. 1. Experimental Results of the Phytochemical constituents of *Artocarpus odoratissimus* seed extract

The phytochemical and antioxidant properties of the *Artocarpus odoratissimus* (Marang) fruit's seed and flesh were investigated [20]. The investigation results indicated that the Marang peel had a higher total phenolic content than the meat. Furthermore, the total flavonoid concentration of the Marang seed was higher than that of the flesh. Many microorganisms, including food-related diseases and clinically significant bacteria, fungi, and protozoa, can have their growth and activity inhibited by plant-derived phenolics, such as phenolic acids, flavonoids, and tannins [21]. The said species of *Artocarpus* is extensively utilized in traditional medicine in Indonesia due to its various pharmacological qualities in practically all plant portions. The review findings indicate that the fruit's peel, seeds, and flesh have antioxidant properties. The peel also possesses antidiabetic and anticancer properties due to its phenol and flavonoid content, which are known to suppress the action of the alpha-glucosidase enzyme. The seeds possess antimicrobial properties due to their flavonoid concentration [22].

Flavonoids are potent antioxidants detected in both extracts, play a crucial role in protecting cells from oxidative stress, and may contribute to the seed extract's potential health benefits. Many flavonoids have additionally demonstrated anti-infective properties by forming complexes with various proteins inside bacterial cell walls or extracellular proteins, eventually killing [23].

Tannins in the seed extract indicate the metabolite's astringent and antibacterial properties. Previous research has examined tannic acid's effects on various bacterial species, including Gram-positive (mostly *Staphylococcus aureus*) and Gram-negative (primarily *Escherichia coli*). The potential of tannins to penetrate bacterial cell walls and reach the interior membrane, along with their ability to disrupt cell metabolism and ultimately cause the cell's death, account for the antibacterial properties of these compounds [24]. Supporting those as mentioned earlier, a study using chestnut tannin on chicken performance discovered that 1,000 mg/kg of tannins reduced the number of harmful bacteria in the small intestine, including *Escherichia coli*. Furthermore, it has been demonstrated that tannins decrease the quantity of bacteria in the small intestine and colon of chickens [25].

The extract contains terpenoids, which have been shown to have a variety of biological

purposes have reported that these metabolites possess antibacterial solid activity, demonstrating bacteriostatic and bactericidal effects on various infections [26].

Alkaloids and polyphenols have demonstrated potent antibacterial action among the secondary metabolites examined [27]. Promising results have been obtained from research investigating the antibacterial mechanism of natural alkaloids derived from *Artocarpus* plants, indicating their potential efficacy in treating bacterial infections. Alkaloids, nitrogen-containing chemicals found in plants, have antibacterial properties by impeding the creation of bacterial cell walls, modifying the permeability of cell membranes, disturbing bacterial metabolism, and interfering with the synthesis of nucleic acids and proteins [28]. Research has demonstrated the antibacterial properties of *Artocarpus* extracts against *Methicillin-Resistant Staphylococcus aureus* (MRSA) strains, suggesting its effectiveness in fighting against bacteria resistant to treatment [29]. Furthermore, the presence of steroids and saponins in the seed extract promotes the antibacterial activity and mechanism of the *Artocarpus odoratissimus* (Marang) fruit.

3.2 Antimicrobial Activity of *Artocarpus odoratissimus* Seed Extract

The Zone of Inhibition (ZOI) was the basis for determining the bacteria's susceptibility and the antimicrobial potential of *A. odoratissimus* seed extract against soil microorganisms, particularly *Escherichia coli* and *Staphylococcus aureus*.

Table 2 showed the susceptibility of *Escherichia coli* to the various treatments impregnated in the sensitivity discs. The sensi disc impregnated with *Artocarpus odoratissimus* seed methanol and ethanol seed extracts produced an average of 10.33- and 9.33-mm zone of inhibitions against *E. coli*, indicating resistance. While the ampicillin sensitivity disc had the highest average zone of inhibition against the test organism (20.6 mm), it was interpreted as susceptible. The disc loaded with distilled water as the negative control has a 0 mm zone of inhibition and a resistant interpretation. The result is supported by the study that most *Escherichia coli* species are resistant [30]. *Escherichia coli* multidrug resistance is a concerning global issue due to the bacterial species' remarkable ability to acquire resistance genes, primarily through horizontal gene transfer [31].

Table 2. Susceptibility of *Escherichia coli*

Treatments Impregnated in the SensiDisk	Zone of inhibition (in millimeters)				
	Plate 1	Plate 2	Plate 3	Average	Interpretation
<i>A.odoratissimus</i> Methanol Seed Extract	10	12	9	10.33	Resistant
<i>A.odoratissimus</i> Methanol Seed Extract	10	8	10	9.33	Resistant
Ampicillin (Positive Control) 30ug	19	22	21	20.6	Susceptible
Distilled Water (Negative Control)	0	0	0	0	Resistant

Table 3. Susceptibility of *Staphylococcus aureus*

Treatments Impregnated in the SensiDisk	Zone of inhibition (in millimeters)				
	Plate 1	Plate 2	Plate 3	Average	Interpretation
<i>A.odoratissimus</i> Methanol Seed Extract	14	11	10	11.67	Resistant
<i>A.odoratissimus</i> Methanol Seed Extract	13	12	11	12	Resistant
Ampicillin (Positive Control) 30ug	14	17	15	15.33	Susceptible
Distilled Water (Negative Control)	0	0	0	0	Resistant

The susceptibility of *Staphylococcus aureus*, the test soil microorganism, to the various treatments impregnated in the sensitivity discs is shown in Table 2. Sensi discs containing *Artocarpus odoratissimus* seed methanol and ethanol extracts have an average zone of inhibition of 11.67- and 12 mm, respectively, indicating that the test microorganisms are resistant to the extract. The positive control, oxacillin sensi disc, demonstrated the most significant zone of inhibition, 15.13mm long, and is susceptible to *Staphylococcus aureus*. The disc with distilled water showed a 0 mm zone of inhibition, indicating that the test microorganisms resist the negative control.

Although the level of bacterial inhibition is low, the flavonoid content of the seed extract still contributes to its antimicrobial potential because the following mechanisms are well known for the effectiveness of flavonoids as antibacterial agents against a variety of pathogenic microorganisms: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and formation of biofilms [32].

4. CONCLUSION

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents known to exhibit medicinal and physiological activities. The presence of phytochemicals such as flavonoids, tannins, phenolics, saponins, steroids, terpenoids, and alkaloids was found through analysis of the plant extracts.

Based on the zone of inhibition, seed extracts from *Artocarpus odoratissimus* exhibited limited antibacterial activity against soil microorganisms, such as *Staphylococcus aureus* and *Escherichia coli*. Both soil bacteria, nevertheless, continue to be resistant to the extract. The impact can arise from secondary chemicals present in the extract. More research is required to assess the extract's effectiveness in various indications, including the presence of particular phytochemicals. Other researchers could use different test species to compare the antibacterial capabilities of the extract.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author hereby declares that artificial intelligence (AI) tools and technologies have been utilized in this study.

The following points outline the role and limitations of AI in this study:

1. Quilbot for Chrome – was utilized for grammar and plagiarism checking.
2. Gemini Apps- was utilized for expanding vocabulary.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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