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PHYTOCHEMICAL SCREENING OF *Artocarpus odoratissimus* (MARANG) SEED EXTRACT AND ITS ANTIMICROBIAL POTENTIAL AGAINST *Staphylococcus* *aureus* & *Escherichia coli*

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 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.

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Keywords: phytochemical screening, *Artocarpus odoratissimus*, antimicrobial potential, soil

14 **1. INTRODUCTION**

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

19 Two of the most widespread bacterial species in many soil ecosystems are *Escherichia coli*
20 and *Staphylococcus aureus*. These two bacterial species cause invasive health-related
21 infections in human populations [2]. *Escherichia coli* has been found in tropical and
22 subtropical soils [3], of which their survival rate is influenced by high and low temperatures
23 [4], limited moisture [5], and variation in soil texture [6]. On the other hand, *Staphylococcus*
24 *aureus* found in different land uses, and their concentrations are associated with varying
25 soil properties[7].

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

30 *Artocarpus odoratissimus*, a native fruit of Southeast Asia, including the Philippines, is one
31 potential antibacterial agent that may be useful in controlling *Staphylococcus aureus* and
32 *Escherichia coli* infections. In some areas, the fruit is known as marang or tarap. Its fruits
33 and leaves are antibacterial. A chemical profiling study on marang conducted in Malaysia
34 identified several phytochemicals that have been shown to support the potency of its
35 antibacterial properties [10]. According to a separate study, the genus *Artocarpus*, which
36 includes marang, contains secondary metabolites such as flavonoids, which have some
37 antibacterial activity [11].

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

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46 **2. MATERIAL AND METHODS**

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48 **Study Design.** This laboratory experiment evaluated the qualitative phytochemical
49 components and the antibacterial activity of the crude ethanolic and methanolic extracts of
50 *Artocarpus odoratissimus* against a selection of soil bacteria. The microbiology laboratory at
51 San Pedro Hospital is where the test bacteria were obtained. At San Pedro College, the
52 study was conducted in the science laboratories.

53 **Ethanol Extraction of Plant Extract.** 85 grams of powdered dried seeds were used to
54 make the extracts, which were then macerated with 500 mL of 95% ethanol in an
55 Erlenmeyer container, covered with aluminum foil, and allowed to settle for 4 days while
56 being stirred intermittently. The filtrate (1) and residue were then produced by filtering the
57 mixture through paper. The residue was then macerated once more (remacerated) with 250
58 mL of 95% ethanol, and the erlenmeyer was then covered with aluminum foil and left for 2
59 days while being stirred occasionally. The material underwent filtration to create filtrate after

60 two days (2). In order to obtain a thick extract, the filtrates (1) and (2) were combined and
61 evaporated using a rotary evaporator [12].

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

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

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

72 Test for Tannins. 20 mL of water was used to boil the extract before it was filtered. **FeCl₃**
73 was added in a few drops to the sample. If the color appears greenish-brown or black-blue,
74 tannin was said to react favorably.

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

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

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

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

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

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

93 **Disc diffusion method.** A stock solution was prepared by dissolving 0.1 g of the extract
94 with 100 mL of their respective solvents (distilled water and absolute ethanol) to produce a
95 final 100 mg/mL concentration. The stock solution was diluted to 50 and 100 mg/mL of
96 extract. 20 μ L of each dilution was impregnated into sterile, 6 mm diameter blank discs.
97 Distilled water and ethanol-loaded discs were negative controls for aqueous and ethanol
98 extracts. Ampicillin for *E. coli* and oxacillin disc for *S. aureus* are positive controls. All discs
99 were thoroughly dried before the application on the bacterial lawn. The susceptibility of the
100 test organisms to *Artocarpus odoratissimus* seed extract was estimated by measuring the
101 diameter of zone inhibition in millimeters. The values average three replicates (NARMS, C.,

102 2008). Tables of the American Society for Microbiology, 2016 were used to obtain a
103 standard qualitative report of sensitive (S), intermediate (I), or resistant (R).

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105 **3. RESULTS AND DISCUSSION**
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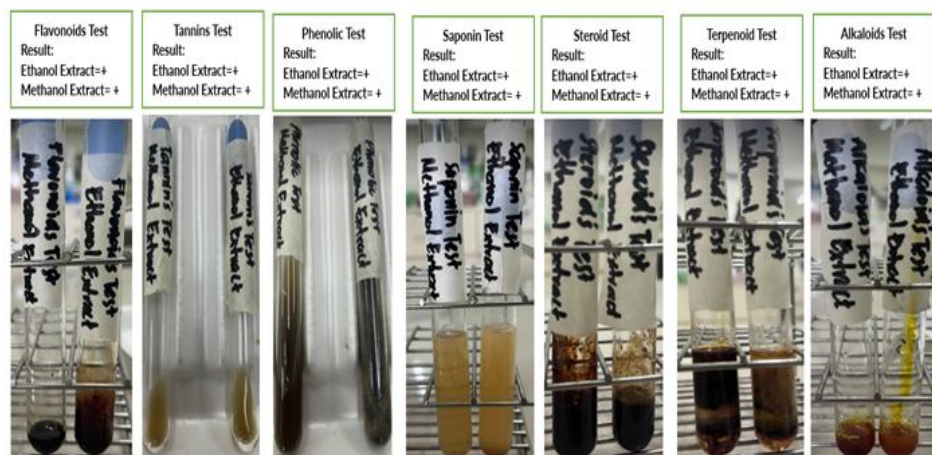
107 **Qualitative Phytochemical Profile of *Artocarpus odoratissimus* (Marang) seed extract.**

108 By utilizing methanol and ethanol extracts from the *Artocarpus odoratissimus*
109 (Marang) seed, a qualitative phytochemical screening process was used to detect the
110 presence or absence of several secondary metabolites. As shown in Table 1, the Marang
111 seed extract's rich phytochemical profile is suggested by flavonoids, tannins, polyphenols,
112 saponins, steroids, terpenoids, and alkaloids.

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

(+) - indicates presence of secondary metabolites
(-) - indicates absence of secondary metabolites

113
114 Table 1. Phytochemical constituents of *Artocarpus odoratissimus* seed extract
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117 Figure 1. Experimental Results of the Phytochemical constituents of *Artocarpus*
118 *odoratissimus* seed extract
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120 The investigation findings are supported by literature indicating abundant secondary
121 metabolite chemicals in *Artocarpus* plants [17]. Some *Artocarpus* species were reported to
122 possess high concentrations of flavonoids, phenols, steroids, tannins, saponins, and
123 triterpenoids [18]. For instance, the *Adoratissimuslacucha* plant, which is native to North
124 Sumatra, Indonesia, and is a related species of *Artocarpus odoratissimus*, has secondary

125 metabolites and bioactive chemicals that give it the ability to serve as an antidiarrheal,
126 immunostimulant, anticholesterol, and hepatoprotective agent[31].

127 The phytochemical and antioxidant properties of the *Artocarpus odoratissimus*
128 (Marang) fruit's seed and flesh were investigated [19]. The investigation results indicated
129 that the Marang peel had a higher total phenolic content than the meat. Furthermore, the
130 total flavonoid concentration of the Marang seed was higher than that of the flesh. Many
131 microorganisms, including food-related diseases and clinically significant bacteria, fungi, and
132 protozoa, can have their growth and activity inhibited by plant-derived phenolics, such as
133 phenolic acids, flavonoids, and tannins [20]. The said species of *Artocarpus* is extensively
134 utilized in traditional medicine in Indonesia due to its various pharmacological qualities in
135 practically all plant portions. The review findings indicate that the fruit's peel, seeds, and flesh
136 have antioxidant properties. The peel also possesses antidiabetic and anticancer properties
137 due to its phenol and flavonoid content, which are known to suppress the action of the alpha-
138 glucosidase enzyme. The seeds possess antimicrobial properties due to their flavonoid
139 concentration[32].

140 Flavonoids are potent antioxidants detected in both extracts, play a crucial role in
141 protecting cells from oxidative stress, and may contribute to the seed extract's potential
142 health benefits. Many flavonoids have additionally demonstrated anti-infective properties by
143 forming complexes with various proteins inside bacterial cell walls or extracellular proteins,
144 eventually killing [21].

145 Tannins in the seed extract indicate the metabolite's astringent and antibacterial
146 properties. Previous research has examined tannic acid's effects on various bacterial
147 species, including Gram-positive (mostly *Staphylococcus aureus*) and Gram-negative
148 (primarily *Escherichia coli*). The potential of tannins to penetrate bacterial cell walls and
149 reach the interior membrane, along with their ability to disrupt cell metabolism and ultimately
150 cause the cell's death, account for the antibacterial properties of these compounds [22].
151 Supporting those as mentioned earlier, a study using chestnut tannin on chicken
152 performance discovered that 1,000 mg/kg of tannins reduced the number of harmful bacteria
153 in the small intestine, including *Escherichia coli*. Furthermore, it has been demonstrated that
154 tannins decrease the quantity of bacteria in the small intestine and colon of chickens [23].

155 The extract contains terpenoids, which have been shown to have a variety of
156 biological purposes have reported that these metabolites possess antibacterial solid activity,
157 demonstrating bacteriostatic and bactericidal effects on various infections [24].

158 Alkaloids and polyphenols have demonstrated potent antibacterial action among the
159 secondary metabolites examined [25]. Promising results have been obtained from research
160 investigating the antibacterial mechanism of natural alkaloids derived from *Artocarpus*
161 plants, indicating their potential efficacy in treating bacterial infections. Alkaloids, nitrogen-
162 containing chemicals found in plants, have antibacterial properties by impeding the creation
163 of bacterial cell walls, modifying the permeability of cell membranes, disturbing bacterial
164 metabolism, and interfering with the synthesis of nucleic acids and proteins [26]. Research
165 has demonstrated the antibacterial properties of *Artocarpus* extracts against *Methicillin-*
166 *Resistant Staphylococcus aureus* (MRSA) strains, suggesting its effectiveness in fighting
167 against bacteria resistant to treatment [27]. Furthermore, the presence of steroids and
168 saponins in the seed extract promotes the antibacterial activity and mechanism of the
169 *Artocarpus odoratissimus* (Marang) fruit.

170 **Antimicrobial Activity of *Artocarpus odoratissimus* seed extract.**

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

174 Table 1 showed the susceptibility of *Escherichia coli* to the various treatments
 175 impregnated in the sensitivity discs. The sensi disc impregnated with *Artocarpus*
 176 *odoratissimus* seed methanol and ethanol seed extracts produced an average of 10.33- and
 177 9.33-mm zone of inhibitions against *E. coli*, indicating resistance. While the ampicillin
 178 sensitivity disc had the highest average zone of inhibition against the test organism (20.6
 179 mm), it was interpreted as susceptible. The disc loaded with distilled water as the negative
 180 control has a 0 mm zone of inhibition and a resistant interpretation. The result is supported
 181 by the study that most *Escherichia coli* species are resistant [28]. *Escherichia coli* multidrug
 182 resistance is a concerning global issue due to the bacterial species' remarkable ability to
 183 acquire resistance genes, primarily through horizontal gene transfer [29].

Treatments impregnated in the SensiDisk	Zone of Inhibition (in millimeters)				
	Plate1	Plate2	Plate3	Average	Interpretation
A.odoratissimus methanol seed extract	10	12	9	10.33	Resistant
A.odoratissimus ethanol 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

184
 185 Table 2. Susceptibility of *Escherichia coli*
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187 The susceptibility of *Staphylococcus aureus*, the test soil microorganism, to the
 188 various treatments impregnated in the sensitivity discs is shown in Table 2. Sensi discs
 189 containing *Artocarpus odoratissimus* seed methanol and ethanol extracts have an average
 190 zone of inhibition of 11.67- and 12 mm, respectively, indicating that the test microorganisms
 191 are resistant to the extract. The positive control, oxacillin sensi disc, demonstrated the most
 192 significant zone of inhibition, 15.13mm long, and is susceptible to *Staphylococcus aureus*.
 193 The disc with distilled water showed a 0 mm zone of inhibition, indicating that the test
 194 microorganisms resist the negative control.

195 Although the level of bacterial inhibition is low, the flavonoid content of the seed
 196 extract still contributes to its antimicrobial potential because the following mechanisms are
 197 well known for the effectiveness of flavonoids as antibacterial agents against a variety of
 198 pathogenic microorganisms: inhibition of nucleic acid synthesis, inhibition of cytoplasmic
 199 membrane function, inhibition of energy metabolism, inhibition of the attachment and
 200 formation of biofilms [30].

Treatments impregnated in the SensiDisk	Zone of Inhibition (in millimeters)				Interpretation
	Plate1	Plate2	Plate3	Average	
A.odoratissimus methanol seed extract	14	11	10	11.67	Resistant
A.odoratissimus ethanol seed extract	13	12	11	12	Resistant
Oxacillin (Positive Control) 30ug	14	17	15	15.33	Susceptible
Distilled Water (Negative Control)	0	0	0	0	Resistant

Table 3. Susceptibility of *Staphylococcus aureus*

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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.

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228 **COMPETING INTERESTS**

229

230 The authors affirm that there are no conflicts of interest about the publication of this paper
231 entitled "Phytochemical Screening of *Artocarpus odoratissimus* (Marang) Seed Extract and
232 Its Antimicrobial Potential Against Selected Soil Bacteria."

233 We do not have any financial or personal affiliations with individuals or organizations that
234 could exert undue influence on our work. There is no monetary benefit for any author
235 involved in the publication of this research.

236

237 **AUTHORS' CONTRIBUTIONS**

238

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

241

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243 Disclaimer (Artificial intelligence)

244 Option 1:

245 Author(s) hereby declare that NO generative AI technologies such as Large Language
246 Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during
247 writing or editing of manuscripts.

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249 Option 2:

250 Author(s) hereby declare that generative AI technologies such as Large Language Models,
251 etc have been used during writing or editing of manuscripts. This explanation will include list
252 the name, version, model, and source of the generative AI technology and as well as the all
253 input prompts provided to a generative AI technology

254

255 Details of the AI usage are given below:

256 1.

257 2.

258 3.

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