INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 1, p. 443-448, 2017

RESEARCH PAPER OPEN ACCESS

Antibacterial property of *Atuna racemosa* Rafin. Chrysobalanaceae shell and kernel extracts (Aqueous, Methanol, Ethyl Acetate, and Decoction)

Jinebeth S. Pacaña[1], Van Ryan Kristopher R. Galarpe*[2]

[1]*Chemistry Department, University of Science and Technology of Southern Philippines, Cagayan de Oro, Philippines*

[2]*Department of Environmental Science & Technology, University of Science and Technology of Southern Philippines, Cagayan de Oro, Philippines*

Key words: *Atuna racemosa*, Antibacterial, Extracts

http://dx.doi.org/10.12692/ijb/11.1.443-448 Article published on July 31, 2017

Abstract

This research evaluated the antibacterial potential of the aqueous, ethyl acetate, methanol, and decocted extracts of the shell and kernel of *Atuna racemosa* Rafin. Chrysobalanaceae (tabon-tabon). The antimicrobial screening was done against *Escherichia coli* and *Staphylococcus aureus* by paper disc diffusion method. The *A. racemosa* shell and kernel showed resistant to intermediate antimicrobial activity against *E. coli* and *S. aureus* in aqueous extracts with mean zone of inhibition of 7.7 mm and 9.8 mm, ethyl acetate extracts with 9.2 mm and 12.8 mm, methanol extracts with 9.5 mm and 13.2 mm, and decoction extracts with 7.3 mm and 11.0 mm, respectively. Ethyl acetate extracts with the highest antibacterial activity against *E. coli* obtained minimum inhibitory concentration values of 0.11375 mg/mL in shell and 2.92 mg/mL in kernel for both bacterial strains. Methanol extracts with the highest antibacterial activity against *S. aureus* obtained minimum inhibitory concentration values of 0.81375 mg/mL in shell for both test organisms, and 8.57 mg/mL for *E. coli* and 2.138 mg/mL for *S. aureus* in kernel. Overall, the ethyl acetate and methanol extracts of *A. racemosa* kernel showed good antibacterial potential against bacterial strains. Further investigation is needed to determine the bioactive components present in these extracts.

* **Corresponding Author:** Van Ryan Kristopher R. Galarpe ✉ vanryangalarpe@gmail.com

Introduction

Studies on Philippines indigenous and other local plants phytochemical profile, toxicological property, and antimicrobial potency had grown research interest recently (Peteros and Uy, 2010; Penecilla and Magno, 2011; Valle *et al*., 2015; Uy and Garcia, 2015; Uy and Villazorda, 2015; Latayada and Uy, 2016). These local plants may provide therapeutic efficacy against common illnesses like diarrhea, fever, and others. The use of plant based medicine may help indigenous communities cutting the cost of medical intervention in urban areas. Although much research is required in this field, few studies cited antibacterial property of these local plants.

Common plants studied in the Philippines showing antibacterial property were guava,Indian mango, watercress, moringa, wild tea, lemon, orange, garlic, and onion (Penecilla and Magno, 2011). In Northern Mindanao, Philippines phytochemical profiles and antioxidant activity of selected indigenous vegetables leaves and stalks were studied (Baang *et al*., 2015). Another local study focused on the antioxidative capacities and phytochemicals of selected fruit peels (Palmes and Del Rosario, 2012) and herbal vines (Licayan *et al*., 2016). In light of the present literature no published study locally on *Atuna racemosa* Rafin. Chrysobalanaceae extracts antibacterial property was recorded. This is considered important owing to the plants common use as spices and is widely used throughout the Pacific region.

One of the locally grown plants is *A. racemosa* Rafin. Chrysobalanaceae commonly used as spice and herb in cooking. The main use is of the cotyledons to extract ant anti-inflammatory massage oil and a putty to caulk boats in Samoa (Prance, 2004). Typically the seed is being used for cooking and the thick shell is commonly thrown. Studies elsewhere on *A. racemosa* had shown its pharmacological application (Buenz, 2006; Buenz *et al*., 2007a; Buenz, 2007b). Distinctively only the study of Buenz *et al*. (2007c) identified *A. racemosa* kernel ethanolic extract to have antibacterial potential against *Staphylococcus aureus*. Although other studies on the Chrysobalanaceae family were investigated for its antimicrobial property (Feitosa *et al*., 2012; Silva *et al*, 2012) no specific literature investigated the antibacterial potential of both shell and kernel of *A. racemosa* multiple extracts.

Overall, it can be inferred that locally grown *A. racemosa* may have relatively comparable pharmacological applications especially against specific bacterial strains. To extrapolate a more detailed observation this study was conducted on *A. racemosa* kernel and shell antibacterial potential. Its efficacy was determined from four extracts namely, aqueous, methanol, ethyl acetate, and decoction against two strains of bacteria.

Materials and methods

Sample Preparation

The fruit samples were washed thoroughly with running water, cut lengthwise into quarters, and simply get the seed through spoon. The shell and kernel were washed thoroughly, cut into pieces and dried at room temperature for five days with proper air ventilation. It was pulverized to fine powder using a laboratory scale mill or blender.

Preparation of Extracts

Decocted Extracts.

In a one liter beaker, 50g of sample was placed and added with 800 mL distilled water. The mixture was bought to boiling for 30 minutes. The decocted extract was filtered and stored in the refrigerator at 4^{o}C until use.

Aqueous Extracts.

A one is to five (1:5) ratio of the weight of the sample to the volume of the extracting solvent was used in the extraction. About 100 g *A. racemosa* shell and kernel were soaked in 500 mL distilled water at room temperature for 48 h. The *A. racemosa* shell and kernel aqueous extracts were filtered and concentrated in rotary evaporator at 95^{o}C. Then, it was stored in the refrigerator at 4^{o}C until use.

Ethyl acetate Extracts.

About 100 g *A. racemosa* shell/kernel were soaked in 500 mL ethyl acetate at room temperature for 48 h.

The *A. racemosa* shell/kernel ethyl acetate extracts were filtered and concentrated in rotary evaporator at 70°C. Then, it was stored in the refrigerator at 4°C until use.

Methanolic Extracts.

About 100 g *A. racemosa* shell/kernel were soaked in 500mL methanol at room temperature for 48 h. The *A. racemosa* shell/kernel methanolic extracts were filtered and concentrated in rotary evaporator at 55°C. Then, it was stored in the refrigerator at 4°C until use.

Antimicrobial Screening

Preparation of Nutrient Agar.

A 28 g of nutrient agar was dissolved in 1L of distilled water and homogenized. Then the solution was sterilized using an autoclave at 15 psi for 35 minutes. Afterwards the solution was allowed to cool and poured into petri dishes. The petri dishes were labeled with numbers for indications of each filter paper disc.

Disc Diffusion Test.

An inoculation loop containing microbial colonies was introduced into the test tube containing the broth and then incubated. Afterwards, another inoculating loop was dipped in the broth and then streaking was done to the culture media. Filter paper discs that were dipped in the different extracts were placed over the medium spread evenly. Then the petri dishes were covered with sterilized papers and were incubated for 24h. The zone of inhibition was examined using a ruler.

Minimum Inhibitory Concentration

A 28g of nutrient broth were added and mixed in one liter of boiled distilled water. About 5mL of this broth was transferred to a series of test tubes, which were sterilized and stored. The *A. racemosa* shell and kernel extracts with the highest susceptibility activity for each bacterium were used for the minimum inhibitory concentration (MIC) test. The extracts were diluted with the broth at different percent volume concentrations. The diluted extracts were added with microorganisms to identify its minimum inhibitory concentration. The last extract-broth ratio solution that did not demonstrate microbial growth was identified as the minimum inhibitory concentration.

The equation below shows how to calculate the MIC values of extracts. Table 1 served as reference for the interpretation of inhibition zone.

Table 1. Interpretation of Zone of Inhibition of Test Cultures.

Evaluation	Diameter of Zone of Inhibition (mm)
Resistant	10 or less
Intermediate	11 to 15
Susceptible	16 or more

Data Analysis

Descriptive as well as inferential statistics were used in analyzing the data obtained from the antimicrobial activity of *A. racemosa* extracts. Two-way ANOVA was used to determine if there was interaction between the different parts, solvents, and methods used for extraction.

Results and discussion

Antibacterial activity of A. racemosa

Table 2 presents the antibacterial potential of the *A. racemosa* shell and kernel extracts against *E. coli* and *S. aureus*, respectively. The ethyl acetate extract seed showed the highest antibacterial potential against *E. coli* with the zone of inhibition ranging from 11 to 14mm and a mean zone of inhibition of 13mm. The ethyl acetate extracts were more effective against *E. coli* because of the presence of cationic peptides and hydrophobic antibacterial agents (Lim, 2013). The cationic peptides interact on the outer membrane of *E. coli*, a gram negative bacterium which has a negative surface charge.

Likewise, the methanol extract of *A. racemosa* kernel showed the highest antibacterial activity against *S. aureus* with the zone of inhibition ranging from 11 to 15mm and mean zone of inhibition of 13.7mm (see Table 2). The observed ranges of antibacterial activity of methanol extract against *S. aureus* can be explained by the presence of various groups of potentially active classes of secondary metabolites. The present findings corroborated with the past study of Buenz *et al.* (2007c) on *A. racemosa* kernel ethanolic extract to have antibacterial potential against *S. aureus*. Extrapolating from this, it can be inferred that alcoholic extracts of *A. racemosa* kernel to have antibacterial potential against *S. aureus*.

On the other hand both the kernel of decocted and aqueous extracts showed antibacterial potential against the tested bacteria. Greater MIC was observed on the kernel of both extracts against *S. aureus* (Table 2). This finding may similarly be beneficial to indigenous communities in which common form of extract preparations either decocting or soaking (aqueous).

Table 2. Antibacterial Activity of *A. racemosa* Shell and Kernel Extracts against *E. coli* and *S. aureus*.

	Mean Zone of Inhibition (mm) ± SD	
Samples	*E. coli*	*S. aureus*
AQUEOUS EXTRACT		
Chloramphenicol	27.7 ± 1.15	27.0 ± 1.00
Distilled Water	0.00 ± 0.00	0.00 ± 0.00
A. racemosa Shell	7.7 ± 1.15	7.7 ± 0.58
A. racemosa Kernel	9.3 ± 1.53	10.3 ± 0.58
ETHYL ACETATE EXTRACT		
Chloramphenicol	27.7 ± 0.58	30.7 ± 1.53
Distilled Water	0.00 ± 0.00	0.00 ± 0.00
Ethyl acetate	7.7 ± 1.15	7.3 ± 0.53
A. racemosa Shell	10.0 ± 1.00	8.3 ± 0.58
A. racemosa Kernel	13.0 ± 1.73	12.7 ± 1.15
METHANOL EXTRACT		
Chloramphenicol	26.7 ± 0.58	30.7 ± 1.53
Distilled Water	0.00 ± 0.00	0.00 ± 0.00
Methanol	7.3 ± 0.58	7.3 ± 0.58
A. racemosa Shell	9.3 ± 0.58	9.7 ± 1.15
A. racemosa Kernel	12.7 ± 1.15	13.7 ± 2.31
DECOCTION EXTRACT		
Chloramphenicol	26.0 ± 1.00	25.7 ± 0.58
Distilled Water	0.00 ± 0.00	0.00 ± 0.00
A. racemosa Shell	7.3 ± 0.58	7.3 ± 0.58
A. racemosa Kernel	12.0 ± 3.00	10.0 ± 1.00

Two-way ANOVA for Antibacterial Activity: plant parts vs. solvents

A two-way ANOVA statistical test was employed to determine whether the antimicrobial activity of the crude extracts was dependent on the solvent used for extraction, and the parts of the *A. racemosa*. Table 3 presents the F-calculated for solvents which were 9.964 for *E. coli* and 7.14815 for *S. aureus*. These F-calculated values were greater than the F-critical value of 3.885. Further, the F-calculated for the types of extracted parts of *A. racemosa* were 20.571 for *E. coli* and 40.3333 *S. aureus*.

The F-calculated values were greater than the F-critical value of 4.747. Consequently both the type of solvent and parts of *A. racemosa* extracts used against *E. coli* and *S. aureus* showed significant difference. Overall, the statistical tests confirm the antibacterial potential of the kernel ethyl acetate extract than other studied extracts.

Table 3. Two-way ANOVA against *E. coli* and *S. aureus* Using Different Solvents and Parts at 95% Significance Level.

Studied parameter	F-calculated	P-value	F-critical	Decision
Against *E. coli*				
Parts	20.571	0.000683	4.747	Significant
Solvents	9.964	0.002818	3.885	Significant
Against S. aureus				
Parts	40.3333	3.66 E -05	4.747	Significant
Solvents	7.14185	0.009031	3.885	Significant

Two-way ANOVA for Antibacterial Activity: parts vs. extraction method

On the other hand Table 4 presents the two-way ANOVA of the antibacterial potential against *E. coli and S. aureus* using the different methods of extraction. Results showed that the F-critical (5.318) for the methods used of extraction was greater than the F-calculated values (1.2564 and 0.67). Thus, there is no significant difference in the antibacterial activities of the *A. racemosa* shell and kernel between the soaking and decoction methods used for extraction for *E. coli* and *S. aureus*. However, there was a significant difference on the antibacterial activities between the parts used against *E. coli* and *S. aureus* where F-calculated (9.2564 and 42.667) was greater than F critical (5.318). Overall, this explains the antibacterial potential of the kernel better than the shell of *A. racemosa*.

Table 4. Two-way ANOVA against *E. coli* and *S. aureus* Using Different Methods at 95% Significance Level.

Studied parameter	F-calculated	P-value	F-critical	Decision
Against *E. coli*				
Parts	9.2564	0.016	5.318	Significant
Methods	1.2564	0.2948	5.318	Not significant
Against S. aureus				
Parts	42.667	0.00018	5.318	Significant
Methods	0.67	0.43785	5.318	Not significant

Minimum Inhibitory Concentration of A. racemosa

The *A. racemosa* shell and kernel extracts with the highest zone of inhibition for each bacterial strain were subjected to MIC tests shown on Table 5 and 6. The methanol extracts of *A. racemosa* shell inhibit the growth of *E. coli* and *S. aureus* until the 6.25% volume concentration of extract (Table 5).

Overall, the methanol extracts of *A. racemosa* kernel inhibit the growth of *E. coli* until 6.25% ratio volume concentration or 8.57 mg/mL and *S. aureus* until 1.60% volume concentration or 2.138mg/mL.

Table 5. MIC Analysis. Methanol Extracts of *A. racemosa* Shell and Seed Against *E. coli* and *S. aureus.*

Percent Volume	*E. coli*		*S. aureus*	
Concentration	Shell	Kernel	Shell	Kernel
50.00	-	-	-	-
25.00	-	-	-	-
12.50	-	-	-	-
6.25	-	-	-	-
3.12	+	+	+	-
1.60	+	+	+	-
0.80	+	+	+	+

Legend: with bacterial growth = +; without bacterial growth = -

On the other hand, the *E. coli* and *S. aureus* were inhibited by the *A. racemosa* ethyl acetate shell extracts until 6.25% volume concentration or MIC value of 0.11375mg/mL (see Table 6). Further, the growth of bacterial strains by the ethyl acetate extracts of *A. racemosa* kernel were inhibited until 1.60% volume concentration or MIC value of 2.92mg/mL.

Table 5. MIC Analysis. Ethyl Acetate Extracts of *A. racemosa* Shell and Seed Against *E. coli* and *S. aureus.*

Percent Volume	*E. coli*		*S. aureus*	
Concentration	Shell	Seed	Shell	Seed
50.00	-	-	-	-
25.00	-	-	-	-
12.50	-	-	-	-
6.25	-	-	-	-
3.12	+	-	+	-
1.60	+	-	+	-
0.80	+	+	+	+

Legend: with bacterial growth = +; without bacterial growth = -

The ethyl acetate and methanol extracts of *A. racemosa* kernel had intermediate effects against *E. coli* and *S. aureus* based on the interpretation of zone of inhibition (Table 5 and 6).

The decocted extract of *A. racemosa* kernel also showed intermediate effect against *E. coli* but only resistant to *S. aureus*. Overall, the *A. racemosa* shell extracts and aqueous kernel extract showed resistant effect against *E. coli* and *S. aureus*.

Conclusion

Antibacterial potential of *A. racemosa* extracts was selective against the test bacteria. The ethyl acetate kernel extract was more potent against *E. coli* (inhibition zone = 13mm). Likewise, the methanol kernel extract was more potent against *S. aureus* (inhibition zone = 13.7 mm). Both aqueous kernel extract of *A. racemosa* was potent against *S. aureus* while decocted kernel extract of *A. racemosa* was more potent against *E. coli.* Overall the kernel ethyl acetate and methanol extracts of *A. racemosa* were selectively potent against the tested bacteria. Present findings may be preliminary and further testing using other strains of bacteria and *A. racemosa* parts may be essential.

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