

Antimicrobial and Phytochemical Properties of *Atuna racemosa* Raf. Kernel Extract

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Received: 22-01-2019

Accepted: 09-07-2019

DOI: 10.18805/IJARE.A-415

ABSTRACT

Atuna racemosa Raf., a forest tree used to season raw fish dish in the Philippines, is proven to have antibacterial properties. The study aimed to help elucidate the significance of using its kernel as a condiment and consequently, increase its value by assessing its antimicrobial and phytochemical properties. The antimicrobial activity of its ethanolic extracts were screened against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* ser. Typhimurium, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* using agar disc diffusion technique. Qualitative phytochemical screening was done and total phenolic content was determined at a concentration of 100 mg dry sample per 1 mL solvent. The extract showed bacterial growth inhibition in *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* while a mild antifungal activity against *Aspergillus niger* was observed. Phytochemical analysis detected the presence of cardiac glycosides, tannins, phenolic compounds and saponins in the extract. It yielded a total phenolic content of 42.7 mg GAE/100 g. This denotes that the kernel of *A. racemosa* has the potential to be classified as a natural food preservative and functional food.

Key words: *Atuna racemosa*, Antibacterial, Antifungal, Phenolic content, Phytochemicals.

INTRODUCTION

Atuna racemosa Raf. is a tree belonging to the family Chrysobalanaceae that is known for its anti-inflammatory properties (Andersson-Dunstan *et al.*, 1997; Perez, 2001; Prance, 2004). Native to parts of the Malesian region (PROSEA, 2016), its kernel is distinctively used as a condiment for raw fish only in Mindanao, Philippines (Prance, 2004). Its use as a condiment dates back to about 1,000 years before present as archeological findings in the form of fishbones in association with halves of *A. racemosa* were excavated in Butuan City, Philippines (Davidson, 2014). Locals use the kernel to eliminate the fishy taste and prevent stomach upsets (Davidson, 2014); however, its use as a food additive is claimed to be attributed more to its antiseptic properties rather than its taste (See, 2017). This was supported by the observed antibacterial property of *A. racemosa* ethanolic extracts against *Staphylococcus aureus* (Buenz *et al.*, 2007). Pacaña and Galarpe (2017b) verified the research and extended the findings to *Escherichia coli* across different extracting solvents. Moreover, methanolic extracts of the kernel have been found to have several phytochemicals, e.g. alkaloids, terpenoids, anthraquinone, flavonoids, saponins, coumarins and tannins (Pacaña and Galarpe, 2017a).

Aside from the toxicity studies conducted (Buenz, 2007; Pacaña and Galarpe, 2017a), no reports on the total phenolic content and antifungal properties of *A. racemosa*

seed extracts were made. Furthermore, earlier studies did not use ethanol as extracting solvent for phytochemical analysis and no broad-spectrum antibacterial screening was conducted. Hence, the study screened the antimicrobial and phytochemical properties of *A. racemosa* kernel ethanolic extracts to help elucidate the significance of using kernel as a condiment and, consequently, increase its value.

MATERIALS AND METHODS

Solvent extraction: *A. racemosa* kernels were extracted based on the modified procedure of Buenz *et al.* (2006). Six hundred grams of *A. racemosa* seeds were macerated and soaked in 1000-mL 70% ethanol for one week at ambient conditions. The supernatant was filtered and its solvent was evaporated under a forced-convection oven at 40°C forming reddish-brown crystalline solids. The dried extracts were reconstituted with 70% ethanol to produce a solution concentration of 100 mg dry sample per 1 mL solvent. The reconstituted extracts were used for antimicrobial assays. Whereas, the solid extracts were used for the phytochemical analysis and total phenolic content determination.

Anti-bacterial screening: The test organisms used in this study are *Bacillus cereus* (BIOTECH 1509), *Pseudomonas aeruginosa* (BIOTECH 1335), *Escherichia coli* (BIOTECH 1634), *Staphylococcus aureus* (BIOTECH 1582) and *Salmonella enterica* ser. Typhimurium (BIOTECH 1826) from the Philippine National Collection of Microorganisms, BIOTECH, University of the Philippines Los Baños. At two

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replicates, preliminary antimicrobial testing was conducted using the agar well method following the Kirby Bauer Method using 200 ppm streptomycin as positive control and 70% ethanol as negative control. Briefly, the test organisms were grown on Tryptic Soy Agar (TSA) for 24 hours and were resuspended in 0.85% NaCl and adjusted to 0.1 MacFarland Standard. With a sterile cotton swab, the suspension was then uniformly swabbed into pre-poured TSA plates containing 20 ml of the medium which were then allowed to completely dry for 10-15 minutes in the laminar flow hood. 8 mm wells were then made using a sterile blue tip. Finally, 100 μ L of the sample was placed on these wells and the plates were then incubated at 37°C for 24 hours and the zone of inhibition was measured.

Anti-fungal screening: The extract was examined for antifungal properties based on the recommendation of *in-vitro* biological reactivity test in the U.S. Pharmacopeia (USP 30-NF 25, 2007) at two replicates. *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* were inoculated in a TS Broth, incubated for 72 hours and adjusted for turbidity that is comparable to 0.5 McFarland standard. Two-hundred microliters of fungal suspension was added in a petri dish containing 20 mL of congealed Potato Dextrose Agar. The plates were then incubated for an hour prior to the addition of the treatments. Ten-millimeter filter paper discs were dipped in the treatments and subsequently placed over the assay plates. Then, the Petri dishes were incubated for 24 hours at 25°C and the zone of inhibition was measured. Clotrimazole and nystatin were used as positive control and 70% ethanol was used as negative control.

Phytochemical screening: Phytochemicals were qualitatively screened using the slightly modified procedures of Harborne (1980), Edeoga *et al.* (2005) and Onwukaeme *et al.* (2007). The presence of tannins was identified through the formation of blue-black or brownish-green precipitate upon dropwise addition of 15% FeCl₃ solution to the plant extract in aqueous solution. Saponins were detected through the occurrence of frothing after the aqueous plant extract was boiled, cooled and vigorously shaken. In determining terpenoids, two milliliters of CHCl₃ was added to the dried extract and then layered with H₂SO₄. A reddish-brown interface indicates presence of terpenoids. Flavonoids were detected by the formation of a pinkish to red-orange solution

upon the addition of magnesium ribbon in the HCl-acidified ethanolic plant extract. Presence of phenolic compounds was shown through the formation of blue-black precipitates with the dropwise addition of 1% FeCl₃ to the aqueous plant extract. Similarly, emergence of blue-black precipitates upon addition of Wagener's reagent to the aqueous plant extract was used as indicator for presence of alkaloids. On the other hand, a Killani-Keller test was performed to detect the presence of cardiac glycosides.

Total phenolic content determination: The total phenolic content was determined using the modified protocol of Ainsworth *et al.* (2007). Supernatant of extracts in methanolic solution was diluted with de-ionized water, neutralized with sodium carbonate solution and mixed with Folin-Ciocalteu phenol reagent. The mixture was incubated at room temperature and the absorbance at 765 nm was determined. A standard curve was made by plotting the absorbance against the concentration of gallic acid, the standard. The slope of regression line and, subsequently, concentration of phenols in the sample were calculated. The total phenolic content was expressed as mg/100 g gallic acid equivalent (GAE) of dry extract.

Statistical analysis: Analysis of variance (ANOVA) and Scheffe's test at 5% level of significance were conducted to determine significant differences between treatments using SAS ver. 9.0.

RESULTS AND DISCUSSION

Anti-bacterial activity: The extract showed bacterial growth inhibition in three out of five test strains (Table 1). The reactivity of the kernel extract to *Pseudomonas aeruginosa* and *Bacillus cereus* is first reported in this paper. Inhibitory activities were present in both gram-positive and gram-negative bacteria, indicating a potential broad-spectrum antibacterial activity. The inhibition zones found on *S. aureus* were observed to be the highest and are comparable to the positive control; whereas, no zone of inhibition was observed in *Escherichia coli* and *Salmonella enterica* ser. Typhimurium. The results agree with the microbial activity against *S. aureus* observed by Buenz *et al.* (2007); however, they contradict the results of Pacaña and Galarpe (2017) that showed activity against *E. coli*. Reactivity of the extract against the test strains of common foodborne bacteria revalidates the antiseptic property of *A. racemosa*. The

Table 1: Antibacterial activity of *A. racemosa* kernel ethanolic extract.

| Species | Zone of inhibition (mm) | | |
|---|-------------------------|--------------------|-------------------|
| | Kernel extract | Streptomycin | 70% Ethanol |
| <i>Bacillus cereus</i> | 15.50 ^b | 27.50 ^a | 0.00 ^c |
| <i>Pseudomonas aeruginosa</i> | 18.33 ^b | 23.50 ^a | 0.00 ^c |
| <i>Escherichia coli</i> | 0.00 ^b | 26.25 ^a | 0.00 ^b |
| <i>Staphylococcus aureus</i> | 28.92 ^a | 30.25 ^a | 0.00 ^b |
| <i>Salmonella enterica</i> ser. Typhimurium | 0.00 ^b | 28.75 ^a | 0.00 ^b |

^{a,b,c} difference in letters within rows indicates significant difference between treatments at 0.05 level of significance.

Table 2: Antifungal activity of *A. racemosa* kernel ethanolic extract.

| Sample/ Control | <i>Aspergillus niger</i> | | | <i>Saccharomyces cerevisiae</i> | | | <i>Candida albicans</i> | | |
|---------------------------|-------------------------------|------------|------------------------|---------------------------------|------------|------------------------|-------------------------------|------------|------------------------|
| | Zone of inhibition (mm) | Reactivity | Inhibitory Activity | Zone of inhibition (mm) | Reactivity | Inhibitory Activity | Zone of inhibition (mm) | Reactivity | Inhibitory Activity |
| Kernel extract | 10.00 | Mild | +++ | 0.00 | None | - | 0.00 | None | - |
| Clotrimazole ¹ | 16.83 | Moderate | +++ | N/A | N/A | N/A | 19.05 | Moderate | +++ |
| Nystatin ¹ | N/A | N/A | N/A | 19.61 | None | +++ | N/A | N/A | N/A |
| 70% Ethanol ² | 0.00 | None | - | 0.00 | None | - | 0.00 | None | - |

Inhibitory activity rating: (+++) complete, (++) partial, (+) slight; (-) negative.

¹Positive control; ²Negative Control.

Table 3: Phytochemical profile of *A. racemosa* kernel ethanolic extract.

| Phytochemicals | Reactivity |
|--------------------|------------|
| Tannins | ++ |
| Saponins | + |
| Flavonoids | - |
| Terpenoids | - |
| Cardiac glycosides | + |
| Phenolic compounds | ++ |
| Alkaloids | - |

(++): Present at high content

(+): Presence

(-): Absence

potential broad-spectrum activity of the extract may prevent growth of bacteria associated to consumption of raw fish. As an example, the extracts may inhibit contamination of *B. cereus* that is reported to infect raw tuna fish (Doménech-Sánchez *et al.*, 2011).

Anti-fungal activity: *A. racemosa* extracts exhibited mild reactivity against *Aspergillus niger*; however, they showed no inhibitory activity against *Saccharomyces cerevisiae* and *Candida albicans* (Table 2). In contrast to the positive control which has an extended zone of inhibition around the specimen, the inhibition zones of the kernel extract were limited under the disc (Fig 1). *Aspergillus* species, which are common food contaminants, have been identified to cause common fungal diseases of freshwater and marine animals, including fishes and prawns, in the Philippines (Leaño, 2001; Santacroce *et al.*, 2008; Chauanan *et al.*, 2014). Hence, the

mild antifungal property of *A. racemosa* could be potentially beneficial in its role as a condiment, preventing fungal contamination and spoilage. The kernel must also be screened against fungi and bacteria isolated directly from fishes eaten raw.

Phytochemicals: The presence of cardiac glycosides from the seed is first reported in this paper. Relatively high contents of phenolic compounds and tannins were detected in the extract; however, contrary to the initial phytochemical screening of the methanolic extracts (Pacaña and Galarpe, 2017a), flavonoids, terpenoids and alkaloids were absent (Table 3). Although methanol is highly toxic compared to ethanol, larger variety of compounds are extracted by methanol (Eloff, 1998), potentially causing the absence of the three phytochemicals in the ethanol extract. The presence and abundance of these phytochemicals will contribute to the development of *A. racemosa* as functional food that contains compounds with health benefits (Premier, 2002). As an example, tannins, a relatively abundant group of phytochemicals in *A. racemosa*, have antioxidant, antimicrobial cardioprotective, anti-cancer and anti-diabetic properties (Smeriglio *et al.*, 2017).

Total phenolic content: A total phenolic content of 42.70 mg GAE/100 g was observed in the *A. racemosa* kernel extract. The results are consistent with the high abundance of phenolic compounds in the qualitative phytochemical screening. Phenolic content is generally linked with high antioxidant capacity that prevents infectious and

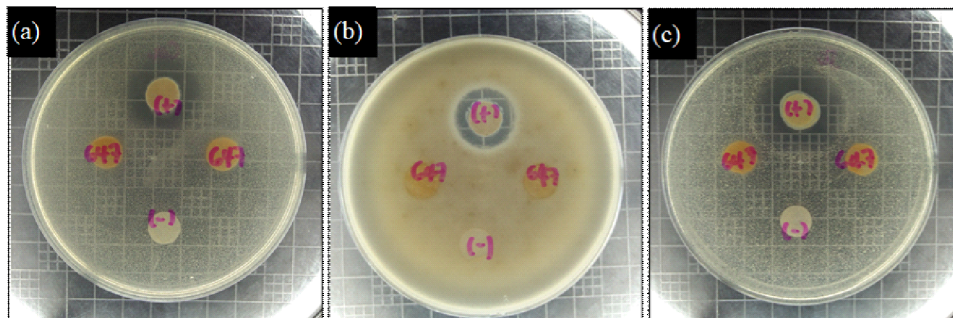


Fig 1: Growth inhibition of (a) *C. albicans*, (b) *A. niger* and (c) *S. cerevisiae* caused by *A. racemosa* kernel extract; (+) and (-) indicates positive and negative control, respectively.

degenerative diseases, inflammation and allergies (Ozcan *et al.*, 2014), increasing the value of *A. racemosa* as a condiment.

CONCLUSION

Atuna racemosa Raf. ethanolic extracts have antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. They also exhibits mild antifungal activity against *Aspergillus niger*. These extracts also contain cardiac glycosides, tannins,

phenolic compounds and saponins with a total phenolic content of 42.7 mg GAE/100 g. Thus, kernels of *A. racemosa* have the potential to be classified as a natural food preservative and functional food.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Science and Technology- Philippine Council for Health Research and Development (DOST-PCHRD) for their support.

REFERENCES

- Ainsworth, E.A. and Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using FolinCiocalteu reagent. *Nature Protocols*. **2**: 875-877.
- Andersson-Dunstan, C., Noreen, Y., Serrano, G., Cox, P.A., Perera, P. and Bohlin, L. (1997). Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosyntheses and rat ear oedema assays. *Journal of Ethnopharmacology*. **57**: 35-56.
- Buenz, E.J. (2007). Mitochondrial involvement in *Atuna racemosa* induced toxicity. *Journal of Ethnopharmacology*. **109**: 304-311.
- Buenz, E.J., Bauer, B.A., Johnson, H.E., Beekman, E.M., Frank, K.L. and Howe, C.L. (2006). Searching historical herbal texts for potential new drugs. *British Medical Journal*. **333**: 1314-1315.
- Buenz, E.J., Tillner, J.E., Limburg, P. and Bauer, B.A. (2007). Antibacterial properties and toxicity of *Atuna racemosa* extract depend on kernel maturity. *Journal of Ethnopharmacology*. **111**: 592-597.
- Chauhan, R., Lone, S.A. and Beigh, A. H. (2014). Pathogenicity of three species of aspergillus (*A. fumigatus*, *A. niger* and *A. sydowii*) on some fresh water fishes. *Life Science Leaflets*. **48**: 65-72.
- Davidson, A. (2014). The Oxford Companion to Food. OUP Oxford, UK, pp. 445-446.
- Doménech-Sánchez, A., Laso, E., Pérez, M.J. and Berrocal C. (2011). Emetic disease caused by *Bacillus cereus* after consumption of tuna fish in a beach club. *Foodborne Pathogens and Disease*. **8**: 835- 837.
- Edeoga, H.O., Okwu, D.E. and Mbaebie B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. **4**: 685-688.
- Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants?. *Journal of Ethnopharmacology*. **60**: 1-8.
- Harborne, J.B. (1980). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London.
- Leaño, E. M. (2001). Fungal diseases. Health management in aquaculture. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines, pp. 43-53.
- Onwukaeme, D.N., Ikuegbwweha, T.B. and Asonye, C.C. (2007). Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of *Pycnanthus angolensis* Weld. Warb. (*Myristicaceae*) on corneal ulcers in rabbits. *Tropical Journal Pharmaceutical Research*. **6**: 725-730.
- Ozcan, T., Akpınar-Bayazit, A., Yılmaz-Ersan, L. and Delikanlı, B. (2014). Phenolics in Human Health. *International Journal of Chemical Engineering and Applications*. **5**: 393-396
- Pacaña, J.M. and Galarpe, V.R.K.R. (2017a). Phytochemical screening and toxicity testing of *Atuna racemosa* Rafin. Chrysobalanaceae shell and seed extracts. *International Journal of Advanced and Applied Sciences*. **4**: 110-115.
- Pacaña, J.M. and Galarpe, V.R.K.R. (2017b). Antibacterial property of *Atuna racemosa* Rafin. Chrysobalanaceae shell and kernel extracts (Aqueous, Methanol, Ethyl Acetate and Decoction). *International Journal of Biosciences*. **11**: 443-448.
- Perez, R.M.G. (2001). Anti-inflammatory activity of compounds isolated from plants. *The Scientific World*. **1**: 713-784.
- Prance G.T. (2004). The uses of *Atuna racemosa* Raf. (*Chrysobalanaceae*) in Samoa. *Economic Botany*. **58**: 470-475.
- Premier, R. (2002). Phytochemical composition: A paradigm shift for food health considerations. *Asia Pacific Journal of Clinical Nutrition*. **11**: 197-201.
- PROSEA. (2016). PlantUse: *Atuna racemosa*. <https://uses.plantnet-project.org/en/Atuna> (PROSEA).
- Santacroce, P.M., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G. and Crescenzo, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Rev Fish Biol Fisheries*. **18**: 99-130.
- See, S.B.O. (2017). Sustainability, inaugural LTB Icon Awards highlight Madrid Fusion Manila. GMA News Online. <http://www.gmanetwork.com/news/lifestyle/food/606170/sustainability-inaugural-ltb-icon-awards-highlight-madrid-fusion-manila/story/>
- Smeriglio, A., Barreca, D., Bellocco, E. and Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal Pharmacology*. **174**: 1244-1262.
- U.S. Pharmacopoeia 30-National Formulary 25. (2007). <87> Biological reactivity Tests, *In vitro*.