Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

QUALITATIVE ASSESSMENT OF THE ANTIMICROBIAL, ANTIOXIDANT, AND PHYTOCHEMICAL PROPERTIES OF THE ETHANOLIC EXTRACTS OF THE INNER BARK OF *Atuna racemosa*

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ARTICLE INFO

Received:
03th November 2018
Received in revised form:
10th Feb 2019
Accepted:
18th Feb 2019
Available online:
28th Feb 2019

Keywords: Phytochemical, antioxidant, anthraquinones, antimicrobial, DPPH

ABSTRACT

Atuna racemosa is a fruit-bearing tree popular in Asia and Polynesia for its medicinal uses. Traditionally, the inner bark of the plant is used to treat hypertension and severe abdominal pain. This study was conducted to determine the phytochemical components, antimicrobial properties and antioxidant properties in the ethanolic extract of the inner bark of the plant. Phytochemical screening reveals the presence of flavonoids, saponins, tannins and alkaloids, and the absence of anthraquinones and cyanogenic-glycosides. Through the DPPH assay, the inner bark extract showed antioxidant potential. The antibacterial assay showed antibacterial activity against *Bacillus subtilis* UPCC129, *Staphylococcus aureus* UPCC1142, *Escherichia coli* UPCC1195, *Klebsiella pneumoniae* UPCC1360, *Salmonella typhimurium* UPCC1368, and *Pseudomonas aeruginosa* UPCC1244. The biochemical components in the plant extract determined by GCMS analysis and identified by comparison with the reference standard found in the NIST library showed the compounds have antimicrobial, antioxidant and other biological properties. These results enhance the medicinal value of the plant as a potential source for pharmaceutical drugs and therapeutics.

To Cite This Article: Jericho Nadayag, Mark Lloyd G. Dapar, Agnes T. Aranas, Roland Anthony R. Mindo, Clint Kenny Cabrido, Muhmin Michael E. Manting et al., (2019), "Qualitative Assessment of the Antimicrobial, Antioxidant, and Phytochemical Properties of the Ethanolic Extracts of the Inner Bark of *Atuna racemosa*", ***Pharmacophore, 10(1),*** *52-59.*

Introduction

Medicinal plants played a major role in different cultures for therapeutic purposes. The Ayurvedic system, Unani system, and Chinese system were among the oldest systems which use plants for medicinal purposes [1]. While conventional drugs serve as effective medicines and therapeutics, poor people who do not have the capability of accessing these drugs, prefer using natural remedies provided by traditional medicine for treating health problems [2]. Currently, over 80% of the world's population rely on plant-derived medicines for their primary health care needs [3].

In traditional medicine, plant species of the family Chrysobalanaceae have been used for various purposes [4]. Some of these plants are said to be hypoglycemic, anti-inflammatory, anti-diarrhea, dysentery, and for the treatment of malaria [5]. One of these is the *Atuna racemosa* tree. This species is used by many indigenous peoples across the Asian-Pacific region for medicinal purposes. It is commonly located on the islands of the Pacific including Malaysia, Borneo, Philippines, Solomon Islands, Fiji, and Samoa [6]. Locally named in the Philippines as tabon-tabon, the fruit kernel is generally utilized as a spice for a raw fish dish called "kinilaw." The medicinal value of *A. racemosa* is in the utilization of the different parts of the plant. In Samoa, the cotyledon of the fruit is made into putty for caulking boats, and teas are prepared from the inner bark to treat severe abdominal pains [7-9]. The fruit kernels are processed into massage oils, while the leaves are used to treat

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swellings and inflammation [8]. The roots are used to treat infection of the soles of the feet. The inner bark is also popular in the Pacific region where the liquid of the inner bark is used as a treatment for hypertension [10]. In this study, we evaluated whether the ethanolic extract from the inner bark of *A. racemosa* has antimicrobial and antioxidant properties. We also look into the presence of phytochemicals in the extract and qualitatively assess the functional properties of these compounds identified by GCMS.

Methodology

The inner barks of *A. racemosa* were collected from the branches and ground into powder. Five hundred grams (500.0 grams) of powdered *A. racemosa* inner bark was soaked in 1.5 L of absolute ethanol for three weeks with stirring. The supernatant was filtered using Whatman filter paper No. 1. The filtrate was concentrated using a rotary evaporator at 45 °C. The crude extract was collected and allowed to completely air-dry at room temperature. The obtained crude extract was used for antimicrobial and antioxidant tests, phytochemical screening and GC-MS analysis.
The antimicrobial assay was done using the agar well diffusion method with the selected test microorganisms from the Microbiological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman. (Fig. 1) The microbial suspensions of gram-negative bacteria were *Escherichia coli* UPCC 1195, *Klebsiella pneumoniae* UPCC 1360, *Pseudomonas aeruginosa* UPCC 1244 and *Salmonella typhimurium* UPCC 1368 while the gram-positive bacteria were *Bacillus subtilis* UPCC 1295 and *Staphylococcus aureus* UPCC 1143.

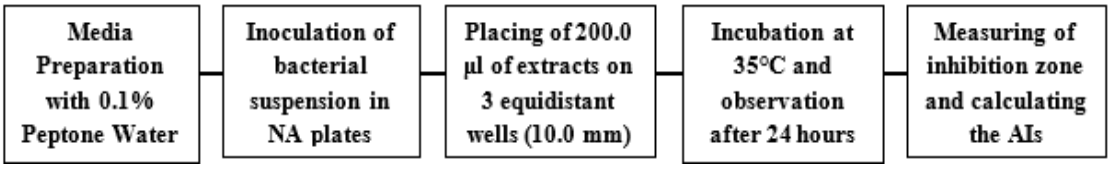

Figure 1. Schematic diagram of the antimicrobial assay using agar well diffusion method.

The phytochemical screening of the ethanolic extract is summarized in Fig. 2. The screening was carried out using the protocol described according to the Laboratory Manual for the UNESCO Sponsored Workshop on the Phytochemical, Microbiological, and Pharmacological Screening of Medicinal Plants [11] at the Department of Chemistry, MSU-IIT. A 3-point scale (+ turbid, ++ moderate and +++ heavy) in scoring was based on the Handbook of Philippine Medicinal Plants [12]. The Free Radical Scavenging Activity (DPPH Assay) protocol was adapted from Jacinto et al. [13], a modification of the procedure from Hou et al. [14]. GCMS analysis was conducted at the Chemistry Analytical Services Laboratory of the Ateneo de Davao University following the modified protocol of Chipiti et al. [15]. With modifications to identify the compounds present in the ethanolic extract of *A. racemosa* to facilitate, identify and quantify several different metabolites present in a plant extract which results in comprehensive coverage of primary metabolic pathways [16].

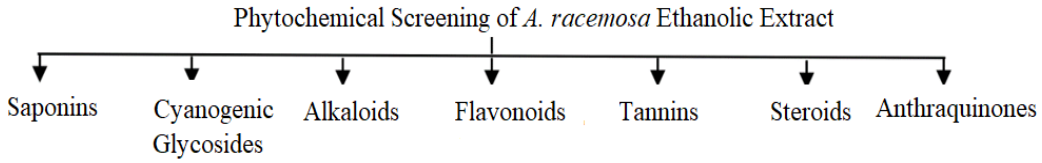

Figure. 2. Schematic diagram of the phytochemical screening of *A.racemosa*

Results and Discussion

The antibacterial properties of the ethanolic extract of *A. racemosa* inner bark against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *K. Pneumonia*, *S. aureus*, and *B. subtilis* are shown in Table 1. The inner bark ethanolic extract showed inhibitory activities to all bacterial isolates. The extract was most effective against all gram-positive bacteria mainly *B. subtilis* and *S.aureus.* The inhibitory activity of the inner bark extract against gram-positive *S. aureus* was similar to the antibacterial property of the fruit kernel extract [17-19]. Among the gram-negative bacteria, the highest inhibition was observed against *K. pneumoniae*. The antibacterial activity shown by the ethanolic extract is similar to the results generated from the methanolic extract of the stem of a related species of Chrysobalanceae, the *Parinari curatellifolia,* where inhibitory activities were also observed against these three bacterial strains [20]. These results indicate a commonality of antibacterial properties among the species under Chrysobalanceae. It is possible that both species have similar properties in the inhibition of proteins, nucleic acid, and membrane phospholipid biosynthesis of bacterial cells [21].
The antibacterial potential by the inner bark ethanolic extract of *A. racemosa* against the gastrointestinal disease-causing bacteria *E.coli* [22], *P. aeruginosa* [23], *S. typhimurium*, *B. subtilis* and *S. aureus* [24] may explain the use of the inner bark as a remedy for abdominal pain in the lower intestine by the Samoans [7].

Table 1. Antimicrobial screening of *A. racemosa* inner bark ethanolic extract against different bacterial strains with chloramphenicol as a negative control.

Test Organism	*Sample*	Clearing Zone			AI
		1	2	3	
E. coli	*A. racemosa*inner bark extract	15	16	16	0.6
	Chloramphenicol disc	27			3.5
P. aeruginosa	*A. racemosa*inner bark extract	16	16	16	0.6
	Chloramphenicol disc	15			1.5
S. typhimurium	*A. racemosa*inner bark extract	16	16	16	0.6
	Chloramphenicol disc	30			4
K. pneumoniae	*A. racemosa*inner bark extract	14	14	14	0.7
	Chloramphenicol disc	38			5.3
S. aureus	*A. racemosa*inner bark extract	20	21	21	1.1
	Chloramphenicol disc	33			4.5
B. subtilis	*A. racemosa*inner bark extract	21	22	22	1.2
	Chloramphenicol disc	20			2.3

Phytochemical Screening

The phytochemicals detected in the inner bark ethanolic extract of *A. racemosa* (Table 2) show the presence of abundant flavonoids, saponins, tannins, and steroids, but fewer alkaloids. Cyanogenic-glycosides and anthraquinones were not detected.

Table 2. Phytochemicals identified in the inner bark ethanolic extract of *A. racemosa* [no presence (-), in small quantities (+), in moderate quantities (++), in large quantities (+++)]

Extract	***Flavonoids***	***Alkaloids***	***Cyanogenic-glycosides***	***Anthraquinones***	***Saponins***	***Tannins***	***Steroids***
A. racemosa	+++	+	-	-	+++	+++	+++

Studies have shown that flavonoids are known to possess biological activities such as antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, antidepressant, and antimicrobial activities [19]. They also exhibit cardioprotective, anti-diabetic and anti-aging properties [25]. Saponins are known to have hypocholesterolemic, anti-coagulant, anticarcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory and antioxidant activities [26]. Saponins also exhibit antibacterial activity by causing leakage of proteins and certain enzymes from the bacterial cell [27]. Tannins from bark extracts revealed to have antidiarrhoeic, antiulcer, antileprotic, antigoitrogenic, antitumor, analgesic, and anti-inflammatory activities [28]. They also exhibit antibacterial activity by inhibiting cell wall synthesis through the formation of irreversible complexes with proline-rich proteins. Steroids are known to have antibacterial, anti-tumor, hepatoprotective, anti-inflammatory, antifungal, antidiarrhoeic, cardiotonic and immunosuppressive activities [29]. The presence of these phytochemicals and their high concentrations in the inner bark ethanolic extract of *A. racemosa* may explain the traditional remedy of the inner bark as an anti-inflammatory and antibacterial remedy for severe abdominal pain [9]. Alkaloids have been reported to exert analgesic, antibacterial and antispasmodic activities [19]. Although in small concentrations, the presence of these phytochemical groups in the inner bark ethanolic extract of *A. racemosa* contributes to the effectivity of the extract as a remedy especially it's antibacterial and analgesic properties which also correlates with the use of the inner bark extract as a remedy to severe abdominal pain. Cyanogenic glycosides when consumed, release lethal hydrogen cyanide into the systematic circulation of the body [30] thus the absence of these compounds in the inner bark extract may indicate its safety for consumption since the traditional method of using the inner bark extract as a remedy is by oral consumption [9].

Other studies have also reported the presence of similar phytochemicals in the seeds and fruit shell of the plant; however, a great number of observed phytochemicals vary in every plant parts. These results indicate variations in chemical characteristics and polarities of compounds in different plant parts that are soluble in particular solvents [19].

Using the DPPH as a scavenging assay method, the IC_{50} values for the ethanolic extract and ascorbic acid were 33 ppm and 1.74 ppm, respectively (Fig. 3). While ascorbic acid is more effective as an antioxidant, the results show the extract has strong antioxidant properties. These could be due to the detected phytochemicals such as flavonoids and tannins (Table 2). These phenolics are known not only having pharmacological properties but as antioxidants [31, 32].

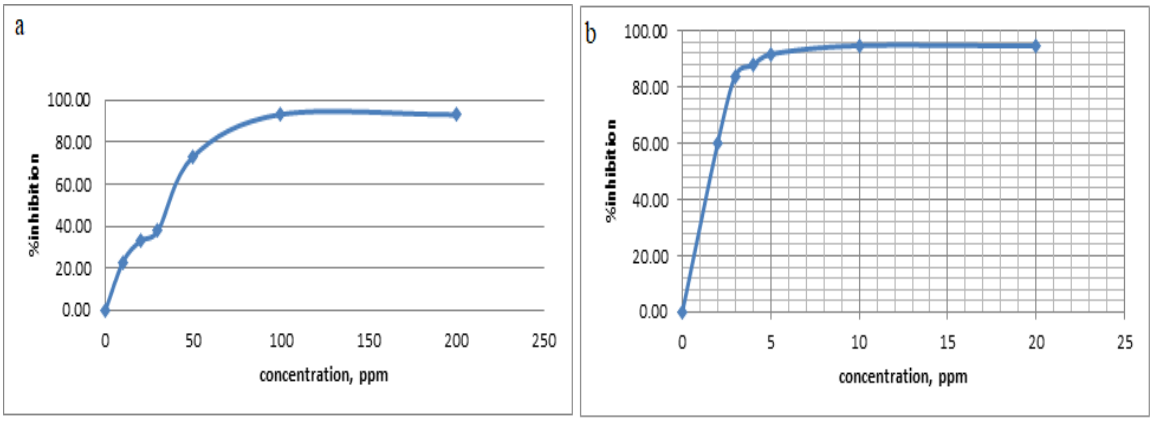

Figure 3. Antioxidant activity in different concentrations of *A. racemosa* inner bark extract (**a**) and ascorbic acid (**b**) measured by DPPH analysis method.

Table 3 and Figure 4 show the compounds from the extract of the inner bark of *A. racemosa* identified by direct comparison of the mass spectrum of analytes at particular retention times to the reference standard found in the National Institute of Standards and Technology (NIST) library. Twenty-five out of 26 compounds has a similarity index (SI) above 80% indicating that the similarities of the detected compounds are similar in molecular structure when compared to that of the reference standard found in NIST library [33, 34].

Table 3. Bioactive phytocompounds detected in the hexane extract of *A. racemosa* by GC-MS Analysis.

Compound	Name of Compound	SI	MW	Biological Properties
1	2- Ethylhexanol	97	130	Antifungal [35] Antimicrobial [35]
2	2,6-Di-tert-butyl-p-benzoquinone	82	220	Antioxidant [36] Antibacterial [37]
3	Heneicosane	93	296	Cytotoxic [38] Antibacterial [38, 39]
4	Pentadecane	97	212	Antibacterial [40, 41]
5	Phenol,2,4-bis(1,1-dimethylethyl)-	96	206	Antifungal [42] Antioxidant [43, 44] Anti-inflammatory, Antibacterial [45]
6	Heneicosane	95	296	Cytotoxic [38] Antibacterial[38, 39]
7	1-Pentadecene	92	210	Antibacterial [46, 47]
8	Hexadecane	97	226	Antioxidant, Antifungal, Antibacterial [35,47,48]
9	2,6,10-Trimethylpentadecane	93	254	Antioxidant [49]
10	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	95	234	Antioxidant[50, 51]
11	2,6,10,15-Tetramethylheptadecane	96	296	No reported activity
12	2,6,10,14-tetramethylhexadecane	95	282	No reported activity
13	2-Hexyl-1-decanol	93	242	No reported activity
14	Heptadecanoic acid, ethyl ester	91	298	Anticancer [52]
15	2,6,10,15-Tetramethylheptadecane	91	296	No reported activity
16	1-Iodohexadecane	89	352	Antifungal [53]
17	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	84	362	Anti-inflammatory, Antistress, Antitumor, Antioxidant [54]
18	Hexadecanoic acid, ethyl ester	95	284	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Hemolytic, Antiandrogenic, reductase inhibitor, Antibacterial, Antifungal [55]
19	Hexadecane	95	226	Antioxidant, Antifungal, Antibacterial [35,47,48]

20	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	74	276	Antioxidan[56]
21	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3.α,4a.β,6a.α,10a.β,10b.β)]-	87	290	No reported activity
22	Heptadecanoic acid, ethyl ester	92	298	Anticancer [52]
23	Phytol	94	296	Cytotoxic, Anti-neoceptive, Antioxidant [38] Anticancer, antimicrobial [55,57] Anti-inflammatory [38, 55,57] Diuretic, stimulant, Antimalarial, Antifungal [55] Antiasthmatic [58]
24	Linoleic acid ethyl ester	94	308	Hypocholesterolemic, Nematicidal, Antiarthriti, Hepatoprotective, Antiandrogenic, 5-alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne [59]
25	E,E,Z,-1,3,12-Nonadecatriene-5,14-diol	84	294	Antimicrobial [60-62]
26	Octadecanoic acid, ethyl ester	92	312	Antimicrobial [59]

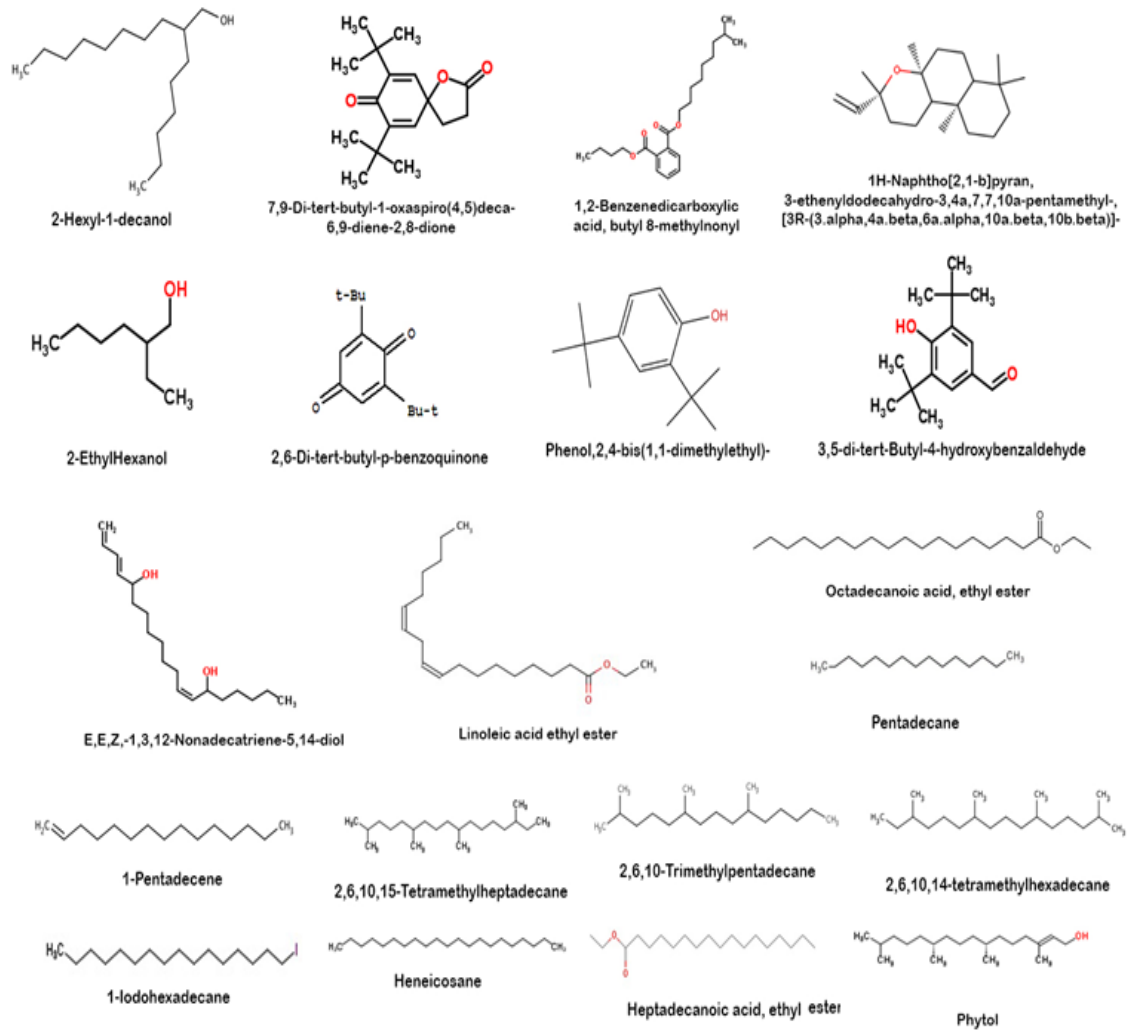

Figure 4. Chemical structure of biochemical compounds observed from the inner bark extract of *A. racemosa* through GCMS analysis.

The compounds isolated from the hexane extract of the inner bark and their studied biological properties particularly their antimicrobial and antioxidant properties can be argued to explain the use of the inner bark as a remedy to treat selected health issues [7, 9, 10]. The use of the inner bark of *A. racemosa* in ethnomedicine indicates its potential for its use in pharmaceutical medicine and therapeutics, especially as an antimicrobial agent and antioxidant.

Conclusion

The results of this study showed the antimicrobial and antioxidant potentials of the ethanolic extract of the inner bark of *A. racemosa.* The presence and qualitative identification of bioactive phytochemicals present and their biological properties enhance the medicinal value of the plant as a potential source for pharmaceutical drugs and therapeutics. The presence of those compounds with antibacterial, antioxidants, anticancer, and cytotoxic properties might have a great impact on the development of drugs that are not only effective but have therapeutic effects.

Acknowledgement

The authors would like to acknowledge the Premier Institute of Science and Mathematics (PRISM) of MSU-Iligan Institute of Technology, Chemistry Department of the College of Science and Mathematics, MSU-IIT, the Microbiological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman, and Chemistry Analytical and Research Laboratory of the Ateneo de Davao University in Davao City, Philippines for the assistance provided in the conduct of this study.

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