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Antibacterial activity of the lyophilized aqueous leaf extract of the Philippine green-leafed *Acalypha amentacea* Roxb. (Maslakot-Ambulong) against selected human bacterial pathogens

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ABSTRACT

Aims: The specific aim of this study was to evaluate for the first time the phytochemical constituents, functional group assignment, and antibacterial activities of the Philippine green-leafed *Acalypha amentacea* Roxb. (Maslakot-Ambulong), a wildcrafted medicinal plant of local traditional healers in the southern most region of Mindoro province.
Methodology and results: Aqueous leaf extracts of *A. amentacea* Roxb. were lyophilized and subjected to qualitative phytochemical screening and FT-IR analysis. The antibacterial activity of the plant using agar-well diffusion assay revealed highest Zone of Inhibition (ZOI) in 500 mg/mL concentration for *Staphylococcus aureus* (21.78 mm), *Escherichia coli,* (21.36 mm), *Serratia marcescens* (21.90 mm), *Klebsiella pneumoniae* (21.44 mm), and *Enterococcus faecalis* (20.52 mm) among other concentrations suggesting a dose dependent bioactivity. Also, compared to the antibiotic Rifampicin, *A. amentacea* Roxb. demonstrated better bioactivity against all the selected bacteria except *S. aureus* (p<0.05) and comparable to Ofloxacin when against *E. faecalis*. The minimum inhibitory concentration (MIC) of the extract was found to be at 15.6 mg/mL for all the bacteria except for *S. marcescens* with 31.25 mg/mL as MIC. The bioactivity of the plant may be accounted to the presence of alkaloid, phenol, flavonoid, tannin, and saponin which were supported by its functional groups like carboxylic acid, alcohols, amine, conjugated alkene, aromatic esters, and alkyl aryl ether.
Conclusion, significance and impact of study: The results of this investigation, proved that *A. amentacea* Roxb. has bioactive antibacterial principles against the selected microorganisms. This also confirms its potentiality as a new source of antibacterial agents.

Keywords: Acalypha amentacea Roxb., antibacterial, traditional medicine, functional groups, phytochemicals

INTRODUCTION

Multi-drug resistant strains of bacteria are becoming more prevalent globally, and their continuous evolution makes them less susceptible to various antibiotics. This phenomenon has resulted in the high specter of untreatable bacterial infections leading to an escalating number of morbidities among humans (Obeidat *et al.*, 2012).

Commonly, there are commercial and synthetic antibiotics that are being applied to address bacterial infections. However, these synthetic antibiotics are widely associated with unfavorable effects on the host, such as allergic reactions and hypersensitivity. Because of the upsurge in the common side effects caused by many synthetic antibiotics which contributed in the incidence of multidrug-resistant bacteria, scientists have put the spotlight on researches for various endemic plants as a source of novel antimicrobial agents (Pratap Gowd *et al.*, 2012). Nature has been the source of medicinal agents for thousands of years, and an impressive number of drugs have been isolated from natural sources wherein many of these derived on traditional medicines (Cragg and Newman, 2001). Based on several studies, medicinal plants, including those traditional and endemic plants, are used by almost 80% of the world's population for their basic and daily health care and living because of their availability.

There has been a change in thinking globally, with a growing tendency to "GO NATURAL" (Carounanidy *et al.*, 2007). The World Health Organization has estimated that 4 billion people are using herbal medicines for some

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aspect of primary health care (Gossell-Williams *et al.*, 2006). This change is because the produced plant-based medicines are natural products which are non-narcotic, bio-degradable, possess minimum environmental hazards, have less adverse effects, and are readily available and affordable (Kannan *et al.*, 2009).

Meanwhile, the province of Mindoro which is located in the northwest of the geographic center of the Philippines with a breadth of approximately 90 and 177 km respectively with total area covers 9,826.5 km^2, making this triangular land mass the seventh largest island in the archipelago (Kasberg, 1994) has a broader flora source particularly endemic traditional herbal medicine.

In Bulalacao Oriental Mindoro, local traditional "Tagalog," "Bisaya," and indigenous healers have popularly known to utilize various traditional medicinal plants and herbs as an immediate remedy to common illnesses. A total of 143 plants and two other natural products have been recorded and documented in the area (Sebastian *et al.*, 2013). However, a lot of these herbal plants have not yet formally and scientifically evaluated and examined for their pharmaceutical capacities to address specific disease and illness.

The green-leafed *A. amentacea* Roxb. has genus *Acalypha* from the family of *Euphorbiaceae*, a wildcrafted plant locally known as "Maslakot- Ambulong" is one of the many traditional medicinal plants used by older "Tagalog," "Bisaya," and by the indigenous "Hanunuo Mangyan*"* healers. The plant is a shrub, sometimes becoming more tree-like, usually growing around two meters tall. The plant could also have dark or bright red, red-green leaves, which are often mottled or variegated with various shades of red, dark pink, white, or bronzy green (Clarke and Thaman, 1993). The locals have known *A. ametacea* Roxb. as an immediate remedy for ringworms (Postma, 2005). Other than the information mentioned on the medicinal use of the plant, no other further investigation has been done for this species, particularly on its antimicrobial properties.

The genus *Acalypha* comprises of 570 species having *wilkesiana* as the most explored species in terms of antibacterial and antimicrobial potentials. In other countries, people also traditionally using *Acalypha* species except for *Amentacea*, for treatment and reported to possess antimicrobial properties (Seebaluck *et al.*, 2015).

This exploration for a new potential source of the plant-based antibacterial agent from *A. amentacea* Roxb. against selected pathogenic bacteria could aid the problem of antibacterial resistance and serve as a natural medicine to help the human body to fight against infectious diseases. It will also assist the increasing need for effective antibiotics that can manage complications present in vulnerable patients.

Thus, this study focused on the first evaluation of phytochemicals, functional groups, and antibacterial activity of the Philippine green-leafed *A. amentacea* Roxb. against selected bacterial pathogens. Specifically, the study has determined the zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) of the different treatment preparations of the lyophilized aqueous leaf extract of the plant against selected bacterial pathogens and compared them to antibiotic controls.

MATERIALS AND METHODS

Collection, preparation, and identification of the plant sample

Fresh leaves of the wildly crafted *A. amentacea* Roxb. were collected at *Sitio* Ambulong, San Roque, Bulalacao, Oriental Mindoro, Philippines. The collected leaves were washed thoroughly with distilled water to remove foreign dirt, dust, and other contaminants. The cleaned leaves were then put aside and stored in a cool and dry place until use. The leaf sample of the plant was sent to the National Institute of Biology in the University of the Philippines- Diliman for proper identification and authentication. The active cultures of the indicator bacterial strains were provided by the Center for Life Sciences Research of the Polytechnic University of the Philippines, a culture collection affiliate of the National Institute of Molecular Biology and Applied Microbiology (BIOTECH), University of the Philippines–Los Baños (UPLB), Laguna, Philippines. The bacterial strains used in the study with their respective accession numbers are reflected in Table 1.

Table 1: Selected strains of bacterial pathogens used with identified accession numbers.

Bacterial Strains	Accession Number
Staphylococcus aureus (+)	BIOTECH 1582
Escherichia coli (-)	BIOTECH1634
Serratia marcescens (-)	BIOTECH 1748
Klebsiella pneumoniae (-)	BIOTECH 1754
Enterococcus faecalis (+)	BIOTECH 10348

(+) Gram-positive bacterial strain, (-) Gram-negative bacterial strain

Aqueous extract preparation

Two (2) kg of *A. amentacea* Roxb. leaves were dried using laboratory oven at 65 °C for 4 h. The dehydrated leaves were cut and crushed into a fine powder using a blender and macerated with water with a ratio of 1:5 w/v wherein 300 *g* of the plant powder were mixed with 1500 mL of sterile distilled water in a container for 48 h. The homogenates were filtered using Whatman filter paper No. 2 and preserved aseptically in sealed bottles at 4 °C until further use (Premanath and Devi, 2011).

Lyophilization of the aqueous leaf extract

Lyophilization was done to prevent severe degradation of the plant chemicals and compounds that are responsible for their bioactivities (Chang *et al.*, 2006).

The aqueous leaf extract was placed in a 500 mL sterile screw-capped glass bottles (2/3 full) and fitted in

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the canisters of the lyophilizer. The extract of *A. amentacea* Roxb. was prepared and cleaned through initial flash freezing in liquid nitrogen before the actual lyophilization. The lyophilization process was set and run for 80 h. The lyophilized extract was sealed with parafilm to prevent water uptake and stored in cool and dry area. Freeze-drying of the extract was done at the National Chemistry Instrumentation Center–Department of Chemistry, School of Science and Engineering in Ateneo De Manila University.

Phytochemical analyses plant's lyophilized extract

The lyophilized aqueous leaf extract of *A. amentacea* Roxb. was subjected to qualitative phytochemical screening to detect biological constituents (Daffodil *et al.*, 2014), as summarized in Table 2. Presence or absence of phytochemicals in the extracts was determined by color reactions of the compounds with specific reagents/dyes.

Table 2: Qualitative phytochemical screening procedures.

Phyto-chemicals	Test	Reagent and Chemicals Used	Positive Result
Alkaloid	Mayer's Test	Hydrochloric acid, Mayer's reagent	White Precipitate
Flavonoid	Shindo's Test	Magnesium Turnings, Hydrochloric Acid	Red or orange-red color
Phenol	Ferric Chloride Test	Ferric Chloride	Bluish green or red color
Saponin	Foam Test	Water	Copious lather formation
Tannin	Lead Acetate Test	Lead Acetate	White Precipitate

FT-IR analysis

Ten (10) mg of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. was mixed with KBr salt using a mortar and pestle and compressed into a thin pellet. The pelleted extract was loaded in the Thermo Scientific Nicolet 6700 for analysis. Characteristic peaks and their functional groups were identified and recorded. The analysis was done at the De La Salle University-Chemistry Department, Manila Philippines.

Treatment preparations

The lyophilized aqueous leaf extract was removed from the sealed-glass container with a sterile spatula and lightly pounded with mortar and pestle to obtain the powdered form. Five (5) grams of the extract was dissolved in 10 mL distilled water (5000 mg/10 mL) and subjected to vortex mixer. From the initial concentration, 1000 µL was taken to obtain 500 mg/mL concentration and was serially diluted to obtain other treatment concentrations. The prepared treatments for the plant were: Treatment 1: 500 mg/mL; Treatment 2: 250 mg/mL; Treatment 3: 125 mg/mL; and Treatment 4: 62.5 mg/mL while Ofloxacin (5 mcg/disc) and Rifampicin (5 mcg/disc) antibiotic discs (positive) and distilled water (negative) were used as the controls.

Preparation and sterilization of media

All the culture media used in the experiment were obtained in dehydrated-powdered form and mixed with an appropriate volume of distilled water according to the manufacturers' instructions. The weighed quantity of each medium was dissolved in water in an Erlenmeyer flask and mixed thoroughly with a magnetic bar on a magnetic stirrer. Culture media were sterilized by autoclaving at 121 °C and 15 pounds per square inch for 15 min and then cooled at 45-50 °C before dispensing into sterile Petri dishes. The plates were allowed to solidify in a Biosafety cabinet (ESCO Streamline, Singapore). On the other hand, the glass materials used were also sterilized by autoclaving at 121 °C and 15 pounds per square for 15 min. After autoclaving, the glassware was brought out and allowed to cool down properly before use (Ugoh *et al.*, 2014).

Antibacterial assay

The antibacterial property of the plant extract was evaluated using agar well diffusion technique with some modifications (Ugoh *et al.*, 2014).

Eight (8) mm wells were bored into the solid Mueller-Hinton agar plates previously seeded with 24-h standardized cultures of pathogens adjusted to match 0.5 McFarland standard (nearly equal to 1.5 x10^8 CFU/mL) and spread onto the plate with sterile cotton applicator by the streak-plate method. Each well was carefully filled with 50 µL of each of the prepared treatments from the plant extract and the controls. The treatments were: T1=500 mg/mL; T2=250 mg/mL; T3=125 mg/mL; T4=62.5 mg/mL; Positive Controls (Ofloxacin and Rifampicin); and Negative Control (Distilled Water). The assay was performed in a biosafety cabinet Class 2 (ESCO Streamline, Singapore) to prevent potential contaminations.

The plates were allowed to stand undisturbed for one hour to allow proper absorption into the medium before the plates were incubated at 37 °C for 24 h in an incubator. The plates were observed for the zone of inhibition (ZOI). The effects of the extracts on the test organism were compared with that of standard antibiotics, Ofloxacin and Rifampicin as positive controls. All assays were done in triplicates and the results were expressed as mean±SD.

Minimum inhibitory concentration (MIC) of the extract against the bacterial strains

Minimum inhibitory concentrations (MIC) were based on the method of Silva *et al.* (2013) with some modifications. MICs were determined by broth microdilution method wherein 96-microwells were filled with 100 µL of Mueller-

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Hinton broth. From the stock solution of 500 mg/mL of the plant extract, 100 µL of the solution was obtained and serially diluted in the microwells using micropipette so that the final concentrations were 50 mg/100µL (500 mg/mL), 25 mg/100µL (250 mg/mL), 12.5 mg/100µL (125 mg/mL), 6.25 mg/100µL (62.5 mg/mL), 3.125 mg/100µL (31.25 mg/mL), 1.56 mg/100µL (15.6 mg/mL), 0.78 mg/100 µL (7.8 mg/mL), and 0.39 mg/100µL (3.9 mg/mL). All the treatment wells except for the negative control, were added with adjusted broth culture of the selected bacteria equivalent to .5 McFarland standards. The wells were incubated at 37 °C for 24 h. The lowest concentrations without visible growth or biofilm formation defined as the plant extract's MICs.

Data analysis

The zone of inhibition (ZOI) results were expressed as means (n=3) and analyzed for possible difference among the treatments and controls using Single-Factor ANOVA at 0.05 level of significance. Scheffe test was employed in determining where the differences occurred.

RESULTS

The result of the phytochemical screening revealed the following metabolites as present in the lyophilized aqueous leaf extracts of *A. amentacea* Roxb. (Table 3). The subject plant affirmed the presence of alkaloid, phenol, tannin, saponin, and flavonoid.

Table 3: Phytochemical analysis of the lyophilized aqueous leaf extract of *A. amentacea* Roxb.

Phytochemical	Result
Alkaloid	(+)
Phenol	(+)
Tannin	(+)
Saponin	(+)
Flavonoid	(+)

Note: (+) present; (-) absent

Functional group analysis plays a vital role in understanding the overall physicochemical properties of the extract. In the present study, functional groups of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. were illustrated as O-H Alcohol, Phenols, and Carboxylic acid (stretch), N-H Aliphatic Primary Amine, Primary Amine, and Amine salt (stretch), C=0 Aliphatic Ketone, Conjugated acid and Conjugated Aldehyde (stretch), C=C Conjugated Alkene, and Cyclic Alkene (stretch), N-H amine (bending), S=O Sulfone (stretch), C-N Aromatic Amine (stretch), C-O Aromatic Ester, Alkyl Aryl Ether (stretch), C-O Primary and Secondary Alcohols (stretch), S=O Sulfoxide (stretch), C- Cl and C-Br Halo Compounds (stretch) shown in Table 4 and Figure 1.

Table 4: FTIR peak values and functional groups of the lyophilized aqueous leaf extract

Characteristic Absorption (cm^{-1})	Functional Group	Bond
	Alcohol(stretch)	O-H
3402.82	Aliphatic primary amine(stretch)	N-H
	Primary amine(stretch)	N-H
2966.00	Carboxylic acid(stretch)	O-H
2936.49	Amine Salt(stretch)	N-H
	Aliphatic Ketone(stretch)	
1705.42	Conjugated acid(stretch)	C=O
	Conjugated aldehyde(stretch)	
	Conjugated Alkene(stretch)	C=C
1606.62	Cyclic alkene(stretch)	C=C
	Amine/bending	N-H
1417.65	Carboxylic acid(bending)	O-H
	Alcohol(bending)	O-H
1319.47	Phenol/bending	O-H
	Sulfone(stretch)	S=O
	Aromatic ester	C-O
1268.41	Alkyl aryl ether(stretch)	C-O
	Aromatic amine(stretch)	C-N
1119.75	Secondary alcohol(stretch)	C-O
1068.43	Sulfoxide(stretch)	S=O
	Primary alcohol(stretch)	C-O
770.81		C-Cl
658.66	Halo compound(stretch)	C-Br
553.56		C-Br

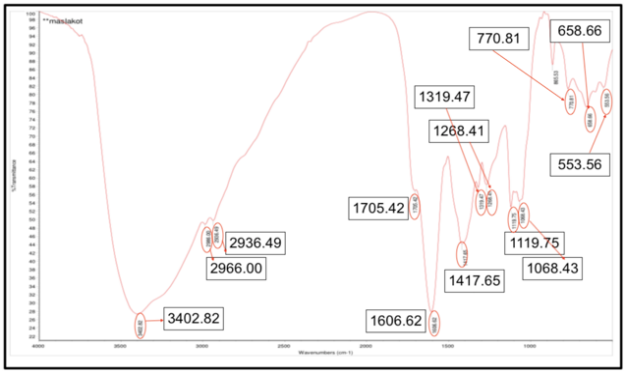

Figure 1: The FT-IR spectrum of the lyophilized aqueous leaf extract of *A. amentacea* Roxb.

This study has revealed the susceptibility of the selected bacteria to the lyophilized aqueous leaf extract of *A. amentacea* Roxb. as shown in Figures 2-6. It was observed that all the prepared treatment concentrations of the plant extract including the antibiotic controls inhibited the selected human pathogens. Results also revealed that Treatment 1 (500 mg/mL) testified the highest ZOI (in mm) of 21.78, 21.36, 21.90, 21.44, and 20.52 for *S. aureus, E. coli, S. marcescens, K. pneumoniae* and *E.*

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faecalis respectively compared to the other prepared treatment concentrations for the plant. Interestingly, ZOIs (in mm) of 12.43, 11.62, 12.06, 10.62, and 9.94 for *S. aureus, E. coli, S. marcescens, K. pneumoniae* and *E. faecalis* respectively were evident at the lowest prepared treatment concentration (62.5 mg/mL) of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. confirming its positive bioactivity. On the other hand, the positive controls such as Ofloxacin and Rifampicin demonstrated ZOIs (in mm) of 39.31, 39, 34.08, 33.17, 23.79; and 39.06, 9.63, 9.56, 10.71, 19.41 for *S. aureus, E. coli, S. marcescens, K. pneumoniae* and *E. faecalis* respectively.

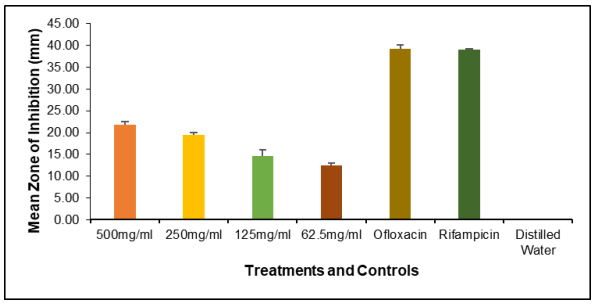

Figure 2: Antibacterial activity of the lyophilized aqueous leaf extract of *A.amentacea* Roxb. against *S. aureus.* (All values are expressed as mean; n=3)

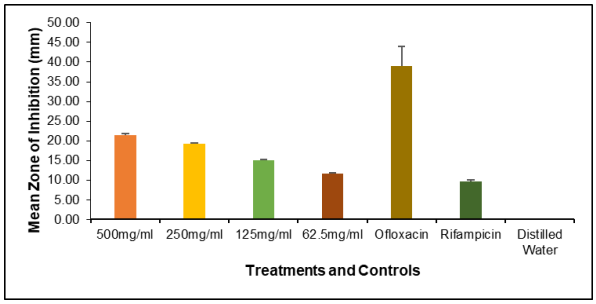

Figure 3: Antibacterial activity of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against *E. coli.* (All values are expressed as mean; n=3)

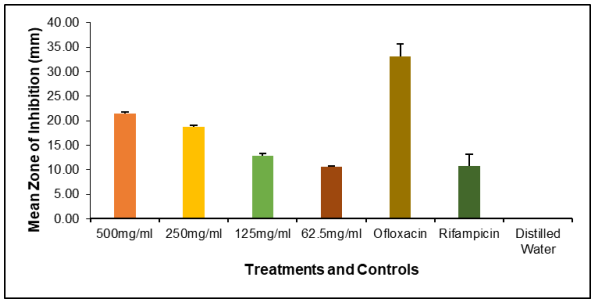

Figure 4: Antibacterial activity of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against *S. marcescens.* (All values are expressed as mean; n=3)

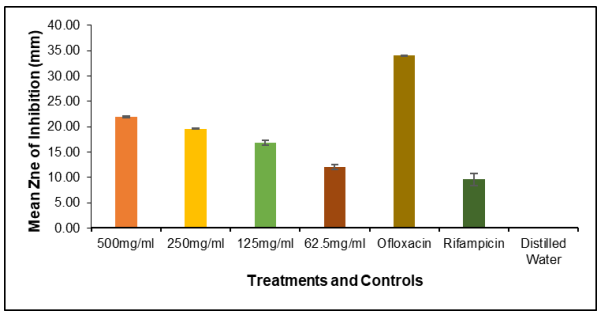

Figure 5: Antibacterial activity of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against *K. pneumoniae.* (All values are expressed as mean; n=3)

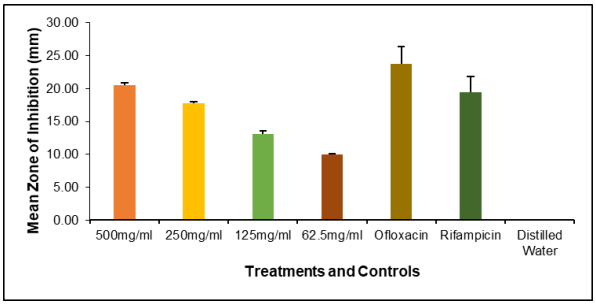

Figure 6: Antibacterial activity of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against *E. faecalis.* (All values are expressed as mean; n=3)

This study affirmed 15.6 mg/mL as the minimum inhibitory concentration (MIC) of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against *S. aureus*, *E. coli*, *K. pneumoniae* and *E. faecalis* while 31.25 mg/mL was obtained for *S. marcescens* as shown in Table 5.

Table 5: Result of the Minimum Inhibitory Concentration (MIC) of the lyophilized aqueous leaf extract of *A. amentacea* Roxb. against the selected pathogenic bacteria.

Organisms	MIC Value
S. aureus (BIOTECH 1582)	15.6 mg/mL
*E. coli (*BIOTECH1634)	15.6 mg/mL
S. marcescens (BIOTECH1748)	31.25 mg/mL
K. pneumoniae (BIOTECH1754)	15.6 mg/mL
E. faecalis (BIOTECH10348)	15.6 mg/mL

DISCUSSION

Gberikon *et al.* (2015) stated that plants with flavonoids, alkaloids, saponin, tannin, steroids, and glycosides affirmed potent antibacterial activity. These phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as defending agents against external stress and pathogenic threats (Tepe *et al.*, 2005). The phytochemical contents of the plant such as terpenoids, alkaloids, and flavonoids could

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demonstrate different bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties (Okarter and Liu, 2010). Also, alkaloids, tannins, and flavonoids when present in plants exhibit anti-cariogenic effects through various modes of action, including bactericidal effects on oral bacteria, prevention of adherence of bacteria to the tooth surfaces, inhibition of glucan production, and inhibition of amylases (Parimala Devi and Ramasubramaniaraja, 2010). On the other hand, the presence of essential phytochemicals in *A. amentacea* like alkaloids, saponins, tannins, flavonoids, phenols, and steroids is also evident to previously reported phytochemicals for *Acalypha wilkesiana* (Kingsley and Marshall, 2014).

The alkaloids in plants serve as complex heterocyclic nitrogenous compounds commonly found to possess antimicrobial properties. These alkaloids are quite useful also against viral and protozoan infections. The mechanism of action of these alkaloids is due to their ability to intercalate with DNA (Cowan, 1999). Meanwhile, saponins, which are considered as amphipathic glycosides and may be mono-or polydesmodic are believed to be responsible for immunostimulant and antinociceptive (pain-relieving) properties (Nah *et al.*, 2000). Similarly, tannins are well known for their antimicrobial and antioxidant activities (Riviere *et al.*, 2009). Several studies have reported that certain tannins are considered to be potential cytotoxic and antineoplastic agents (Poojary *et al.*, 2015).

Meanwhile, several flavonoid derivatives were reported to be effective antimicrobial substances against different microorganisms. Their mode of activity may be due to their capacity to complex with extracellular and soluble proteins as well as to complex with the bacterial cell wall. Also, flavonoids being more lipophilic may also disrupt microbial membranes (Poojary *et al.*, 2015).

Results of the presence of the various functional groups in the plant have also confirmed the existence of the revealed phytochemicals in the plant. For instance, the presence of phenolic compounds and flavonoids was due to the alcohols, phenol, primary and secondary alcohols and aromatic amines (O-H, C-N, C-O) in the plant (Saxena and Saxena, 2012). Meanwhile, the aqueous plant extract with bonds of stretching O-H , bending N-H and O-H, and stretching C=C or the carboxylic acid and alcohols, amine, and conjugated alkene can also confirm the presence of alkaloids, flavonoids and phenols. In addition, saponin in plant extracts was supported by its stretching bond of O-H alcohols, phenols, and carboxylic acid and C-O aromatic ester and alkyl aryl ether (Jabamalairaj *et al.*, 2015).

Interestingly, FT-IR analysis of the plant has established the presence of various biologically active functional groups which confirmed that the plant possesses bioactive phytochemicals that might help in the bioactivities of the plant including bactericidal and antimicrobial activities. The results also affirmed a significant difference among the treatments of *A. amentacea* Roxb. and the controls for the ZOIs ($F=391.1567$; $p=0.000$) in *S. aureus* (BIOTECH1582) wherein Ofloxacin obtained the highest ZOI. In *E. coli (BIOTECH1634)*, the significant difference of ZOIs (in mm) was also observed ($F=38.745$; $p=0.000$) but despite the highest ZOI of Ofloxacin, treatments 1 and 2 of *A.amentacea* Roxb. demonstrated higher ZOIs (in mm) (21.36, 19.15, 15.07) compared to Rifampicin. Also, the other lower concentrations (3 and 4) of the plant testified comparable ZOIs to Rifampicin as identified through the Scheffe test.

Interestingly, for *S. marcescens* (BIOTECH1748), treatments 1 to 4 of the *A. amentacea* Roxb. testified higher ZOIs to Rifampicin which caused significant difference ($F=3.88.443$; $p=0.000$) but Ofloxacin has still the highest ZOI. Meanwhile, there was also a significant difference in the ZOIs in *K. pneumoniae* wherein Ofloxacin had the highest ZOI (33.17 mm) followed by Treatments 1 (21.44 mm), 2 (18.79 mm) and 3 (12.76 mm). These prepared treatments of *A. amentacea* also worked better in inhibiting the growth of *K.pnuemoniae* compared to the antibiotic Rifampicin.

There was a difference among the ZOIs of all the treatments and control ($F=35.871$; $p=0.000$) in *E. faecalis* (BIOTECH10348), but surprisingly, it was revealed through the Scheffe test that the difference did not occur among treatment 1 (500 mg/mL) of the *A. amentacea* Roxb. and the two antibiotics so as between treatment 2 (250 mg/mL) and Rifampicin. The present findings support the comparability of the 500 mg/mL of the plant to both antibiotics and its 250 mg/mL to Rifampicin.

According to Seebaluck *et al.* (2015), plants from *Acalypha* genus has affirmed wide-arrays of biological activities such as antimicrobial, antidiabetic, antioxidant, anti-inflammatory, anticancer, anti-venom, analgesic, anthelmintic, hepatoprotective, laxative, and wound healing. Also, *Acalypha* genus has a pronounced presence of the polyphenol derivatives gallic acid, corilagin, and geraniin that can be isolated from the leaves (Adesina *et al.*, 2013). The presence of these metabolites may be accounted to the bioactivity of *A. amentacea* Roxb. towards the inhibition of the selected human pathogenic bacteria.

In addition, based on the presented results for MICs, it can be deduced that the lyophilized aqueous leaf extract of *A. amentacea* Roxb. testified to have stronger antibacterial activity against *S. aureus* (BIOTECH1582), *E. coli* (BIOTECH1634), *K. pneumoniae* (BIOTECH1754), and *E. faecalis* (BIOTECH10348) compared to *S. marcescens* (BIOTECH1748).

Interestingly, when compared to the MICs reported for the aqueous extract of the *A. wilkesiana* against *S. aureus* (50 mg/mL), *E. coli* (25 mg/mL), *E. faecalis* (25 mg/mL) and *K. pneumoniae* (25 mg/mL) (Haruna *et al.*, 2013), the lyophilized aqueous leaf extract of *A. amentacea* Roxb. affirmed lower MIC values which revealed more potent antibacterial activity against the said pathogens.

The promising bioactivity of the *A. amentacea* Roxb. against the selected human pathogenic bacteria which resulted to the disruption of their growth is parallel to the several studies of the other species of the *Acalypha*

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genus plants which previously reported to have antimicrobial and antibacterial potentials. In the study of Seebaluck *et al.* (2015), *Acalypha* genus plants viz. *A. alnifolia Klein ex* Wild*, A. fruticose Forsk., A. lanceolate* Wild*, A. macrostachya* Jacq., *A. ornate* Hochst. ex *A.* Rich., and *A. siamensis* Oliv. ex Gage have confirmed to have antibacterial and antimicrobial activities.

CONCLUSION

The lyophilized aqueous leaf extract of *A. amentacea* Roxb. affirmed antibacterial activity against the selected human pathogenic bacteria. The higher the concentration of the plant extract the higher its ZOI. This means that *A. amentacea* Roxb. is dose-dependent. Also, *A. amentacea* Roxb. demonstrated better bioactivity to all the selected human bacterial pathogens except *S. aureus* compared to the antibiotic control Rifampicin and appeared comparable to Ofloxacin when against *E. faecalis.*

The bioactivity of the plant may be due to the presence of its important functional groups which confirmed several phytochemicals with specific mode of actions that may affected the bacterial growth like the intercalating capacity with DNA of its alkaloid and the capability of its flavonoid to complex with extracellular and soluble proteins as well in the bacterial cell wall. Flavonoid is considered lipophilic and may also disrupt microbial membranes.

To the best knowledge of the researchers, this is the first report on the bactericidal activity, phytochemicals and functional group assignment of the Philippine green-leafed *A. amentacea* Roxb. leaves. In this study, the search to counter the challenge posed by resistant strains of bacteria have proven yielding results as the investigation of this unexplored traditional medicine has demonstrated enormous potent therapeutical application. Further tests shall be conducted to determine the antibacterial effects of the plant extract against other clinically-important bacterial pathogen, including multi-drug resistant strains.

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