Antibacterial activity of the lyophilized aqueous leaf extract of the Philippine green-leaved 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-leaved 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 km², 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-leaved 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 “Hanunu 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. amentacea 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-leaved 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- Dilliman 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 Philippine 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 (-)                        | BIOTECH 1634 |
| 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.
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 paraffin 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.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Reagent and Chemicals Used</th>
<th>Positive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Mayer’s Test</td>
<td>Hydrochloric acid, Mayer’s reagent, Magnesium</td>
<td>White Precipitate</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shindo’s Test</td>
<td>Turnings, Hydrochloric Acid</td>
<td>Red or orange-red color</td>
</tr>
<tr>
<td>Phenol</td>
<td>Chloride Test</td>
<td>Ferric Chloride</td>
<td>Bluish green color</td>
</tr>
<tr>
<td>Saponin</td>
<td>Foam Test</td>
<td>Water</td>
<td>Copious lather formation</td>
</tr>
<tr>
<td>Tannin</td>
<td>Acetate Test</td>
<td>Lead Acetate</td>
<td>White Precipitate</td>
</tr>
</tbody>
</table>

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-
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.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>(+)</td>
</tr>
<tr>
<td>Phenol</td>
<td>(+)</td>
</tr>
<tr>
<td>Tannin</td>
<td>(+)</td>
</tr>
<tr>
<td>Saponin</td>
<td>(+)</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Note: (+) present; (-) absent

Functional group analysis plays a vital role in understanding the overall physicochemical properties of the plant 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=O Aliphatic Ketone, Conjugated acid and Conjugated Aldehyde (stretch), C=O 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- CI 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Absorption (cm⁻¹)</th>
<th>Functional Group</th>
<th>Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol(stretch)</td>
<td>3402.82</td>
<td>Aliphatic primary</td>
<td>O-H</td>
</tr>
<tr>
<td>amine(stretch)</td>
<td></td>
<td>Primary amine(stretch)</td>
<td>N-H</td>
</tr>
<tr>
<td>Carboxylic acid(stretch)</td>
<td>2966.00</td>
<td></td>
<td>O-H</td>
</tr>
<tr>
<td>Aliphatic Ketone(stretch)</td>
<td>2936.49</td>
<td></td>
<td>N-H</td>
</tr>
<tr>
<td>Conjugated acid(stretch)</td>
<td>1705.42</td>
<td>Conjugated aldehyde(stretch)</td>
<td>C=O</td>
</tr>
<tr>
<td>Conjugated Alkene(stretch)</td>
<td></td>
<td>Conjugated Alkene(stretch)</td>
<td>C=O</td>
</tr>
<tr>
<td>Cyclic aliphatic(stretch)</td>
<td>1606.62</td>
<td>Amine(bending)</td>
<td>C=O</td>
</tr>
<tr>
<td>Alcohol(bending)</td>
<td>1417.65</td>
<td>Phenol(bending)</td>
<td>O-H</td>
</tr>
<tr>
<td>Sulfone(stretch)</td>
<td>1319.47</td>
<td>Aromatic ester</td>
<td>C-O</td>
</tr>
<tr>
<td>Alkyl aryl ether(stretch)</td>
<td>1268.41</td>
<td>Aromatic amine(stretch)</td>
<td>C-N</td>
</tr>
<tr>
<td>Secondary alcohol(stretch)</td>
<td>1119.75</td>
<td></td>
<td>C-O</td>
</tr>
<tr>
<td>Sulfoxide(stretch)</td>
<td>1068.43</td>
<td>Primary alcohol(stretch)</td>
<td>C-O</td>
</tr>
<tr>
<td>Halo compound(stretch)</td>
<td>770.81</td>
<td>C-Cl</td>
<td></td>
</tr>
<tr>
<td>553.56</td>
<td></td>
<td>C-Br</td>
<td></td>
</tr>
</tbody>
</table>

**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.*
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.

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.

**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
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-carcinogenic 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 amphiphatic glycosides and may be mono-or polydextronic 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=O 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.pneumoniae 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, anti-diabetic, 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.
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-leaved 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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