

Antibacterial Activity and Phytochemical Screening of Selected Folkloric Medicinal Plants of Maggok, Hungduan, Ifugao, Cordillera Administrative Region, Philippines

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Abstract- Indigenous knowledge on medicinal plants has been transferred from one generation to generation by the elderlies in every community. These medicinal plants have been used by the Tuwalis in Maggok, Hungduan, Ifugao in curing different diseases. This study aimed to evaluate the antibacterial activity of the ten folkloric medicinal plants against five human pathogenic bacteria and determine the phytochemicals present on the ethanolic leaf extracts with antibacterial potentials.

The antibacterial activity of the ethanolic leaf extracts were evaluated using disc diffusion or Kirby Bauer method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus*. Commercially available antibiotics were used as positive controls like Erythromycin (10µg/disc), Ampicillin (10µg/disc), Streptomycin (10µg/disc), and Gentamicin (30µg/disc). The antibacterial assay was performed in triplicates. Preliminary phytochemical screening was done using standard methods.

Results revealed that *Centella asiatica* (L.) Urban showed an active reaction against *Staphylococcus aureus* with a mean zone of inhibition of 16 mm. *Vaccinium myrtilloides* (Bl.) Miq. manifested a partially active reaction against *Pseudomonas aeruginosa* with a mean zone of inhibition of 12 mm, *Rubus fraxinifolius* Hayata also showed a partially active against *Bacillus cereus* with a mean zone of inhibition of 11 mm. Only three out of ten plant samples possessed antibacterial potentials. Ethanolic leaf extracts of the three plant samples with antibacterial potential contained flavonoids, phytosterols, and phenolic compounds. Results of these findings revealed that the antibacterial activity is due to the phytochemicals present.

Index Terms- antibacterial, phytochemical screening, folkloric, medicinal plants, Maggok

I. INTRODUCTION

The occurrences of diseases and increase in prices of synthetic medicines have resulted to the demand for discovery of less expensive and more potent sources of drugs. Plants are best sources of potent drugs (Balangcod et al, 2012). Plants have been proven to be most useful in the treatment of diseases; they provide an important source of all the world's pharmaceuticals; and are now occupying important position in allopathic and herbal medicines, homoeopathy and aromatherapy (Ayayi et al, 2011).

Search for new antibacterial agents should be continued by the screening of many plant families (Parekh and Chanda, 2006). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003) and that plant extracts according to Nascimiento (2000) have great potential as antimicrobial compound against microorganisms. Thus, there is a continuous and urgent need to discover plants with antimicrobial activities with these diverse chemical structures and novel mechanisms of actions (Vital and Rivera, 2011).

The medicinal value of plants lies in the bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds that produce a definite physiological action on the human body (Nostro, 2000).

Philippines is rich in biodiversity of plant species where only small portion of the species were investigated in detail (Galvez, 2015). Scientific understanding of medicinal plants remains largely unexplored and pharmacological investigation of the Philippine flora only gained momentum recently (Vital and Rivera, 2011), thus, this study.

II. MATERIALS AND METHODS

Sample Collection and Authentication

Fresh leaves of *Medinilla pendula* Merr., *Rubus fraxinifolius* Hayata, *Melastoma polyanthum* Blume, *Desmodium sequax* Wall., *Astilbe rivularis* Buch.-Ham ex. D. Don, *Drymaria cordata* (L.) Willd. ex J.A. Schultes, *Allium odoratum* L., *Physalis minima* L., *Centella asiatica* (L.) Urban, and *Vaccinium myrtilloides* (Bl.) Miq. were collected from Maggok, Hungduan, Ifugao. The plant samples were identified by Dr. Teodora D. Balangcod, a Botanist from the Department of Biology, University of the Philippines-Baguio, Baguio City, Cordillera Administrative Region (CAR), Philippines.

Preparation of Extracts

Leaves of plant samples were air dried for a period of three weeks. These air-dried leaves were grinded and pulverized using a mechanical grinder. Two hundred grams of the ground samples were placed in a 3-L capacity bottle. Then 95 % ethyl alcohol was added until the plant samples were completely submerged. These amber bottles were covered and set aside and stored at room temperature for 48 hours. The plant samples were filtered

using a Buchner funnel with gentle suction. Each filtrate was then concentrated in a rotary evaporator until approximately 20% of the filtrate was left. These extracts were used both for the phytochemical screening of secondary metabolites and antibacterial activity.

Antibacterial Assay

Kirby-Bauer method was used to conduct the antibacterial assay (Lalitha, 2005; Quinto & Santos, 2005; Righi et al., 2013). Five bacterial strains, namely: gram negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*; and gram positive *Staphylococcus aureus*, and *Bacillus cereus* were used as test bacteria. The bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were obtained at the Natural Sciences Research Unit (NSRU), Saint Louis University, Baguio City. Other bacterial strains were donated by a botanist friend.

A. Preparation of Microbial Inocula

The test microorganisms were inoculated in petri dishes using sterile saline solution and compared with McFarland standards to give an approximate concentration of 9.0 to 1.2 x 10⁹ cells per ml (Quinto & Santos, 2005).

B. Kirby-Bauer Method

Agar petri plates were prepared using 20 ml of sterile Mueller-Hilton agar. Bacterial colonies from a 24-hour culture were suspended in sterile normal and standardized with 0.5 M McFarland solution. The surfaces of the petri plates were inoculated using sterile swab cotton that contains the standardized saline suspension of bacteria and these were allowed to dry for a period of 15 minutes. Sample stock solutions were delivered to a previously prepared sterile paper disks, 6.0 mm in diameter. Paper discs were allowed to dry and were put on the agar surface. Commercially available antibiotics were used as positive controls. These include: Erythromycin (10µg/disc), Ampicillin (10µg/disc), Streptomycin (10µg/disc), and Gentamicin (30µg/disc). Zones of inhibitions were determined by measuring clearing zones in millimeters (mm) using a ruler. The measurement was done after incubation for 24 hours at 37 °C. The antibacterial assay was performed in triplicates.

Phytochemical screening

Ethanol leaf extracts prepared were analyzed for the presence of alkaloids, glycosides, saponins, phytosterols, tannins, flavonoids, terpenoids and phenolic compounds based on the studies of Himesh, et al. (2011) and Tiwari, et al. (2011).

III. RESULTS AND DISCUSSION

The use of ethnopharmacological knowledge is one attractive way to reduce empiricism and enhance the probability of success in new drug finding efforts (Patwardhan, 2005). Thus, studying plant based antimicrobial properties provides additional information in developing natural antibiotics and discovering the alternative of antimicrobial drugs for the treatment of infectious diseases (Saad et al., 2012).

Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility (Kumar et al., 2006). Antibacterial screening of the ten plant samples (Table 1) revealed that *Centella asiatica* (L.) Urban showed an active reaction against *Staphylococcus aureus* with a mean zone of inhibition of 16 mm. *Vaccinium myrtilloides* (Bl.) Miq. showed a partially active reaction against *Pseudomonas aeruginosa* with a mean zone of inhibition of 12 mm. *Rubus fraxinifolius* Hayata also showed a partially active against *Bacillus cereus* with a mean zone of inhibition of 11 mm. No ethanolic extract exhibited a zone of inhibition against *Escherichia coli* and *Salmonella typhimurium*.

There were three out of ten plants or 30% of the total plants studied possessed antibacterial activity with varying effects that can be inferred from their mean zones of inhibition which range from partially active to active (11-16 mm) based on the general standards (Guevarra et al, 2005).

The susceptibility test of the antibiotics used against the test organisms (Table 3) revealed that the control (ethanol) has no zone of inhibition. This indicates the absence of suppression on bacteria involved. The reference antibiotics such as Erythromycin, Ampicillin, Streptomycin and Gentamicin displayed positive results against the five bacteria. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000). All antibiotics have high zones of inhibition regarded to be very active as against the five bacteria except for Ampicillin that has a lower zone of inhibition of 11 mm against *Staphylococcus aureus* which is regarded as partially active.

Table 1. Antibacterial Activity of the Ten Plant Samples

Plant Samples	Mean Zone of Inhibition of Test Microorganisms (mm)				
	Gram Negative Bacteria			Gram Positive Bacteria	
	EC	PA	ST	BC	SA
<i>Medinilla pendula</i> Merr.	-	-	-	-	-
<i>Rubus fraxinifolius</i> Hayata	-	-	-	11	-
<i>Melastoma polyanthum</i>	-	-	-	-	-
<i>Desmodium sequax</i> Wall.	-	-	-	-	-
<i>Astilbe rivularis</i> Buch.-Ham ex. D. Don	-	-	-	-	-
<i>Drymaria cordata</i> (L.) Willd. Ex J.A. Schultes	-	-	-	-	-
<i>Allium odoratum</i> L.	-	-	-	-	-
<i>Physalis minima</i> (L.)	-	-	-	-	-

<i>Centella asiatica</i> (L.) Urban	-	-	-	-	16
<i>Vaccinium myrtoides</i> (Bl.) Miq.	-	12	-	-	-

Legend: Inhibition zones expressed in diameter, (-)-inactive, (+)-partially active, (++)-active; EC- *Escherichia coli*, PA- *Pseudomonas aeruginosa*, ST- *Salmonella typhimurium*, BC- *Bacillus cereus*, SA- *Staphylococcus aureus*

Table 2. Standard zones of inhibition and corresponding inferences (Guevara et al., 2005)

Zones of inhibition	Inferences
< 10 mm	Maybe expressed as inactive
10-13 mm	Partially active
14-19 mm	Active
> 19 mm	Very active

Table 3. Result of the Susceptibility Test of Antibiotics used against the Test Organisms

Antibiotics/Control	Mean Zone of Inhibition of Test Microorganisms (mm)				
	Gram Negative Bacteria			Gram Positive Bacteria	
	EC	PA	ST	BC	SA
Ethanol (Control)	-	-	-	-	-
References					
Erythromycin(10 µg/disc)	21	38	37	35	35
Ampicillin (10 µg/disc)	30	40	36	50	11
Streptomycin (10 µg/disc)	30	34	38	35	35
Gentamicin (30 µg/disc)	30	40	35	35	33

Legend: Inhibition zones expressed in diameter, (-)-inactive, (+)-partially active, (++)-active; EC- *Escherichia coli*, PA- *Pseudomonas aeruginosa*, ST- *Salmonella typhimurium*, BC- *Bacillus cereus*, SA- *Staphylococcus aureus*

Most antibacterial medicinal plants attack gram positive strains while few are active against gram negative bacteria (Herrera et al., 1996; Meng et al., 2000; Scrinivasan et al., 2001). The findings showed higher activity on gram positive bacteria than gram negative bacteria (Table 3). One of the several unique characteristics of gram negative bacteria is the outer membrane of the cell that is responsible for protecting the bacteria from destruction of the inner membrane or cell wall (peptidoglycan). Outer membranes of the gram negative bacteria provide a selective barrier to external molecules and that prevent the release of metabolite-binding proteins and hydrolytic enzymes (nucleases, alkaline phosphatase) found in the periplasmic space between the outer surface of the inner (plasma) membrane and the inner surface of the outer membrane (Baron, 2008). As gram positive possesses single layer boundary, it is being attacked easily (Prabha & Vashantha, 2012).

Phytochemical analysis of the three plant samples with potential antibacterial activity revealed that the three ethanolic leaf extracts contain flavonoids, phytosterols, and phenolic compounds (Table 4). Tannins were present in both ethanolic leaf extract of *Rubus fraxinifolius* Hayata and *Vaccinium myrtoides* (Bl.) Miq. Alkaloids were found in the ethanolic leaf extract of *Rubus fraxinifolius* Hayata.

In the study, the presence of the secondary metabolites in the leaves of the three plants with antibacterial potentials is responsible of their antimicrobial properties. Flavonoids have

been recognized as potential natural sources of antimicrobial drugs (Friedman, 2007). It can exert antibacterial activities through multiple mechanisms such as disruption of cytoplasmic membrane, inhibition of nucleic acid synthesis, inhibition of energy metabolism, inhibition of cell wall synthesis, and inhibition of cell membrane synthesis (Cushnie & Lamb, 2011). Their activity is due to their ability to react with extracellular and soluble proteins and complex bacterial cell walls leading to the death of the bacteria (Cowan, 2002).

The presence of alkaloids in the solvent fraction could be well correlated with the antimicrobial activities (Ramkumar et al., 2007). Elisabetsky and Campos (2006) reported that alkaloids are used by the plants as defense mechanism against pathogens and predators. Kovaceric (2004) explained that the mechanism of antibacterial action of alkaloids is attributed to their ability to intercalate with DNA, causes inhibition of enzymes (esterase, DNA-, RNA-polymerase), and inhibition of cell respiration.

Tannins are secondary metabolites responsible for antimicrobial properties in various plants (Chung, 1998). According to Stefanović, et al. (2012), the antimicrobial effects of the plants studied are attributed to the presence of tannins in the ethanol extracts. Their mode of antimicrobial action is related to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins, because of a property known as astringency.

The antibacterial activity of phenolic compounds is exerted by multiple functions, primarily due to its ability to act as a nonionic surface-active agent therefore disrupting the lipid-protein interface (Greenberg et al., 2008).

Table 4. Phytochemical Analysis of the Three Plant Samples with Potential Antibacterial Activity

Plant Samples	Alkaloids	Glycosides	Saponins	Phyto-Sterols	Tannins	Flavonoids	Terpenoids	Phenolic Compounds
<i>Rubus fraxinifolius</i> Hayata	+	-	-	+	+	+	-	+
<i>Centella asiatica</i> (L.) Urban	-	-	-	+	-	+	-	+
<i>Vaccinium myrtilloides</i> (Bl.) Miq.	-	-	-	+	+	+	-	+

Legend: (+) - presence of the bioactive component; (-) - absence of the bioactive component

IV. CONCLUSIONS

The clamor for new and less expensive plant-based medicinal plants is the trend of the time. Based on the results generated from the study, only three namely *Centella asiatica* L. Urban, *Rubus fraxinifolius* Hayata and *Vaccinium myrtilloides* (Bl.) Miq. have antibacterial potentials. The antibacterial potential is attributed to the secondary metabolites present that could provide baseline information. Also, the three plants can be developed further as plant-based antibacterial drugs.

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