Indian Journal of Traditional Knowledge
Vol. 12 (4), October 2013, pp. 630-637

Antibacterial potential of some medicinal plants of the Cordillera Region, Philippines

*Rosemary M.Gutierrez[1], Ronan Baculi[1], Nardo Pastor[2], Timothy Puma-at[3] & Teodora Balangcod[1]

[1]Department of Biology, College of Science, University of the Philippines Baguio, [2]Cordillera Career Development College, Buyagan La Trindad, Benguet, [3]Mt. Province State Polytechnic College, Bontoc, Mt. Province
E-mail: rosegutz@gmail.com

Received 10.01.13, revised 08.05.13

Since plants have been the source of a large percentage of prescription drugs, it is very important that the antimicrobial properties of medicinal plants be investigated because these plants could be a resource for new and alternative treatment for some common infectious diseases. Eleven medicinal plants of the Cordillera Region, in the Philippines were evaluated for their antimicrobial properties by the standard disc diffusion assay method using test bacterial organisms: *Escherichia coli*, *Bacillus subtilis*, *Staphyloccocus aureus* and *Proteus vulgaris,* while test fungal organisms used were *Candida albicans* and *Aspergillus flavus.* Only four plants namely, *Agathis dammara* , *Eupatorium triplenerve*, *Citrus aurantifolia* and *Tithonia divserifolia* were found to have antibacterial properties. The Minimum Inhibitory Concentrations (MICs) of these plant extracts against the four test organisms were also determined. Results of MIC determination revealed that the gram positive organisms, *Bacillus subtilis* and *Staphylococcus aureus*, were more sensitive to the four plant extracts, since they scored the lowest value of MIC which ranged from 62.5 to 125 µg/ml. The gram negative organisms *Escherichia coli* and *Proteus vulgaris* scored higher MIC values ranging from 62.5 - 250 µg/ml. Phytochemical screening of the four plants with antibacterial activities were also undertaken.

Keywords: Antimicrobial, Medicinal plants, Disc diffusion assay, Minimum inhibitory concentrations, Phytochemical analyses, Zones of inhibition

IPC Int. Cl.[8]: A61K 36/00, A01D 7/64, A01D 1/00, A01D 11/00, A01D 12/00, A01D 18/00, A01D 20/00, C12Q, C12P, B30B 3/00, G01N 13/00, C23F 11/00, C23F 13/00, C23F 15/00, G01N, C12N, C12M

In developing countries like the Philippines, people from remote communities use folk medicine in treating infectious diseases. People from the communities of the Cordilleras in the Northern part of the Philippines, for instance use herbal drugs not only as an alternative medicine but some of them who cannot afford to buy expensive pharmaceuticals use these herbal drugs as their only source of medication against diseases[1]. Because of the alarming increase of new and re-emerging diseases, as well as resistance to drugs, there is a continuous need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action.

In 1989, a book on Common Medicinal Plants of the Cordillera Region was written by Co and published by CHESTCORE (Community Health Education, Services and Training in the Cordillera Region) Baguio City, Philippines[2]. This book was intended for Community based Health Programs (CBHPs) operating in the Cordilleras of Northern Luzon, and primarily it was for the ancestral domain of a distinct ethno-linguistic group of minority Filipinos collectively known as *Igorots*. The book is divided into three parts; the first part is a compendium of Common Medicinal Plants in the Cordillera and dealt with a total of 122 species entries. The second part dealt with collection, storage, preparation and usage of herbal drugs. The third part of this book dealt with Major constituents- Pharmacological and Clinical Research. However, some of the common medicinal plants listed in this book had no pharmacological and clinical research noted.

Although many studies have already been done on the determination of antimicrobial properties of many natural products like plant extracts, continued research on this area would still be promising because of some phenomena like spread of multiple drug resistance, emergence of new diseases and re-emergence of previously existing diseases[3-5]. Scientists should constantly be in search for plants and other natural products that may have the promise or potential for drug discovery.

*Corresponding author

The knowledge gained in this study may add to the relevant data of information on the indigenous materials that could be of great value to the field of medicine and at the same time to be of help to the community based programs of the Cordillera Region in the Philippines. This study was undertaken to evaluate the antimicrobial activity of some common medicinal plants of the Cordillera Region using the disc diffusion assay method, and to determine the minimum inhibitory concentration (MIC) of these plant extracts. This research might open new avenues that may pave the way to the discovery of new drugs against the common diseases prevalent in the region.

Materials and methods

Test organisms

Four bacterial strains, namely: gram-negative *Escherichia coli* and *Proteus vulgaris*; gram-positive *Staphylococcus aureus* and *Bacillus subtilis* were used as test bacteria. *Candida albicans* and *Aspergillus flavus* were used as test fungi. Strains of *Candida albicans* and *Aspergillus flavus* were purchased from the National Institute of Biotechnology (BIOTECH, UP Los Banos); while the bacterial strains used were obtained from the Microbiology Laboratory of University of the Philippines Baguio.

Collection and identification of plant materials

Medicinal plants listed in the book of Co[2] with no known pharmacological and clinical research were selected for the study (Table 1). The plants were collected from Baguio City and various sites of the Cordillera Region, Philippines: Banaue, Ifugao (N16° 55.1333', E121° 3.5333'), Barlig, Mt. Province (N17° 2.3995', E121° 8.6116') Bayyo, Mt. Province (N16° 59.866', E121° 1.1505'), Atok, Benguet (N16° 35.0', E120° 42.0'), Bontoc, Mt. Province (N17° 5.2333', E120° 58.5333'), Ambiong, La Trinidad, Benguet (N16° 25.9812', E120° 36.4448') and Baguio City (N16° 24.5989', E120° 35.8869'). The plants were identified by Dr. Teodora Balangcod, Botanist from the Department of Biology. Specimen collections were done and were deposited in the UP Baguio Herbarium.

Preparation of plant extracts

The plant extracts (except for *Citrus aurantifolia*) were prepared using a modified method[6]. Plant leaves were washed with tap water and air dried for 24 hrs, after which they were oven dried for 2-3 days. Dried leaves were powdered using a mortar and pestle. For each plant, 100 gm of powdered leaves were soaked at room temperature for 24 hrs in 500 ml 95% methanol and filtered in Whatman filter paper No 1. Each filtrate was then concentrated in a rotary evaporator until approximately 20% of the filtrate was left. The concentrations of the crude extracts were dissolved in 95% methanol to obtain concentrations of 80 mg/ml which were subsequently diluted to obtain, 40mg/ml, 20 mg/ml and 10 mg/ml respectively. For *Citrus aurantifolia* the fresh juice extracted from the fruit of the plant was used as 100% pure extract and subsequently diluted with sterile water to give 50% extract, and 25% extract.

Culture media

Nutrient Agar (NA) medium was used for the propagation of the four bacterial species while Potato Dextrose Agar (PDA) medium was used for the propagation of fungi. Disc diffusion assays were carried out using Mueller-Hinton agar media. These culture media were prepared according to the recommendation of the manufacturer (Hi-media and Beckton Dickinson).

Preparation of microbial inocula

The test microorganisms were inoculated into sterile saline solution and compared with McFarland standards to give an approximate concentration of 9.0 to 1.2 x 10^9 cells per ml[7].

Phytochemincal analyses

Plant extracts that gave antimicrobial properties were submitted to the Natural Science Research Unit (NSRU) of St. Louis University, Baguio City for phytochemical analyses. Several qualitative phytochemical analyses (Table 3) were used to test for the presence of the following: alkaloids, steroids, anthraquinones, flavonoids, saponins, tannins and polyphenols, as well as cyanogenic glycoside.

Antibacterial assay

The modified disc diffusion assay or the Kirby-Bauer method was used for the antibacterial assays[7-8]. Ten (10) ml of Mueller – Hinton (MH) medium were poured into each sterile Petri plate, which served as the base agar. Then MH soft agar, previously inoculated with 0.5 ml of bacterial suspension were overlaid over the top agar and allowed to solidify for approximately thirty minutes. All bacterial strains or bacterial inocula were grown in Nutrient Agar medium for 24 hrs at 37° C. Bacterial growth was adjusted according to the McFarland standard. Then sterile paper discs (6 mm in diameter) were placed on agar to load 20 µl of each concentrations used. For positive control, commercially available

antibiotic discs were used. These were the following: (ampicillin, 10 µg/disc; streptomycin, 10 µg/disc, and Erythromycin, 10 µmg/disc and Kanamycin, 30µg/disc). As negative control, 95% methanol was used. Zones of inhibitions were determined by measuring clearing zones in millimeters (mm) with the aid of a ruler after incubation for 24 hrs at 37° C. All antibacterial assays were performed in triplicate.

Antifungal assays

Candida albicans and *Aspergillus. flavus* were grown in Sabouraud Dextrose Broth for 72 hrs at 25 to 28°C. SDA plates were used in the agar diffusion experiments. Fungal suspensions in saline solution were adjusted to 10^7 cells/ml. Grisovin with a concentration of 1000 µg/ml was used as positive control and 98 % methanol was used as positive control. Zones of inhibition were determined after incubation for 48 - 72 hrs at 25- 27°C. All tests were performed in triplicate[8].

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration or MIC for the plants which gave positive results for the antibacterial assays were evaluated by the broth dilution method[7].

Statistical analyses

Antimicrobial activity was assessed using the following rating system: (1) < 10 mm zone of inhibition maybe expressed as inactive, (2) 10-13 mm zone of inhibition is partially active; (3) 14-19 mm zone of inhibition is active and (4) > 19 mm zone of inhibition is very active[7].

To confirm the results of the antibacterial assays, one sample t-tests (at $\alpha = .05$) were used to analyze the data on zones of inhibition with test values equal to 10 mm (interpreted as partially active), 13 mm (interpreted as active) and 19 mm (interpreted as very active). The average of the zones of inhibition of the plant extracts against the test organisms were computed and subjected to the t-tests to confirm their interpretation. If the average falls between 10-13 mm with the impression as partially active, they were tested whether they were significantly greater than the test value of 10 mm by calculating the P values. If the P values calculated were less than .05, then the plant extract was partially active, otherwise it was inactive. If the average of the zones of inhibition were between 14 -19 mm with the impression of active, they were tested whether they were significantly greater than the test value of 13 mm. If the calculated P values were less than .05, the plant extract was considered active, otherwise it is partially active. If the average of the zones of inhibition were greater than 19 mm with the impression of very active, they were tested whether they were significantly greater than 19; if the calculated P values were less than .05, the plant extract was considered very active, otherwise it is active.

Results

The results presented in Table 1 are averages of several trials of our disc diffusion assays. It was

Table 1— Antimicrobial activity of the common medicinal plants from the Cordillera region, Philippines against different test organisms compared to well known antibiotics.

Scientific name	Test Microorganisms					
	EC	BS	PV	SA	CA	AF
Agathis dammara (Lamb.) Rich. & A. Rich.	+	++	+++	+	-	-
Cyperus kyllingiformis Lye	-	-	-	-	-	-
Ageratum conyzoides (L.) L	-	-	-	-	-	-
Drymaria cordata Willd. Ex Schult	-	-	-	-	-	-
Amaranthus gracilis Desf.	-	-	-	-	-	-
Sonchus arvensis L.	-	-	-	-	-	-
Artocarpus heterophyllus Lam.	-	-	-	-	-	-
Cordyline fruticosa (L.) A. Chev	-	-	-	-	-	-
Eupatorium triplenerve L.	++	++	++	++	-	-
Tithonia diversifolia (Hemsl.) A. Gray	+++	+++	++	++	-	-
Citrus aurantifolia (Christm.) Swingle	++	+++	+++	++	-	-
Positive Controls						
Ampicillin (10 µg/disc)	+++	++	+++	+++	nd	nd
Kanamycin (30 µg/disc)	+++	++	++	++	nd	nd
Penicillin (10 µg/disc)	+++	++	+++	+++	nd	nd
Gersovin (100 µg/ml)	nd	nd	nd	nd	+++	+++

Legend: Inhibition zones expressed in diameter: (-) no inhibition, (+) 10-13mm, (++) 14-19 mm, (+++) 19-21 mm, nd, not determined; EC , *Escherichia coli*, BS, *Bacillus subtilis*, PV, *Proteus vulgaris*, SA, *Staphylococcus aureus,* CA, *Candida albicans* (C.P Robin), AF, *Aspergillus flavus (*J. H. F. Link)

generally observed that the higher the concentration of the plant extract, the greater the diameter of the clearing zones observed (data not shown). Only four plant extracts out of the eleven plant extracts studied, namely *Agathis dammara*, *Eupatorium triplenerve*, *Tithonia diversifolia* and *Citrus aurantifolia* demonstrated a partially active, active and very active antibacterial activities against the four test bacteria used, while no antifungal activity was observed in all of the 11 plants evaluated. The mean zone of inhibition demonstrated by the *Agathis dammara* extract at 80 mg/ml was 15.75 mm which can be interpreted as active against all the test organisms and this was comparable to the mean zone of inhibition, (15.55 mm), of the positive control used which was Kanamycin. Highest antibacterial activity of the *Agathis dammara* extract was observed against *P. vulgaris*, with an average of 20 mm at the highest concentration used (80 mg/ml) which can be interpreted as very active compared to the positive control which only gave an average zone of inhibition of 10 mm. Statistical analyses using the one - sample t- test confirmed that the plant extract at 80 mg/ml have active to partially active antibacterial activity against the four test bacteria used.

The mean zone of inhibition of the *Eupatorium triplenerve* extract was 12.17 mm at the highest concentration of 80 mg/ml used against all test organisms. However, compared to the positive control, the antibiotic Penicillin (10 µg/disc) gave a very active antibacterial activity with an average zone of inhibition of 28.16 mm against all test organisms. The mean of all zones of inhibition for all *Eupatorium triplenerve* plant extract concentrations used was calculated to be 10.95 mm, and statistical analyses revealed that it was significantly greater than the test value used and therefore can be interpreted as having partially active antibacterial activity against all test organisms used in this study.

The *Tithonia diversifolia* extract gave very active antibacterial activities against *E. coli* (average diameter of zone of inhibition was 20.33 mm) and *B. subtilis* (average diameter of zone of inhibition was 23 mm) at the highest concentration of 80 mg/ml. However, the positive control, Ampicillin (10 µg/disc) gave an average diameter of zone of inhibition of 39.66 mm for *E coli* as test organism and 16 mm only for *B subtilis* as test organism. Therefore highest zone of inhibition for the *Tithonia diversifolia* extract was against *B. subtilis*, since it gave even greater zone of inhibition than the positive control used. The overall average of zones of inhibition for all concentrations of sunflower extract used was 19.19 mm. Statistical analyses confirmed that it has very active antibacterial activity against both *E. coli* and *B subtilis* and active antibacterial activity against *P. vulgaris* and *S. aureus*.

The fruit juice of *Citrus aurantifolia* gave highest activity against *P. vulgaris* (average diameter of zone of inhibition was 17.67 mm) and *Bacillus subtilis* (average diameter of zone of inhibition was 16.33 mm) for the 100% pure *juice* extract. The positive control Penicillin (10 µg/disc) gave an average diameter of zone of inhibition of 12 mm and 14 mm respectively against *P. vulgaris* and *B. subtilis.* The average diameter of zones of inhibition of all concentrations of the *Citrus aurantifolia* extract for all test organisms used was 12.74 mm which was comparable to the mean diameter of zone of inhibition of the positive control used, which was 14.16 mm (Penicillin, 10 µg/disc). Statistical analyses confirmed that the *Citrus aurantifolia* extract was active against *B. subtilis* and *P vulgaris* and partially active against both *E. coli* and *S. aureus*.

The lowest concentration of a drug that prevents the growth of an organism *in vitro* is called the minimum inhibitory concentration or MIC[9]. This is usually determined using serial dilution test tube preparations in Mueller Hinton broth and inoculated by the test organisms. Results of the MIC determination are shown in Table 2. These results revealed that *Bacillus subtilis* and *Staphylococcus aureus*, both gram positive organisms were more sensitive to the four plant extracts, since they scored the lowest value of MICs which ranged from 62.5 to 125 µg/ml. The two other test organisms, gram negative *Escherichia coli* and *Proteus vulgaris* scored higher than the gram positive organisms in terms of their MICs which ranged from 62.5 to 250 µg/ml.

The results that the four plant extracts gave partially active, active and very active antibacterial activities against the four test organisms prompted us to submit

Table 2—Minimum inhibitory concentrations (MICs) of the plant extracts with antibacterial properties against the test organisms in µg/ml

	Test Organisms			
Plants	BS	EC	PV	SA
Tithonia diversifolia	62.5	250	250	62.5
Agathis dammara	62.5	125	125	125
Eupatorium triplenerve	62.5	125	125	62.5
Citrus aurantifolia	62.5	62.5	125	62.5

Legend: EC, *Escherichia coli*, BS, *Bacillus subtilis*, PV, *Proteus vulgaris*, SA, *Staphylococcus aureus*

the four crude plant extracts to the Natural Science Research Unit (NSRU) of St. Louis University, Baguio City, where the crude plant extracts were subjected to qualitative phytochemical screening. Results of the phytochemical screening are shown in Table 3. This table indicates that the physiologically active constituents commonly present in the four plant extracts with antibacterial properties are the following phytochemicals: alkaloids, steroids, saponins, tannins and polyphenolic compounds. *Agathis dammara* on the other hand, had anthraquinones and flavonoids specifically leucoanthocyanins and cyanidin.

Discussion

The antibacterial action of the plant extracts used in this study might be similar to antibiotics or antibacterial drugs that either inhibit the growth or kill pathogenic microorganisms since we obtained partially active, active and very active antibacterial activities of the four plant extracts against different test organisms. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA[10]. Moreover, the mode of action of steroids which was present in the four plants with antibacterial property, is that they directly damage cell membranes. This action suggests the loss of viability that maybe caused by the steroids' damage to the membranes with the loss of cytoplasmic constituents[11]. Anthraquinone which is present in the *Agathis dammara* plant is a member of the quinone family. They are a good source of free radicals, and they are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. Probable targets in the microbial cell are surface-exposed adhesions, cell wall polypeptides and membrane bound enzymes. Quinones also render substrates unavailable to the microorganism[10]. Flavonoids, on the other hand, are widely distributed in the plant kingdom. DNA synthesis was strongly inhibited by flavonoids in *Proteus vulgaris* while RNA synthesis was most affected in *Staphylococcus aureus*. The B ring of the flavonoids may play a role in the intercalation or hydrogen bonding with the stacking of nucleic acid bases and this may explain the inhibitory action on DNA and RNA synthesis[12]. Furthermore, flavonoids can cause cytoplasmic membrane damage by rendering it leaky to internal solutes. They also increase the permeability of the inner bacterial membrane and a dissipation of the membrane potential preventing ATP synthesis and membrane transport and motility[13]. In this study, three plant extracts gave a positive test for flavonoids and these are *Tithonia diversifolia*, *Citrus aurantifolia* and *Agathis dammara* plant extracts. Moreover, all four plants studied with antibacterial activity tested positive for saponins. The antibacterial activity of saponins may be attributed to their interactions with membrane sterols, especially the pore formation on

Table 3— Results of phytochemical analyses of the four plants that exhibited antibacterial properties against the test organisms used

Constituents/Plants	*A. dammara*	*T. diversifolia*	*E. triplenerve*	*C. aurantifolia*
Alkaloids				
Dragendorff's test	+	-	+	-
Mayer's test	++	-	+	+
Steroids				
Keller-Killiani	+	+	+	+
Lieberman-Buchard	-	+	+	+
Kedde test	-	-	-	-
Anthraquinones				
Borntrager's test	+	-	-	-
Modified Borntrager' s test	-	-	-	-
Flavonoids				
Bate and Metcalf	+	+	-	-
Wilstatter "cyanidin"	-	-	+	-
Saponins				
Froth test	+	-	-	-
Liebermann-Buchard	-	+	+	+
Tannins and Polyphenols				
Gelatin Test	-	-	+	-
Ferric Chloride test	+	+	+	+
Cyanogenic glycoside				
Guignard test	-	-	-	-

membranes[14]. However the interaction between saponins and membranes seems to be complicated because it would depend on the composition of the target membrane, the type of side chain, and the nature of the aglycone to which these are attached[15]. Tannins are water-soluble polymeric phenolics that precipitate proteins[16]. In a review of the antimicrobial properties of tannins, it was documented that tannins can be toxic to filamentous fungi, yeasts, and bacteria[17]. The different mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Condensed tannins have been determined to bind to cell walls of ruminal bacteria, preventing growth and protease activity. One of their molecular actions is to complex with proteins through so-called non-specific forces such as hydrogen bonding and hydrophobic forces as well as by covalent bond formation. All four plants evaluated have positive results for polyphenolic compounds using the ferric chloride test.

Secondary metabolites are molecules produced by organisms especially plants that have no obvious role for normal growth and development. They are produced in small amounts and may be widespread or restricted to particular plants groups or species, or even plant parts. The general groups of secondary metabolites produced by plants include terpenoids, glycosides, phenolics, and alkaloids[18]. These secondary metabolites usually enhance plant's protection against predation and competition, and are responsible for use of plants in traditional medicines.

The resins that are found in the *Agathis dammara* plant contain aromatic chemicals called terpenes which is responsible for their volatility and their being flammable. Resins also have been reported to have antibiotic properties that protect trees by fighting infections caused by losing a branch or an invasive parasite like that of a beetle. For humans, resins have possible medicinal properties because it has been used in tea as a stimulant, also as diuretic and it is also being used as treatment for intestinal parasites[19]. This present study has proven that the *Agathis dammara* plant extract has broad antibacterial activity since it was partially active against gram negative as well as gram positive bacteria.

A study reported that *Eupatorium triplenerve* contains a coumarin called hernarin (7-mehtoxycoumarin) which may help explain why the plant is used in herbal medicine as an anti-tumor remedy. This chemical was proven to be toxic to cancer cells including multi-drug resistant cancer and leukemic cells[20].

Moreover, another study reported the antimicrobial properties of terpenoids from the air dried flowers of *T. diversifolia* collected from Cavite City, Philippines[4]. Through NMR spectroscopy the authors were able to isolate five active terpenoid components namely: tagitinin C, fatty acid esters of fardiol, squalene, and a mixture of stigmasterol and sitosterol. Antimicrobial tests revealed that tagitinin C was moderately active against *S. aureus* and *C. albicans;* slightly active against *E. coli*, *P. aeruginosa* and *T. metagrophytes* and inactive against *B. subtilis* and *A. niger.* Our present study indicates a broad antibacterial activity for the *T. diversifolia* extract since it was very active against both *E coli* and *B. subtilis*, and active against *P. vulgais* and *S. aureus*.

Citrus aurantifolia is of significance among the people of the Cordilleras in the Philippines because it is usually used in folkloric medicine to treat nausea and fainting, headache, malaria, and sore throat. In addition, it can also be used in cleaning wounds. Plant parts usually involved among these treatments are rind, leaves, bark, roots, juice and stem[2]. A more recent study reported the antibacterial activity of lime or *Citrus aurantifolia* and other natural spices against a multiple drug resistant *Escherichia coli*[21]. This was consistent with the findings of this study since *C. aurantifolia* or lime juice exhibited partially active zone of inhibition against *E. coli.* Moreover, our MIC determination experiments revealed that among all the plant extracts used in our study, *C. aurantifolia* juice was the most effective since the test organisms *E. coli*, *B subtilis* and *S. aureus* were inhibited at a lower concentration of 62.5 µg/ml, suggesting again a broad antibacterial activity of the juice extract.

The results of the MIC determination experiments indicate that the gram positive organisms *B. subtilis* and *S. aureus* gave a general trend of relatively lower MIC values, (62.5 µg/ml for *B.subtilis* for all the extracts) and 62.5 to 125 µg/ml for *S. aureus*. These gram positive organisms were more sensitive to the extracts used since it gave low MIC values. The gram negative organisms *E. coli* and *P. vulgaris* however were more resistant to the plant extracts that were used since generally they require higher concentrations of the plant extract (125 to 250 µg/ml) in order to inhibit their growth, except for the

C. aurantifolia juice which had an MIC of 62.5 µg/ml for *E. coli*. The results of this present study were consistent with previous studies wherein gram negative organisms also showed greater resistance to other extracts from sponges and from honey[21-22]. A possible explanation given by some authors was that these gram negative organisms are surrounded by more complex cellular envelope, and therefore exhibit more permeability barrier so that they can afford greater resistance to many antibiotics[22-23].

No antifungal activity was observed in all of the 11 plant species evaluated in this present study. One possible explanation for this result is that fungi are generally more resistant to antimicrobials because of their strong and rigid cell walls. This observation was also noted in marine sponges wherein no antifungal activity was observed in the nine crude marine sponge extracts studied[22]. This does not mean that the plant extracts that were used in this present study have no antifungal activity. The antifungal activity of sunflower terpenoids have been earlier reported[4&24]. A mixture of two diterpene acids, kaurenoic and angeloylgrandifloric, was the most potent inhibitor of hyphal growth. Selective breeding to increase the amounts of these terpenoids in sunflower could improve the natural resistance to fungal pathogens. Flavonoids of *Citrus aurantifolia* were also reported to have antifungal activity against phytopathogenic and human pathogenic fungi[25]. Moreover, the antifungal activity of *Eupatorium* leaf extracts using petroleum ether as extraction medium has also been reported[26]. However, not much has been reported on the antifungal activity of *Agathis dammara* leaf extract so it would be interesting to work on this in future studies. No antifungal activity was observed in this present study for all the plant extracts used; this might also be due to lesser susceptibility of the plant extracts against the test organisms used or probably methanol was not efficient as an extraction medium to test for antifungal activity.

In conclusion, the results of this study suggest that the four plant extracts that gave antibacterial properties may act as alternative to chemical or synthetic bactericides since it was proven that they can control or inhibit the growth of pathogenic organisms. This study has therefore validated the use of these four plant extracts in folkloric medicine in the indigenous communities of the Cordillera Region in the Philippines. The practice of indigenous peoples of the Cordilleras or *Igorots* to use plant extracts to treat diseases like malaria and sore throat and the use of plants for wound healing, headaches, nausea and fainting have really a scientific basis. Further pharmacological studies are therefore recommended like t purification of the major active plant constituents, that will help to elucidate their chemical structures and their specific mode of actions or mechanisms against common pathogenic organisms. The results of this present study confirmed that the Cordillera Region in the Philippines is a rich reservoir of plants that have medicinal uses that are potential sources for the discovery of active biological compounds that may further be explored for drug development in the future.

Acknowledgement

The financial support of the Commission on Higher Education – Grants in Aid Project (CHED- GIA) in the Philippines is hereby acknowledged. The authors are very much grateful to the support given to them by their respective institutions: The University of the Philippines Baguio (UPB), The Cordillera Career Development College (CCDC) and The Mt. Province State Polytechnic College (MPSPC). Assistance of the CHED-Zonal Research Center Office at St. Louis University, Baguio City, headed by Dr. Gaston Kibiten and Mr. Carl Johnson Anacin is also highly appreciated. Special thank you is extended to Professor Rizavel Addawe, of the Department of Mathematics and Computer Science, UP Baguio, for the statistical analyses. Authors are also grateful to the assistance given by Ms. Lea Dee Ojadas, Ms. Mary Rhovian Bakkal and Ms. Socorro Martha Megay Daupan in the laboratory experiments and field collections. This work is humbly dedicated to the late Mr. Leonardo Co, whose book "The Common Medicinal Plants of the Cordillera Region" served as inspiration for the authors to pursue this microbiological investigation on the antibacterial potential of different medicinal plants.

References

1 Tan ML, Community herbal; tapping medicinal plants, (1985) (Retrieved from https://idl- bnc.idrc.ca/dspace/ bitstream/ 123456789/24728/1/110937.pdf, accessed October 28, 2008.

2 Co LL, *Common medicinal plants of the cordillera region* (Northern Luzon Philippines), [Community Health Education, Services and Training in the Cordillera Region (CHESTCORE), Baguio City], 1989.

3 Lirio LG, Hermano ML & Fonatanilla MQ, Antibacterial activity of medicinal plants from the Philippines, *Pharmaceut Biol*, 35 3(1998) 57-359.

4 Ragasa CY, De Luna RD & Hilofina JG, Antimicrobial terpenoids from *Pterocarpus indicus*, *Nat Prod Res*, 19(4) (2005) 305-309.

5 Hansel CG & Lagare VB, Antimicrobial screening of 10 Maranao medicinal plants: A preliminary study. *Mindanao Journal*. Retrieved from http://www.msumain.edu.ph/ mindanaojournal/pdf/mj4-2005.pdf (2005)

6 Alade PI & Irobi O, Antimicrobial activities of crude leaf extracts of *Acalypha wikensiana*, *J Ethnopharmacol*. 39 (1993)171-174.

7 Quinto E & Santos M, Microbiology, In: edited by Guevarra BQ, (Espana Manila. Philippines: University of Santo Tomas Publishing House), 2005.

8 Ertuk O, Kati H, Yayli N, & Demirgag Z, Antimicrobial properties of `*Silene multifida* (Adams) Rohrb. plant extracts, *Turkey J Biol*, 30 (2006)17-21.

9 Nester EW, Anderson DG, Roberts CE Jr, Pearsall NN, Nester MT *et al.*, Microbiology, A Human Perspective, IV edn, (McGraw Hill Higher Education), 2004.

10 Cowan MM, Plant Products as Antimicrobial Agents, *Cl Microbiol Rev*, 12(4) (1999) 564–582.

11 Smith RF & Shay DE, Steroid lysis of protoplasts and effects of stabilizers and steroid antagonists, *Appl Environ Microbiol*, 13(5) (1965)706-712.

12 Cushnie TPT & Lamb AJ, Detection of galangin-induced cytoplasmic membrane damage in *Staphylococcus aureus* by measuring potassium loss*, J Ethnopharmacol,* 101 (2005) 243–248.

13 Mirzoeva OK, Grishanin RN & Calder PC, Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria, *Microbiol Res,*152 (1997) 239–246.

14 Olekszek WA, Hoagland RE & Zalblotowicz RM, Ecological significance of plant saponins, Principles and Practices of Plant Ecology, (CRC Press LLC, Boca. Raton, FL), 1999, 451-465.

15 Gee JM, Wortley GM, Johnson IT, Price KR, Rutten AAJ *et al.,* Effect of sapnins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations in vitro*, Toxicol in Vitro*, 10 (1996) 117-128.

16 Reed JD, Nutritional toxicology of tannins and related polyphenols in forage legumes, *J Animal Sci,*73 (1995) 1516-1528.

17 Scalbert A, Antimicrobial properties of tannins, *Phytochemistry*, 30(12) (1991) 3875-3883.

18 Hopkins W, Introduction to Plant Physiology, (New York: John Wiley and Sons, Inc.), 1999, 493.

19 Johnson TL, Amber: Arboral Gold, University of Kentucky http://www.uky.edu/AS/Geology/webdogs/amber/welcome.html; accessed March 8, 2010.

20 Watanabe, Coumarin and flavone derivatives from estragon and thyme as inhibitors of chemical mediator release from RBL-2H3 Cells, *Biosci Biotechnol Biochem*, 69(1) (2005) 1-6.

21 Rahman S, Parves AK, Islam R & Kahn MH, Antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh, *Ann Cl Microbiol Antimicrob,*10 (2011) 10.

22 Pangan AC, Uy FA & Oclarit JM, Antimicrobial properties of some marine sponges (*Porifera*) from Mactan, Cebu, Philippines, *The Philippine Scientist*, 44 (2007) 35-45.

23 Viveiros M, Dupont M, Rodrigues L, Couto I, Davin-Regli A. *et al.,* Antibiotic stress, genetic response and altered permeability of *E. coli, Plos One*, 2 (4) (2007) 365.

24 Picman AK, Schneider EF & Ger-henzon J, Antifungal activities of sunflower terpenoids, *Biochem Syst Ecol,* 18 5 (1990) 325-328.

25 Johann S, Smana Jr A, Pizzolatti MG, Schripsema J, Braz-Filho R. *et al.*, Complete 1H and 13C NMR assignments and anti fungal activity of two 8-hydroxy flavonoids in mixture, *Anais da Academia Brasileira de Ciências*, 79(2) (2007):215-22.

26 Gupta M, Mazumber UK, Chaudhuri I, Chadudhusi RK, Bose P *et al*., Antimicrobial activity of *Eupatorium ayapana, Fitoterapia*, 73(2) (2002) 168-70.