Phytochemical screening and Antibacterial activity of selected medicinal plants of Bayabas, Sablan, Benguet Province, Cordillera Administrative Region, Luzon, Philippines

Teodora D Balangcod¹, Vilma L. Vallejo², Melba Patacsil², Orlando Apostol², Lianne Marie Victoria A. Laruan⁴, Jayjay Manuel¹, Sonny Cortez⁵, & Rosemary M. Gutierrez¹

¹Department of Biology, College of Science, University of the Philippines Baguio,  
²Department of Physical Sciences, College of Science, University of the Philippines Baguio,  
³Department of Biology, College of Arts and Sciences, University of the Cordilleras,  
⁴Department of Pharmacy, School of Natural Sciences, Saint Louis University,  
⁵Natural Sciences Department, College of Arts and Sciences, Baguio Central University  
E-mail: tdbalangcod@yahoo.com

Received 22.02.12, revised 20.06.12

The traditional knowledge on medicinal plants, when confirmed by antimicrobial and phytochemical studies, can lead to the development of drugs and plant-based medicine. Twenty selected plants that are used for the treatment of various ailments by the Ibaloi tribe in Bayabas, Sablan were tested for antibacterial activity and analysed for phytochemical constituents. A modified Kirby-Bauer method was used to test the antibacterial potential of the plants against gram-positive and gram-negative bacteria such as Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Pseudomonas aeruginosa. Results showed that out of the twenty plant samples, seven showed positive results in varying degrees. Methanol extracts from the same twenty plants were analysed for their secondary metabolites using standard methods. The extracts were macerated with 95% methanol and were tested for the presence or absence of saponins, alkaloids, glycosides, anthraquinones, steroids, flavonoids and tannins. Bidens pilosa gave positive results for all secondary metabolites while the other plants contained one or two metabolites. The presence of the secondary metabolites can contribute to the antibacterial potential of the plants. The information derived herein provides scientific basis for the ethno-botanical knowledge of the local community. The phytochemicals of the plants when developed further, can offer a less-expensive treatment for various ailments.

Keywords: Bayabas, Sablan, Ethnobotany, Ibaloi tribe, Plant-based medicine, Secondary metabolites, Traditional knowledge  
IPC Int. Cl.⁸: C01, C07, C08, A61K 36/00

The prevalence of diseases, some brought by climate change, and the increasing prices of medicine have resulted in the demand for discovery of less expensive but more potent sources of drugs. Plants are one of the best sources of potent drugs. In fact, most plant-based medicines that are developed by pharmaceutical companies have their beginnings in ethnomedicine. The traditional knowledge on medicinal plants that is inherent within local communities is a very important source of information that continually provides the present-day herbal remedies. In recent years, researches in various countries have been directed towards discovering the medicinal uses of plants through phytochemical and antimicrobial screening¹⁰.

Essentially, this is the initial step towards discovering and harnessing the potential of plants to be developed into drugs. In the Philippines, the Department of Science and Technology has proclaimed ten medicinal plants¹⁰ which are products of research and some are already in the market in the form of food supplements and herbal drugs.

As diseases and health problems are experienced by the human population, the need for discovery of potent drugs from plant resources continues to be in demand. The value of traditional knowledge on medicinal plants is being recognized and hence researches are directed towards the local communities for answer to the increasing health problems¹¹-¹⁵. The objectives of this study are three-fold: (1) to perform antibacterial screening of selected plants that are

*Corresponding author
being used by the local community in Sablan as medicine for various ailments specifically ailments that are bacteria-related; (2) to determine the secondary metabolites present in the medicinal plants through phytochemical screening and (3) to validate the ethnomedicinal knowledge of the local community in Bayabas, Sablan through antibacterial screening methods.

The establishment of a scientific basis for the claimed medicinal properties of the plants used by local communities is important. This not only provide scientific information for continued use of plant as curative agents for a certain ailment or ailments but also unlock opportunities for discovery and development of new plant-based drugs that are less expensive yet equally or more potent.

**Methodology**

Prior to the conduct of the study, a prior informed consent was sought from the local community of Bayabas, Sablan, Benguet Province. Twenty plants which were acknowledged by the local residents to have medicinal value were tested for their antibacterial property and phytochemical contents. These plants, traditionally used by the local community as folk medicine for generations are listed in Table 1. Samples of these twenty plants were collected, air dried for two weeks and finely chopped. These were placed in sealed containers until ready for use.

**Preparation of extracts**

For each plant, 100 gm of the finely chopped samples were macerated using 95% methanol for 24 hours. The mixtures were then filtered and concentrated in a water bath with a maintained temperature of 50°C until 20% of the filtrate was left. These extracts were used both for the phytochemical screening of secondary metabolites and antibacterial activity.

**Phytochemical screening**

Phytochemical screening was performed following the method of Guevara et al. (2005). The metabolites to be determined were: glycosides, tannins alkaloids, steroids anthraquinones, flavonoids, and saponins.

**Antibacterial screening**

The antibacterial screening was conducted following a modified Kirby-Bauer method and the selected plants were screened against gram-positive and gram-negative bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The bacteria used for the

---

**Table 1—Traditionally used medicinal plants of Bayabas, Sablan, Benguet province and the secondary metabolites present in methanol extracts**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Alkaloids</th>
<th>Steroids</th>
<th>Anthra-quinones</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ageratum conyzoides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Artemisia vulgaris</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. Bidens pilosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. Blumea balsamifera</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5. Cassia occidentalis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. Centella asiatica</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7. Coffea arabica</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8. Cymbopogon citratus</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. Eleusine indica</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10. Gliricidia sepium</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11. Hibiscus rosa-sinensis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12. Imperata cylindrica</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13. Jatropha curcas</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14. Livistona rotundifolia</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15. Mangifera indica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16. Mimosa pudica</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17. Psidium guajava</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18. Tinospora ramphii</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19. Tithonia diversifolia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20. Vitex negundo</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
study were obtained from the Natural Science Research Institute of the University of the Philippines Diliman. All the organisms were maintained at 4°C in slants of nutrient agar. All the glassware and other materials needed for antibacterial screening were thoroughly sterilized.

**Preparation of the inoculum**

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test-tubes containing nutrient agar. The test-tubes were incubated for 24 hrs at 37°C. The obtained microorganisms were standardized using normal saline solution to obtain a uniform population density of the bacteria.

**Preparation of media and zone of inhibition**

The Kirby-Bauer method or test otherwise known as the disk-diffusion method is the most widely used antibiotic susceptibility test in determining the kind of antibiotics to be used in treating an infection. This method relies on inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism, and then filter paper discs which are impregnated with a specific concentration of a particular antibiotic, are placed on the medium. The organism will grow on the agar plate while the antibiotic “works” to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a zone of inhibition can be observed and measured to determine the susceptibility to an antibiotic for that particular organism.

In the present study, two modified Mueller-Hinton agar were prepared. The first, which is called base agar, was prepared by following the concentration as instructed on the label. This mixture was poured on the Petri dishes and allowed to solidify. The second preparation was with half of the quantity of Mueller-Hinton agar diluted with the same amount of distilled water as prescribed for the base agar. The second modified preparation is the soft agar. This solution was mixed with the saline solution which has been suspended with bacteria and then poured over the top of the base agar. The soft agar plus bacteria suspension was allowed to solidify. Dried and sterilized filter paper discs were then impregnated with known amount of the plant extracts using micropipette. The discs were then arranged on the nutrient agar medium using sterilized forceps. Antibiotic discs such as kanamycin, streptomycin, vancomycin and chloramphenicol were used as positive control while discs soaked with methanol were used as negative control. These were also arranged on the petri plates which were seeded with the microorganisms. The plates were then incubated at 37°C for 24 hours. After 24 hours, the antibacterial activity of the test samples and the antibiotics were determined by measuring the diameter of zone of inhibition in millimeter using a celluloid ruler. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

**Results and discussion**

Phytochemical analysis showed the presence of secondary metabolites such as tannins and glycosides in all 20 plant samples tested (Table 1). Steroids are generally present in most of the 20 plants except in Artemisia vulgaris, Imperata cylindrica and Livistona rotundifolia. Almost all the plants contained alkaloids except in Cassia occidentalis, Coffea arabica, Gliricidia sepium and Hibiscus rosa-sinensis while flavonoids are generally present except for Blumea balsamifera, Centella asiatica and Cymbopogon citratus. Saponins and anthraquinones are present only in few of the plant extracts. Interestingly, Bidens pilosa contain all the secondary metabolites tested. Mangifera indica and Psidium guajava showed positive results to almost all secondary metabolites except saponins.

Antibacterial screening of the 20 plants showed that C. arabica exhibited a zone of inhibition from 10 to 14 mm (Table 2). Based on standards (Guevarra et al16 Table 3), the activity of this plant exhibits antibacterial potentiality from partially active to active against all four test organisms with the highest zone of inhibition observed against S. aureus. Psidium guajava shows a relatively high zone of inhibition in three bacteria namely E. coli, S. aureus and P. aeruginosa. It is inactive against S. typhimurium. For E. coli, only two out of the twenty plants gave a partially active to active zone of inhibitions with Vitex negundo yielding the highest zone of inhibition of 17 mm followed by P. guajava. Six plants, viz. B. pilosa, C. occidentalis, C. arabica, M. indica, P. guajava and Tithonia diversifolia were active against S. aureus. Only two plants, namely C. arabica and P. guajava showed partially active to active zones of inhibition against P. aeruginosa. Likewise,
C. arabica and M. indica showed partially active reaction against S. typhimurium, with zones of inhibition of 12 mm and 11 mm respectively.

There are seven out of twenty plants or 35% of the total plants studied having potential antibacterial activity with varying effects as can be inferred from their zones of inhibition which range from partially active to active (11-17 mm) based on the general standards (Guevarra et al. 16).

Table 4 shows that the antibiotics such as kanamycin, streptomycin, chloramphenicol and vancomycin that were used as positive control have varying susceptibility against the four bacteria. Vancomycin showed very small zones of inhibition while the first three antibiotics have relatively larger zones of inhibition. Interestingly, chloramphenicol showed the highest zones of inhibition against S. aureus and S. typhimurium. Generally, three antibiotics namely kanamycin, streptomycin and chloramphenicol showed antibacterial activities against all four bacteria while vancomycin showed antibacterial potential only against E. coli. The zones of inhibition and their corresponding inferences, based from Guevarra et al.16 were used as the standard.

From among the twenty plants tested for their antibacterial activity, P. guajava showed a broad spectrum antibacterial activity. The activity was relatively strong against the three out of four test-bacteria used. The result for the activity of P. guajava parallels that of some of the earlier findings19-21. The authors concluded that P. guajava showed strong antibacterial activity against different bacteria. Next to P. guajava is C. arabica which is active against S. aureus, P. aeruginosa and S. typhimurium.

The activity exhibited by P. guajava can be due to the secondary metabolites that it contains. The secondary metabolites detected in this plant are alkaloids, steroids, anthraquinones, flavonoids, tannins and glycosides. These components have been separately reported by various authors to exhibit antibacterial and antioxidant activities21-22. Flavonoids
have been demonstrated as good antioxidants while flavonoids like morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin-3-O-arabinoside, guaijavarin and quercetin have been isolated and found to exhibit good antibacterial activity against some enteric bacteria.

Next to P. guajava is C. arabica. Antibacterial screening of the leaf extracts showed a broad potential antibacterial activity. It has a relatively strong antibacterial activity against S. aureus, with a zone of inhibition of 14 mm. Conversely, it is also partially active against P. aeruginosa and S. typhimurium. Coffea arabica also contains flavonoids and other secondary metabolites such as steroids, anthraquinones, tannins and glycosides as in P. guava. These two plants display a potential to have broad spectrum antibacterial activities. Both plants have comparable effect with streptomycin (Tables 2 & 3).

Mangifera indica is also partially active against the two bacteria such as S. aureus and S. typhimurium. All three plants, P. guajava, C. arabica and M. indica contain similar phytochemicals which probably contribute to their antibacterial potentials.

The other plants tested which also showed potential antibacterial activity to a specific bacteria is Vitex negundo, which exhibited a high zone of inhibition only to E. coli but not for the rest of the test organisms. Similarly, T. diversifolia and C. occidentalis were active only against S. aureus. In terms of the test organism used, the most number of plants with antibacterial potential are against S. aureus. In general, not all the plants claimed to have medicinal properties by the local communities are active against the test organisms. Nonetheless, based from the results of this study, some traditionally used medicinal plants have potential to be developed further as plant-based drugs.

Conclusion

The continuous emergence of diseases, the emergence of drug-resistant organisms and the increasing prices of medicines call for the discovery of new less expensive plant-based medicines. This study has shown that some plants used by the local communities as folk medicine have high potential as antibacterial agents. Conversely, the antibacterial potential can be attributed to the secondary metabolites that are present in varying degrees. Results from the present study can provide baseline information about the antibacterial potential and phytochemical constituents of some traditional medicinal plants. Plants such as P. guajava, M. indica and C. arabica are proven to be of medicinal value due to their antibacterial activity and therefore can be developed further as plant-based drugs.

Acknowledgement

The authors are grateful to the Commission on Higher Learning –Grants in Aid (CHED-GIA) for the financial assistance, Dr. G. Kibiten and Dr. P. Macansantos for their endorsement and support. We also thank the University of the Philippines Baguio, for permitting to use the laboratory facilities for the conduct of experimental part of the study. The local officials of Sablan for allowing to carry out this project; the local residents for sharing their traditional knowledge on medicinal plants. To Vladimir, Eunice, Sheena, Berlie, Kay, Amor, Alice, Kryssa, Ashlyn and Bino and my Botany 109 students, for their help in the collection and processing of the plant samples; to Mark and Jun for the endless errands, we appreciate your help.

References


