Antimicrobial Activity and Phytochemical Screening of Selected Medicinal Plants Used by Mamanwa in Caraga Region

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ABSTRACT

The study was conducted to scientifically validate 15 herbal plants traditionally utilized by the Mamanwa of Mindanao for child health care. Phytochemical screening for alkaloids, anthraquinones, flavonoids, saponins, steroids and tannins was done as well as antimicrobial activity testing of the crude ethanolic and methanolic extracts against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa. Result of Phytochemical analysis showed the presence of anthraquinones in the 14 plants sample except alibangbang leaves, 13 were present with alkaloid and steroid except tagbak tubers and togup bark, 9 plant sample contains flavonoids, 5 plant sample contains saponins and 4 plant sample contains tannins. Antimicrobial result from ethanolic and methanolic extract shows only three herbal plants were found to be inactive with the four test organisms and the rest of the test plants were partially active, active and very active. Alibangbang leaves with ethanolic and methanolic extract showed high affinity or very active with S. aureus, P. aeruginosa and E. coli while lunas bark in ethanolic extract was found to be very active with S. aureus and togup bark in ethanolic extract showed very active with P. aeruginosa.

KEYWORDS

Science, Antimicrobial analysis, bioactive components, phytochemical analysis, Philippines
INTRODUCTION

Herbal medicine involves the use of plants for medicinal purposes. The term “herb” includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. The quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethno-botanical significance for a complete range of biological activities, which range from antibiotic to anticancerous substances. Several plant and herb species used traditionally have potential antimicrobial and antiviral properties (Shelef, 1983; Zaika, 1988) and this has raised the optimism of scientists about the future of phyto-antimicrobial agents (Das, Mujib & De, 1999).

In the Philippines, emphasis has been placed on indigenous plants to produce safe, efficacious, and affordable drugs for primary health care, the impact of new technologies has been tremendous as far as the development of potential drugs especially with the presence of indigenous tribes in Mindanao specifically the Manobo and the Mamanwa, with the latter as the focus of the study.

The Mamanwa live in the northeastern provinces of Surigao and Agusan del Norte and they rely on the subsistence economy which is a hand- to- mouth existence. Most of the food gatherers who move from one place to another depending upon the supply of food found in the place.

Due to hardship, the Mamanwa tend to use herbal medicine for their health and medicinal needs especially for child care. The biggest tribes of Mamanwa are found in Cantugas, Maiinit, Surigao Del Norte having eighty eight (88) families and each family has an minimum of children of seven (7) and a maximum of fifteen (15). Most of the herbal medicines are often used for the cure of stomach- ache, bloody diarrhea, healing wounds, scabies, insect bites, itchiness, to relieve toothache, burns, scalds, eye sores, lowering of fever, headache, skin diseases, asthma, sore throat, relieves cough, cold, diuretic agent, kidney stones, constipation, snake bite, dyspepsia, treat mouth ulcers and tongue blisters and anti-inflammatory agent.

The study was conducted to scientifically validate 15 herbal plants traditionally utilized by the Mamanwa of Mindanao for child health care namely: the Albahaka (Ocimum basilicum), Alibangbang (Bauhinia monandra), Angelika (Bryophyllum pinnatum), Elepante (Elephantopus Scaber Linn.), Gabon (Hilbas (Artemisia vulgaris Linn)), Kalabo (Origanum vulgare), Lunas (Lunasia amara Blanco), Makulibhag (Rabelaisaphilippinensis), Sawan-sawan (Blumea balsamifera), Sinaw-sinaw (Peperomiapellucide), Tagbak (Alpiniaegans K.), Talawatawa (Musseanda philipicca), Tawa-tawa (Euphorbia hirta Linn) and Togup (Artocarpusaltilis) through
the process of phytochemical analysis and antimicrobial assay.

Figure 1. The Mamanwa People

OBJECTIVES OF THE STUDY

This study primarily aimed to identify the ethnomedicinal plants used by the Mamanwa people to further evaluate the presence of the secondary metabolites through phytochemical screening and the potential antimicrobial activity properties of fifteen (15) herbal plants from Figure 2 commonly used by the Mamanwa.

More specifically, the study aimed to:

1. determined the most commonly used Ethnomedicinal plants used by the Mamanwa in Mindanao;
2. determined the phytochemicals found in the selected medicinal plants; and,
3. determined the antimicrobial activities of the selected plants using crude ethanolic and methanolic extracts against the test microorganisms of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*
Figure 2. Mamanwa Herbal Plant Samples
Figure 2. Mamanwa Herbal Plant Samples
METHODOLOGY

Research Design

Identification & Preparation of Plant Samples

Plant Extract

*against bacteria (S. aureus, B. subtilis, P. aeruginosa & E. coli)

Antimicrobial Assay
(Paper Disc Diffusion)

Zone of inhibition

Phytochemical Screening

presence of Phytochemicals
(Inactive partially active, active and very active)
(alkaloids, anthraquinones, flavonoids, saponins, steroids & tannins)

Figure 3. Schematic diagram for the process of the study

Preparation of Plant Samples

Fresh plant leaves and barks were collected at Cantugas, Mainit, Surigao del Norte where the Mamanwa are residing. The plant samples were identified as constantly used for different cure of minor to major diseases by an eighty eight (88) year old herbolaria Nanay Felisa Hubasan and the local people of the Mamanwa tribe. Then, the fresh plant leaves and bark were cut into smaller pieces and weighed about 100g in an Erlenmeyer flask and mixed with analytical grade ethanol and methanol solution and submerged the plant materials and kept soaked for 48 hours. The extracts were then filtered using Whatman #2 filter paper with gentle suction. The flask and plant material was rinsed with fresh portions of alcohol. After washing the plant material was transferred to the funnel, combining the washing of the first filtrate. Gentle suction was applied to complete the collection of the plant extract; then the plant residue was discarded. The filtrate plant extract was concentrated over a steam bath at temperature below 50°C to about 20 ml. The concentrations of the stock plant extract recorded as grams of dried plant material per mL of the extract obtained. The extract was then stored in cold (0 to -5°C) and labeled property with the name of the plant, concentration of the plant and date of extraction.
Antibacterial Assay by Paper Disc Diffusion Method

The bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were swabbed on the different nutrient agar surface using soft tip to evenly distribute the bacteria. Paper discs were dipped into the extract. It was then put onto the surface of the media inoculated with the bacteria respectively, then incubated inversely at 30°C for 48 hours to allow the bacterial growth. Antibacterial activities of the extract were then determined by measuring the respective zone of inhibition in millimeter for three replicates with ethanolic and methanolic extracts were used.

Phytochemical Screening for Secondary Metabolites

The stock plant extract of the different leaves underwent several tests. The phytochemical analysis of the plant extracts was carried out as described by Guevara, et.al (2005). This is the screening for the presence of secondary metabolites such as alkaloids, steroids, anthraquinones, flavonoids, saponins, and tannins by qualitative method.

To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff’s reagent were used to treat 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. Exact 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, to test for presence of tannins, about 0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins. Borntrager’s test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinones. The presence of cardiac glycosides was confirmed by Lieberman’s test, Salkowski test and Keller-Killani test (Culei, 1982; Sofowora, 1993; Trease and Evans, 2002) and cyanogenic glycosides were carried out according to the methods described by Harborne (1973) and Trease and Evans (1983).
RESULTS AND DISCUSSION

The results on the analysis of the antimicrobial activity of the tested herbal plants using ethanolic extract are shown in Table 1. The interpretations with the zone of inhibition has values of <10mm, inactive; 10 -13mm, partially active; 14- 19mm, active; >19mm, very active (Guevara 2005).

Table 1. Antimicrobial Activity of 15 Sample Herbal Plants with Interpretations (Ethanolic Extract)

<table>
<thead>
<tr>
<th>Samples Local &amp; Scientific Name</th>
<th>Zone of Inhibition (mm) and Activity of Sample Herbal Plants (Ethanolic Extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Albahaka (Ocimum basilicium)</td>
<td>14.4 Active</td>
</tr>
<tr>
<td>Alibangbang (Bauhinia monandra)</td>
<td>19.9 Very Active</td>
</tr>
<tr>
<td>Angelika (Bryophyllum pinnatum)</td>
<td>16.7 Active</td>
</tr>
<tr>
<td>Elepante (Elephantopus Scaber Linn.)</td>
<td>13.9 Active</td>
</tr>
<tr>
<td>Gabon (Plectranthi Amboinici Folium)</td>
<td>0 Inactive</td>
</tr>
<tr>
<td>Hilbas (Artemisia vulgaris Linn.)</td>
<td>9.7 Partially Active</td>
</tr>
<tr>
<td>Kalabo (Origanum vulgare)</td>
<td>8.6 Inactive</td>
</tr>
<tr>
<td>Lunas (Lunasia amara Blanco)</td>
<td>19.9 Very Active</td>
</tr>
<tr>
<td>Makulibhag (Rabelaisia philippinensis)</td>
<td>8 Inactive</td>
</tr>
<tr>
<td>Sawan-sawan (Blumeabalsamifera)</td>
<td>12.7 Partially Active</td>
</tr>
<tr>
<td>Sinaw-sinaw (Peperomia pellucide)</td>
<td>0 Inactive</td>
</tr>
<tr>
<td>Tagbak (Alpinia elegans K.)</td>
<td>10.3 Partially Active</td>
</tr>
<tr>
<td>Talawatawa (Muscaenadaphilipica)</td>
<td>14.6 Active</td>
</tr>
<tr>
<td>Tawa-tawa (Euphorbia hirta Linn.)</td>
<td>13.7 Active</td>
</tr>
<tr>
<td>Togup (Artocarpusaltilis)</td>
<td>15.3 Active</td>
</tr>
</tbody>
</table>
Results revealed that, the activity S. aureus, these are (4) plants classified as inactive (Gabon, Kalabo, Makulibhag and Sinaw-sinaw), three (3) herbal plants were partially active (Hilbas, Sawan-sawan and Tagbak), six (6) medicinal plants were classified as active (Albahaka, Angelika, Elepante, Talawatawa, Tawa-tawa and Togup) and two tested plants was very active namely; Alibangbang and Lunas.

For the bacteria P. aeruginosa activity, it was found out that nine (9) herbal plants were inactive (Albahaka, Angelika, Elepante, Gabon, Hilbas, Lunas, Makulibhag, Sinaw-sinaw and Tagbak), two (2) plants are partially active (Kalabo and Talawatawa) and two (2) tested herbal plants are very active (Alibangbang and Togup).

For E.coli microbial activity shows four (4) herbal plants were inactive with this bacteria (Gabon, Kalabo, Makulibhag and Sinaw-sinaw) four (4) plants was partially active (Albahaka, Hilbas, Sawan-sawan and Tagbak), six (6) plants was active (Angelika, Elepante, Lunas, Talawa-tawa, Tawa-tawa and Togup) and only one was very active which was the Alibangbang.

Finally for B. subtilis, four (4) were inactive with this bacteria (Gabon, Kalabo, Makulibhag and Sinaw-sinaw), four (4) herbal plants were partially active (Albahaka, Hilbas, Sawan-sawan and Tagbak), seven (7) medicinal plants were active (Alibangbang, Angelika, Lunas, Talawa-tawa, Tawa-tawa and Togup) and none of this plant was classified as very active.

Table 2. Antimicrobial Activity of 15 Sample Herbal Plants with Interpretations (Methanolic Extract)

<table>
<thead>
<tr>
<th>Samples Local &amp; Scientific Name</th>
<th>Zone of Inhibition (mm) and Activity of Sample Herbal Plants( Methanolic Extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Albahaka (Ocimum basilicum)</td>
<td>17.9 Active</td>
</tr>
<tr>
<td>Alibangbang (Bauhinia monandra)</td>
<td>21.3 Very Active</td>
</tr>
<tr>
<td>Angelika (Bryophyllumpinнатum)</td>
<td>8.9 Inactive</td>
</tr>
<tr>
<td>Elepante (ElephantopusScaber Linn.)</td>
<td>13.2 Partially Active</td>
</tr>
<tr>
<td>Gabon (PlectranthiAmboinici Folium)</td>
<td>0 Inactive</td>
</tr>
<tr>
<td>Hilbas (Artemisia vulgaris Linn.)</td>
<td>7.3 Inactive</td>
</tr>
<tr>
<td>Kalabo (Origanumvulgare)</td>
<td>9.7 Partially Active</td>
</tr>
</tbody>
</table>
The activity against *S. aureus* were six (6) plant samples (Angelika, Gabon, Hilbas, Makulibhag, Sinaw-sinaw and Tagbak) were inactive with *S. aureus*, four (4) are partially active (Elepante, Kalabo, Sawan-sawan and Talawa-tawa), four (4) are active (Albahaka, Lunas, Tawa-tawa and Togup) and only one was found out to be very active which was Alibangbang. Results obtained from the test of *P. aeruginosa* shows nine (9) among the tested plants were inactive (Albahaka, Angelika, Elepante, Gabon, Hilbas, Lunas, Makulibhag, Sinaw-sinaw and Tagbak), two (2) are partially active (Sawan-sawan and Talawa-tawa), three (3) was active (Kalabo, Tawa-tawa and Togup) and Alibangbang was very active with *P. aeruginosa*.

For *E. coli* activity, six (6) are inactive with this test bacteria (Angelika, Gabon, Hilbas, Makulibhag, Sinaw-sinaw and Tagbak), three (3) are partially active (Elepante, Kalabo, and Tawa-tawa), five (5) are active with this organism (Albahaka, Lunas, Sawan-sawan, Talawatawa and Togup) and only one was very active with *E. coli* which was Alibangbang.

Further, five (5) of this herbal plants were inactive with *B. subtilis* (Angelika, Gabon, Hilbas, Makulibhag and Sinaw-sinaw), five (5) were partially active (Elepante, Kalabo, Sawan-sawan, Tagbak and Tawa-tawa), and five (5) were active with *E. coli* antimicrobial activity which were Albahaka, Alibangbang, Lunas, Talawatawa and Togup.

The detection of the active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects. The work described in this study does not consist of a new method of identifying active principles in a given plant extract, a simple qualitative analysis modified by Guervarra, 2005 was used in

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Activity 1</th>
<th>Activity 2</th>
<th>Activity 3</th>
<th>Activity 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunas (<em>Lunasiaamara Blanco</em>)</td>
<td>14.3 Active</td>
<td>7 Inactive</td>
<td>15.7 Active</td>
<td>17.4 Active</td>
</tr>
<tr>
<td>Makulibhag (<em>Rabelaisiphiliippensis</em>)</td>
<td>7 Inactive</td>
<td>0 Inactive</td>
<td>7 Inactive</td>
<td>7.8 Inactive</td>
</tr>
<tr>
<td>Sawan-sawan (<em>Blumeabalsamifera</em>)</td>
<td>11.6 Partially Active</td>
<td>12.7 Partially Active</td>
<td>13.7 Active</td>
<td>11.7 Partially Active</td>
</tr>
<tr>
<td>Sinaw-sinaw (<em>Peperomiapellucide</em>)</td>
<td>0 Inactive</td>
<td>0 Inactive</td>
<td>0 Inactive</td>
<td>0 Inactive</td>
</tr>
<tr>
<td>Tagbak (<em>Alpinialegans K.</em>)</td>
<td>8.8 Inactive</td>
<td>8.8 Inactive</td>
<td>9.3 Inactive</td>
<td>9.6 Partially Active</td>
</tr>
<tr>
<td>Talawatawa (<em>Mussaendaphilipica</em>)</td>
<td>12.7 Partially Active</td>
<td>12 Partially Active</td>
<td>14.1 Active</td>
<td>14 Active</td>
</tr>
<tr>
<td>Tawa-tawa (<em>Euphorbia hirta Linn</em>)</td>
<td>14.7 Active</td>
<td>15.3 Active</td>
<td>12.7 Partially Active</td>
<td>12.4 Partially Active</td>
</tr>
<tr>
<td>Togup (<em>Artocarpusaltitis</em>)</td>
<td>18.7 Active</td>
<td>17.1 Active</td>
<td>15.7 Active</td>
<td>18.5 Active</td>
</tr>
</tbody>
</table>
the identification of the six common classes of phytochemicals, namely, alkaloids, anthraquinones, saponins, tannins, flavonoids and steroids.

The presence of the Phytocompounds found in the Mamanwa medicinal plants is shown in Table 3.

Table 3. Summary on the Presence of the Active Phytocompounds in the Fifteen (15) Medicinal Plants of Mamanwa Tribe

<table>
<thead>
<tr>
<th>Name of Medicinal Plant</th>
<th>Alkaloid</th>
<th>Anthraquinones</th>
<th>Steroid</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavonoid</th>
<th>Total Nos. of Phytocompounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albahaka</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>Alibangbang</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Angelika</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Elepante</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Gabon</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Hilbas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Kalabo</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Lunas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Makulibhag</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Sawan-sawan</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>Sinaw-sinaw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Tagbak</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Talawatawa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Tawa-tawa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Togup</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

Results revealed from Table 3 that two tested plants contains six (6) secondary metabolites, the Albahaka and Sawan-sawan, two tested plants contains five (5) bioactive components –the Elepante and Makulibhag. Four tested plants contain four (4) secondary metabolites namely; Angelika, Lunas, Talawa- tawa and Tawa-tawa, another tested plants contains three(3) bioactive components there were Alibangbang, Hilbas, Gabon, Kalabo, Sinaw-sinaw and Togup. Only one tested plant contains two bioactive components which is the Tagbak.
CONCLUSIONS

In phytochemical screening, six Phytocompounds were identified in terms of the presence of alkaloids, anthraquinones, flavonoids, steroids, tannins and saponins for its bioactive components. The findings of the study show that all of the fifteen Ethnomedicinal plants used by the Mamanwa tribe for child health care contains the least of three bioactive components and has maximum of six bioactive components.

Further, using ethanolic extract three ethnomedicinal plants with no antimicrobial activity namely; gabon, makulibhag and sinaw-sinaw. Using methanolic extract, four ethnomedicinal plants showed no antimicrobial activity namely; angelika, gabon, makulibhag and sinaw-sinaw. The results obtained that most of the ethnomedicinal plants used by the Mamanwa for herbal medicine have antimicrobial activity and have the presence of the secondary metabolites which are indications of its effectiveness as medicinal plants.

RECOMMENDATIONS

Based from the findings, the following are recommended:

Determination of the bioactive compounds present in the fifteen ethnomedicinal plants should undergo IR spectroscopy to identify the functional group(s) present and structural elucidation of the bioactive component using Gas Chromatography- Mass Spectroscopy (GC-MS) to come up of a novel compound(s) which will be beneficial especially in the cure of asthma for children, antioxidant property for cleansing effect and cure for leukemia for children and for the most as an anticancer agent.

It is further recommended that assessment of the bioactive components should be done to unique Mamanwa medicinal plants such as alibangbang leaves, lunas bark and togup bark should undergo bioactivity guided fractionation to identify the responsible molecule that might lead to the cure of anti-cancer activities.

LITERATURE CITED


