Phytochemical and Antibacterial Properties of the Ethanolic Leaf Extract of Merremia Peltata (L.) Merr. and Rubus spp.

Kristine Jay B. Perez, Mark Anthony I. Jose, Edgardo Aranico and Ma. Reina Suzette B. Madamba

Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Ave., Iligan City, 9200 Philippines

**ABSTRACT**

Rubus species have been cultivated and have been used traditionally for therapeutic purposes as an astringent, ophthalmic and restorative. In the Philippines, the leaves of this plant have been used as treatment for cough especially by the Higa-onon tribe in Rogongon, Iligan City. While Merremia peltata (L.) Merr. is a species of flowering vine in the morning glory family, Convolvulaceae and the leaves are used for stomach pains, skin sores and inflammation. This study was to determine the phytochemical and antibacterial properties of ethanolic and water leaf extracts of these two ethnomedicinal plants, Merremia peltata (L.) Merr. and Rubus spp. since not much has been known so far. The Kirby-Bauer disc diffusion method was used to test the antimicrobial activity of the two extracts at 5, 10, 15, 20 and 25 µg/ml concentrations. Four bacterial strains were used and were grown overnight at 37°C in the Mueller-Hinton Agar (MHA) plates with the treated discs. Results showed that both ethanolic and water extracts of Merremia peltata (L.) Merr. and Rubus spp. had inhibitory effect on Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. The ethanolic and water leaf extracts of both plants showed strong to moderate inhibitory activity against the test bacterial strains compared to positive controls. However, the inhibitory effect exhibited by ethanolic extract was significantly higher than that by water extract (p<0.05). Also, the inhibitory activity of both plant extracts on all test isolates showed no significant difference among concentrations. Phytochemical analysis revealed the presence of steroids and flavonoids in both plant samples. Alkaloids were found only in both plant samples. Alkaloids were found only in Merremia peltata (L.) Merr. while tannins were only found in Rubus spp. Thus, this may indicate that both plants have the potential bioactive substances for medicinal purposes as claimed by the Higaonon tribe.

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**INTRODUCTION**

Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or new therapeutic agents, food additives, agrochemicals, and industrial chemicals [2]. Despite the increasing use of synthetic drugs, it persisted as the “treatment of choice” since the therapy with synthetic antibiotics is not always possible because of their high cost as well as toxicity due to their extended use [14]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [7].

Phytochemical analysis of medicinal plants has shown that numerous secondary compounds in plants traditionally used for medicinal purposes have chemical properties effective at treating illness. This was observed in the work done by Wadood *et al.* [20], on the phytochemical analysis of medicinal plants occurring on local area of Mardan, where all extracts used have displayed anti-inflammatory, anti-analgesic, anti-cancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal. Studies like this are important and have commercial interest in both research institutes and pharmaceutical companies for the manufacturing of new drugs for treatment of various diseases.
Moreover, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and reemerging infectious diseases [16, 18]. There is therefore a need to search for more herbal medicines with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs [15].

This study used two plants specifically *Merremia peltata* (L.) Merr. and *Rubus* spp., which has been used ethno-medicinally among the Higa-onon tribe of Rogongon Iligan City as a remedy to various illnesses. The genus *Rubus* is comprised of more than 250 species of shrubs, often with prickles on stems and leaves within this genus from the rose (Rosaceae) family. They are found throughout the world, some are cultivated for their ornamental value, as a useful food source, while others are regarded as weeds [1]. *Rubus* spp. is a spreading, low, spiny shrub, rarely exceeding a meter in height. Leaves are pinnate with 5 to 7 leaflets. Leaflets are smooth or hairy, with lobed margins, obovate or broadly lanceolate, and 2 to 7 centimeters in length. Flowers are borne upon slender, prickly, hairy, 3 to 5 centimeter-long stalks and arise from the uppermost leaf axils. Calyx is hairy. Petals are white, broadly oblong, and constricted toward the base. Berries are red, conically elongated, and 1.5 to 2 centimeters across.

*Merremia peltata* (L.) Merr. is under the family Convolvulaceae known as bindweed or morning glory family. This plant is a coarse and widely spreading woody vine. Stems are 5 or more centimeters thick, and porous. Leaves are alternate, smooth, somewhat rounded, about 20 centimeters wide, those toward the ends of the branchlets being much smaller, heart-shaped at the base, pointed at the tip. Peduncle grows solitary from each of the upper leaf axils, erect and longer than the leaves. Flowers are large, golden-yellow, few to many, or clustered. Sepals are smooth, thick, oblong, 2 centimeters long. Corolla has a wide limb.

Thus, this study was conducted to determine their phytochemical property and investigate the bioactivity of ethanolic and water leaf extracts of *Merremia peltata* (L.) Merr. and *Rubus* spp. through Kirby-Bauer disc diffusion method against Gram-positive and Gram-negative bacteria inorder to assess their ethnomedicinal viability.

**MATERIALS AND METHODS**

*Collection of the Plant Samples:*

The *Merremia peltata* (L.) Merr. and *Rubus* spp. plant samples (Fig. 1) were collected from Rogongon, Iligan City during early morning. Disease free-looking and fresh plants were selected and its leaves were carefully washed with tap water thrice. A total of 700 g plant samples were zip locked inside polyethylene bags. Pressed samples were identified and authenticated at the Herbarium of the Department of Biological Sciences Mindanao State University, Iligan Institute of Technology where voucher specimen was prepared and deposited. The collected samples were hanged for days until sufficiently dried samples were obtained. The dried samples were placed in polyethylene bags ready for further processing in the laboratory.

*Preparation of the Plant Extracts:*

Five hundred (500) grams of the two air-dried plant samples were pounded till its powder state was achieved. Powdered samples were mixed in 100 ml 95% ethanol and enclosed in a sterile, dark container for 72 hours.

*Phytochemical Screening:*

The screening was done to determine the presence of bioactive chemical components in the two plant samples such as alkaloids, antraquinones, cyanogenic glycosides, unsaturated sterols, flavonoids and saponins according to the standard protocols described previously [2, 6, 15].

*Preparation of Microorganism:*

In this study, pure cultures of four different pathogens (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) were obtained from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology, University of the Philippines - Los Baños. Prior to the antimicrobial activity study, the test microorganisms were subcultured on Nutrient Agar (NA) media, incubated at 37°C for 24 hrs then inoculums of the test microorganisms were grown into Nutrient Broth (NB) and adjusted according to Mac Farlands Standard to achieve approximately 1x10⁵ CFU/ml before inoculating to the test media, Mueller-Hinton agar (MHA).

*Preparation of Controls:*

Streptomycin and Chloramphenicol were used as the positive control for the Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) respectively. Standard dosage used in the study for
Streptomycin was 10µg/ml and for Chloramphenicol was 30µg/ml based on the Kirby Bauer Chart for antibacterial susceptibility testing [10]. Ninety-five percent (95%) ethanol was used as the negative control.

Preparation of the Two Leaf Extracts as treatments:
The treatments used were 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml for both Merremia peltata (L.) Merr. and Rubus spp. extracts with three replications. The solvent used for the preparation of the extract was 95% ethanol.

Antimicrobial Activity:
The Kirby-Bauer Disk Diffusion Method [12] which is commonly used method for antimicrobial activity test was employed. Using the sterile micropipette tips, 100 µL or 0.1 mL of bacterial culture in broth were transferred to the MHA plates and was spread using a sterile L- rod aseptically to minimize contamination and erroneous result. Sterile Whatman’s No. 4 filter paper with a 6mm diameter was used as paper discs. The specific filter paper discs with 10 µL each of the test extracts of various concentrations and the controls were aseptically placed into the centre of divisions on each MHA plate using a forceps to test the activity of each extract as an antimicrobial agent. The plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition were measured using a standard ruler by measuring the diameter of the clear zone in the nearest millimeter. This was then subtracted with the diameter of the disc and the result was the measurement considered in this study.

Data Analysis:
In order to determine whether different concentrations of the extracts were significantly different or not with the controls, the average measurement of zone of inhibition for each concentrations were analysed using Analysis of Variance (ANOVA). with 95% level of significance. Post-hoc test was also applied to assess differences within treatments.

Results:
Phytochemical Sreening of the Ethanolic Leaf Extracts of Merremia peltata (L.) Merr. and Rubus spp:
Results of the phytochemical screening (Table 1) showed that only Merremia peltata (L.) Merr. leaf extract had alkaloid while Rubus spp. extract had tannins. Both plants had more steroids and flavonoids except for the two phytochemicals, anthraquinones and cyanogenic glycosides which were absent.

Antimicrobial Activity of the Ethanolic and Water Leaf Extract of Merremia peltata (L.) Merr. and Rubus spp: A. Against Gram-positive (G+) Bacteria:
The Merremia peltata (L.) Merr. and Rubus spp. ethanolic and water leaf extract concentrations: 5µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml had antimicrobial effect on the Gram-positive bacteria S.aureus and B.subtilis as compared to the negative controls: ethanol and water. A mean zone of inhibition of 5.7 mm was observed to be highly significant (p<0.05) for the ethanolic leaf extract of Merremia peltata (L.) Merr against S. aureus and B. subtilis at concentrations 20 ug/ml and 10 ug/ml respectively (Fig.1A). For Fig.1B, the mean zones of inhibition of 4.8 mm against S. aureus and 5.2 mm against B. subtilis were observed to be highly significant (p<0.05) for the ethanolic leaf extract of Rubus spp. The observed results showed higher inhibitory activity than that of the standard antibiotic Streptomycin which has a mean zone of inhibition of 2 mm.

Table 1: Phytochemical Screening Results of Merremia peltata (L.) Merr. and Rubus spp ethanolic leaf extracts.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Merremia peltata (L.) Merr.</th>
<th>Rubus spp.</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>(++)</td>
<td>(+++</td>
</tr>
<tr>
<td>Steroids</td>
<td>(++++)</td>
<td>(++++</td>
</tr>
<tr>
<td>Tannins</td>
<td>(-)</td>
<td>(++)</td>
</tr>
</tbody>
</table>

Legend: (+) presence, (+++) abundant, (++++) very abundant and (-) absence

B. Against Gram-negative (G-) Bacteria:
The measured zones of inhibition around the treated discs placed on the surface of the MHA plates showed that the ethanol and water leaf extracts of Merremia peltata (L.) Merr. and Rubus spp also inhibited the growth of all Gram-negative bacteria. However, their antibacterial effectiveness varied and ethanol extract exhibited more pronounced inhibition than water extract in the present study. Figure 2 showed that the various Merremia peltata (L.) Merr. and Rubus spp. ethanolic and water leaf extract concentrations: 5µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml had antibacterial effect also on the Gram-negative bacteria E. coli and P. aeruginosa as compared to the negative controls: ethanol and water.
Merremia peltata (L.) Merr. showed to be more effective in inhibiting Gram-negative bacteria than Rubus spp. and had the most significant concentrations at 5µg/ml and 15µg/ml for E. coli and P. aeruginosa respectively with mean zones of inhibition of 2.66 mm and 4.66 mm which was observed to be highly significant (p<0.05) for the ethanolic leaf extract (Fig 2A). For Fig. 2B, the mean zone of inhibitions of 4.8 mm against E. coli and 4.7 mm against P. aeruginosa were observed to be highly significant (p<0.05) for the ethanolic leaf extract of Rubus spp. at the same concentration of 25µg/ml. The observed results showed higher inhibitory activity than that of the standard antibiotic Chloramphenicol which has a mean zone of inhibition of 1 mm at 30µg/ml.

Also, based on the results shown in Fig. 2 and 3 in comparing the inhibitory activity between ethanol and water as solvent used for extraction, the solvent ethanol for both M. peltata and Rubus spp. plants showed higher inhibitory activity than water extracts on all Gram-negative and Gram-positive microorganisms used. The inhibitory activity of the ethanolic leaf extracts of the two plants was even higher than that of the positive controls (Streptomycin in G+ bacteria and Chloramphenicol in G- bacteria) used.

Fig. 1: Merremia peltata (L.) Merr. leaf (left) and Rubus spp. leaves (right).

Fig. 2: Zone of inhibition of the ethanolic and water leaf extracts of (A) Merremia peltata (L.) Merr. and (B) Rubus spp. against Gram- positive bacteria S. aureus and B. subtilis. Positive control is 10µg/ml Streptomycin while 95% ethanol and water are the negative controls. The labels (***) (**) (*) mean highly significant, more significant and significant respectively at p<0.05. Data is mean of 3 replicates.
Discussion:
Phytochemical analysis of medicinal plants has shown that numerous secondary compounds in plants traditionally used for medicinal purposes have chemical properties effective at treating illness. Alkaloids are nitrogenous compounds that function in the defense of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics and poisons due to their potent biological activities. In nature, alkaloids exist in large portions in the seeds and roots of plants and often in combination with vegetable acids. Alkaloids have pharmacological applications as anesthetics and CNS stimulants [13]. In this study, *Merremia peltata* (L.) Merr. leaves had an abundant amount of alkaloids but none was found in *Rubus spp.* The presence of alkaloids in *Merremia peltata* (L.) Merr. means that the plant could be used as anesthetics, CNS stimulants, as antioxidant or free radical scavenger [11, 13]. However, in *Rubus spp.*, there are more tannins present but none in *Merremia peltata* (L.) Merr. Accordingly, these tannins have significant antibacterial property which was observed in the work of Clark [3] and Ekpo and Etim [8]. Flavonoids and steroids were found in both plants extracts. The former substance, flavonoids are chemical compounds active against microorganisms. They have been found *in-vitro* to be effective antimicrobial substance against a wide array of microorganisms [4, 9, 19]. This suggests that both *M. peltata* and *Rubus. spp.* may have an active antimicrobial property.

![Fig. 3: Zone of inhibition of the ethanolic and water leaf extracts of (A) *Merremia peltata* (L.) Merr. and (B) *Rubus spp.* against Gram-negative bacteria *E. coli* and *P. aeruginosa.* Positive control is 30μg/ml Chloramphenicol while 95% ethanol and water are the negative controls. The labels (***) (**), (*) mean highly significant, more significant and significant respectively at P<0.05. Data is mean of 3 replicates.](image)

The high presence of the latter component in the two plants, the steroids, is of importance and interest in pharmacy due to sex hormones, corticosteroids and contraceptives [5, 17].

*Merremia peltata* (L.) Merr. and *Rubus spp.* leaf extracts inhibited the growth of all Gram-positive and Gram-negative bacteria, but their effectiveness varied and ethanol extract exhibited higher inhibition than water extract in the present study. This could be because ethanol being an organic solvent could dissolve organic compounds better, hence liberate the active component required for antimicrobial activity since ethanol was
found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material [9, 21]. Also, the presence of higher amounts of polyphenols in ethanolic extracts as compared to aqueous extracts means that the former are more efficient in cell walls and seeds degradation which have nonpolar character and cause polyphenols to be released from cells.

The ethanolic leaf extracts of both plants have higher inhibitory activity compared with the positive controls, Streptomycin and Chloramphenicol against Gram+ (B. subtilis and S. aureus) and Gram- (E. coli and P. aeruginosa) bacteria respectively. This suggests that both ethnomedicinal plants have pharmaceutical potentials against wide arrays of microbes.

**Conclusion:**

The phytochemical screening of *Merremia peltata* (L.) Merr. and *Rubus* spp. ethanolic leaf extracts showed the presence of flavonoids, steroids, alkaloids and tannins except tannins and alkaloids for the former and latter plants respectively. Both ethanolic leaf extracts of the two plants have higher inhibitory activity compared with the positive controls: Streptomycin and Chloramphenicol and the negative control. The study showed that the two leaf extracts for both plants had inhibitory activity on both Gram-positive (S. aureus and B. subtilis) and Gram-negative (E. coli and P. aeruginosa) bacteria however, the effect were varied as seen in the different concentrations employed. Moreover, the ethanolic and not the water extract did show a significant inhibitory activity against the different bacterial strains used. This is maybe attributed to the property of the ethanol as organic solvent itself which could extract better the active intracellular ingredients from the plant material. Overall, the results demonstrate and indicate that the *Merremia peltata* (L.) Merr. and *Rubus* spp. ethanolic and water leaf extracts can be a source of bioactive substances that have a broad spectrum antimicrobial activity. This confirms the traditional use of the leaves of *Merremia peltata* (L.) Merr. and *Rubus* spp. by the Higao-onon tribes in Rogongon, Iligan City, Philippines for the treatment of diseases.

**REFERENCES**


