Assessment of the safety of tawa-tawa (*Euphorbia hirta* L.) decoction as alternative folkloric medicine

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**ABSTRACT**

*Euphorbia hirta* L., plant is a pantropic weed, widely distributed in the Philippines. Folkloric use of tawa-tawa (*E. hirta* L.), as alternative treatment and prevention for various ailments has been promoted and patronized due to the increasing trend in the quest for inexpensive alternative medicines compared to commercial drugs. Nowadays, the traditional decoction preparation has been widely used however, the safety of its oral use against normal microflora, is still ambiguous despite traditional practice hence, this study. The disc diffusion method was used to determine the antibacterial activity against gram positive and gram negative bacteria (standard strains and clinical isolates). Antibacterial activity was tested on *E. coli, S. aureus, B. subtilis* and *P. aeruginosa*. Results show that there was no formation of any zone of inhibition as observed after 24 hrs. Thus, this justifies the traditional use of the plant as decoction treatment for various ailments. Various concentrations of crude extracts diluted in distilled water were also tested for comparison purposes but it yielded the same results as the decoction procedure. Since, the results were negative for any antibacterial potential, the use of tawa-tawa decoction for oral intake should be highly recommended in the treatment of various conditions without the risk of altering the normal flora of microorganisms in the gastrointestinal tract. Hence, it is safe to use.

**Key words:** *Euphorbia hirta* L., tawa-tawa, decoction preparation, disc diffusion method

**Introduction**

Since time immemorial, remedies known to mankind are herbal medicines. Several plants have been exploited including tawa-tawa (*Euphorbia hirta* L.), as alternative treatment and prevention for various ailments. *Euphorbia hirta* L. belongs to the family Euphorbiaceae. It is an annual herb common to tropical countries. It has been known for its medicinal, cultural and ethnobotanical uses (Sofoforowa, 1982). Preparations made for treatment include decoction and crude aqueous/ethanolic extracts. Traditionally, decoction preparation has been used in the treatment of asthma, respiratory tract inflammation, gastrointestinal disorders, bronchial and respiratory diseases, kidney stones, diabetes and in conjunctivitis (Wong-Ting-Fook, 1980; Kokwaro, 1993). It also exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities (Nelofar, et. al, 2006; Kumar, 2010). Several studies revealed that ethanolic extracts proved to be effective against several bacteria tested in vitro but, the oral intake of decoction preparation is more commonly used. However, there are no studies conducted testing the safety of decoction preparation for oral use as it may alter normal microflora present in the gastrointestinal tract. The normal flora of humans consists of eukaryotic fungi and protists, but bacteria are the most numerous and obvious microbial component of the normal flora. In fact, not much is known regarding the nature of the associations between humans and their normal flora but there are thought to be more dynamic interactions rather than associations of mutual indifference. The associations for the most part are mutualistic wherein, the normal flora derive, from their host a steady supply of nutrients, stable environment and protection and transport. The host obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic microbes (Todar, 2011). The known bacteria that are regularly associated with humans are: *E. coli* -common in the mouth, nearly 100% in the lower gastrointestinal tract (GI); is consistent in the small intestine; some strains are pathogenic that can cause neonatal meningitis, intestinal and urinary tract infections; *S. aureus* -common in skin, mouth, pharynx and nearly 100% in the lower GI but, can be a potential pathogen; *P. aeruginosa* -rare in the mouth and pharynx but common in the lower GI; can be an opportunistic pathogen in humans that can invade virtually any tissue; *B. subtilis* -is often used as gram positive equivalent of *E. coli*; is not a human pathogen but it may contaminate food but rarely causes food poisoning; its spores can survive extreme heat during cooking (Ryan, 2004; Todar, 2011).
Moreover, interaction with traditional practitioners revealed that the plant is very popular among them, thus, there is a need to determine its antibacterial potentials which may have implications in the oral use of tawa-tawa decoction preparations. At present, there are no studies conducted substantiating the antibacterial potential of decoction preparations as commonly used in medication. Hence, the goal of this study is to assess the safety of tawa-tawa (Euphorbia hirta L.), decoction as alternative folkloric medicine by evaluating its antibacterial activity against several gram-positive (Staphylococcus aureus; Bacillus subtilis) and negative (Escherichia coli; Pseudomonas aeruginosa) bacterial strains in vitro, that are commonly associated with humans especially, those that are normally found in the gastrointestinal tract. Positive results for antibacterial potentials of decoction preparation may imply that oral intake should not be highly recommended since it can alter the normal flora of microorganisms present in the gastrointestinal tract.

Materials And Methods

Plant collection and sample preparation:

Fresh whole plants of Euphorbia hirta, were collected around Tibanga, Iligan City. The fresh plants were washed and allowed to stand for about 5 minutes to remove remaining water. Varied concentrations of decoction preparation was made by weighing 50g, 100g and 150g respectively and soaking each weighed plant in 500ml of distilled water. This is consistent with the folkloric way of preparing the decoction solution. There were three (3) decoction preparations made 10%, 20% and 30% concentrations respectively. In a 1000ml beaker, the preparation was boiled for about 10 minutes then stored in room temperature before processing. For the crude extract preparation, tawa-tawa plant was crushed using mortar and pestle to obtain pure crude extract, then a 10% concentration was made by adding 9 ml distilled water (dH2O) to 1ml crude extract, for the 20% concentration, 2 ml crude extract was used plus 8 ml dH2O and 30% preparation was done by adding 7 ml dH2O to 3 ml crude extract. A pure crude extract (100%) not diluted in distilled water was also tested for further comparison.

Test microorganisms:

Aseptic technique was done during the entire duration of the study to avoid contaminations. The strains of test organism of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis were obtained from University of the Philippines-Los Baños Biotechnology Laboratory. They were re-isolated and pure cultures were sub-cultured on Nutrient Agar slants. Cultures were then incubated at room temperature that will enable the bacteria to reproduce. After 24 hours of incubation, a bacterial suspension for each test organism was made based on 0.5 Macfarland Turbidity Standard which is presumed to have approximately, 1.5x10^8 cells/ml in screw-capped tubes. A sterile cotton swab was dipped into the tubes containing the bacterial suspension and then streaked unto the surface of the properly labeled Mueller Hinton Agar (MHA) plates. The standard discs for the concentrations and the antibiotic discs (TE and OFX) were aseptically place at standard distance from each other.

Evaluation of antimicrobial property:

Subsequently, the plates were incubated for 24 hours at room temperature. Disc diffusion method was used. The zone of inhibition produced by each disc was measured. The average of the zones of inhibition was calculated. Photographs of the samples were taken.

Results And Discussion

In vitro, antimicrobial activity of different concentrations of tawa-tawa (E. hirta) was studied by disc diffusion method using different concentrations of decoction and crude exact preparation on different microbial strains. Antibacterial activity was tested on E. coli, S. aureus, B. subtilis and P. aeruginosa, respectively. The results of the antibacterial screening of the different concentrations of the decoction preparation on the test isolates are shown in Table 1. The results show that increase in the concentration did not have any effect on the test isolates. In fact, there was no zone of inhibition produced as the decoction preparation was tested on four (4) strains of bacteria (Fig. 1 and 2). Hence, the result is negative for any antibacterial potential. This implies that decoction preparation can be taken safely orally since it did not exhibit any antimicrobial activity that may alter the normal flora of microorganisms in the body. Hence, this supports the safety of the folkloric use of tawa-tawa, decoctions as alternative medicine.
Crude exact preparations of tawa-tawa, diluted in distilled water to attain the same concentrations (10%, 20%, and 30%) as the decoction preparation were also done and tested on different microbial strains present for comparison purposes. The results of the antibacterial screening of the different concentrations of the crude extract preparation in distilled water on the test isolates are shown in Table 2. There was no zone of inhibition produced as the crude exact preparation was tested on four (4) strains of bacteria (Fig. 3 and 4). A pure crude extract (100%) not diluted in distilled water was also tested for further comparison and, yielded the same results. The results were negative for any antibacterial potential justifying the safety of the preparations for oral use.

However, it was observed that pure crude extracts tend to contain other bacteria which are somehow, evident in the plates examined (Fig. 5). This is owing to the fact that the preparation did not undergo filtration and heat procedure (unlike decoction) that will make it purified against other microorganisms. Thus, decoction is more recommended than oral intake of crude extracts.

**Table 2: Antibacterial screening of different concentrations of crude extract of tawa-tawa (E. hirta) diluted in distilled water.**

<table>
<thead>
<tr>
<th>Concentrations of extract (%)</th>
<th>Zones of inhibition (mm)</th>
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<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>100</td>
<td>NI</td>
</tr>
<tr>
<td>30</td>
<td>NI</td>
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<tr>
<td>20</td>
<td>NI</td>
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<td>10</td>
<td>NI</td>
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*NI= No Inhibition*
Fig. 2: *In vitro* test for antimicrobial activity of different concentrations of decoction preparation of tawa-tawa (*E. hirta*) on gram positive (*Staphylococcus aureus; Bacillus subtilis*) bacteria using disc diffusion method. Varied concentrations of the plant decoction: 50g (10%); 100g (20%) and 150g (30%) each soaked in 500ml of distilled water were tested on *S. aureus* (a-c) and *B. subtilis* (d-f); No zone of inhibition produced by tawa-tawa, compared to the control (antibiotic disk of tetracycline (TE); positioned on the rightmost part of the plate).

Normally, the decoction procedure is taken like tea. Moreover, it should be noted that percentage of tannins have been known to be present in plants and that tannins form a vital element of tea. Although, human saliva possess innate antimicrobial properties owing to the presence of trace amounts of hydrogen peroxide and lysozymes that eliminate unwanted or harmful microbes, including bacteria and maintaining their presence at a level that is manageable, it does not contain tanning-binding proteins (mucin) unlike tannin consuming animals that use proline-rich proteins (PRPs) in their saliva to inactivate tannins. The tannins have been known to precipitate proteins (Bate-Smith and Swain, 1962), which in some ruminant animals inhibits the absorption of nutrients from high-tannin grains. In sensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain. With the exception of tea, long-term and excessive use of herbs containing high concentrations of tannins is not recommended (Elvin-Lewis, 1977). Hence, decoction preparation should be consumed in moderate amounts only. It is further recommended to counter possible problems regarding prolong and excessive intake of tannin containing preparations, to add milk or lemon juice to the tea, this helps in reducing or neutralizing the tannins’ adverse actions on the iron intake. Similarly, consuming food that is rich in Vitamin C also helps in neutralizing tannin’s effects on iron adsorption. Tannin has been found to have remedial values on external applications on burns, wounds and it can stop bleeding. When applied internally, tannins affect the walls of the stomach and other digestive parts. In fact, they sour the mucus secretions and contract or squeeze the membranes in such a manner that the secretions from the cells are restricted. Tannins’ have anti-inflammatory effect that helps to control or curb all indications of gastritis, enteritis, oesophagitis and irritating bowel disorders. This action is possible by involving lymph stasis and neutralizing the autolytic enzymes. In most rural areas diarrhea is causes by the irritation of the small intestine hence, conventionally tannins have been used to cure diarrhea (Elvin-Lewis, 1977).
Fig. 3: *In vitro* test for antimicrobial activity of different concentrations of crude extract preparation of tawa-tawa (*E. hirta*) on gram negative (*Escherichia coli*; *Pseudomonas aeruginosa*) bacteria using disc diffusion method. Varied concentrations of the plant crude extract: 10%; 20% and 30% were tested on *E. coli* (a-c) and *P. aeruginosa* (d-f); No zone of inhibition produced by tawa-tawa, compared to the control (antibiotic disk of ofloxin (OFX); positioned on the rightmost part of the plate).

Fig. 4: *In vitro* test for antimicrobial activity of different concentrations of crude extract preparation of tawa-tawa (*E. hirta*) on gram positive (*Staphylococcus aureus*; *Bacillus subtilis*) bacteria using disc diffusion method. Varied concentrations of the plant crude extract: 10%; 20% and 30% each were tested on *S. aureus* (a-c) and *B. subtilis* (d-f); No zone of inhibition produced by tawa-tawa, compared to the control (antibiotic disk of ofloxin (OFX); positioned on the rightmost part of the plate).
Table 3: Antibacterial screening of different concentrations of crude ethanolic extract of tawa-tawa (*E. hirta*).

<table>
<thead>
<tr>
<th>Concentrations of extract (mg/ml)</th>
<th>Zones of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>150</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>5.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>NI</td>
</tr>
</tbody>
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*Values are means of triplicate readings. NI= No Inhibition
<sup>a,b</sup> Values with different superscripts on the same row are significantly different (p=0.05)
*source: Ogbulie et al., 2007*

Fig. 5: *In vitro* test for antimicrobial activity of showing the presence of a different bacteria surrounding the discs (possible contaminants from the crude extract) as indicate by red arrows for each bacterial culture examined. a.) *E. coli*; b.) *P. aeruginosa*; c.) *S. aureus* and d.) *B. subtilis* culture; No zone of inhibition produced by 100% tawa-tawa crude extract.

Furthermore, results obtained were compared to the study performed by Ogbulie et al., 2007 (Table 3) using crude ethanolic extracts of *E. hirta*. This study shows that the ethanolic extract of tawa-tawa (*E. hirta*) inhibited the growth of *E. coli*, *S. aureus*, and *P. aeruginosa*. The extract is noncytotoxic and antibacterial. Thus, the extract contained substances that can inhibit the growth of some microorganisms. As shown in table 3, an increase in the concentration of the extract increased the zone of growth inhibition of the microorganisms tested. These results by Ogbulie et al., 2007 were contrary to the results obtained from decoction preparation. Other studies conducted show that the plant extract was found to contain tannins, alkaloids and flavonoids which may be responsible for antibacterial properties. Other secondary metabolites like coumarines and terpenes and a number of substances such as gallic acid, quercetin, phenols, phyto-sterols, alcohols have been reported in the plant (Kerharo and Adam, 1974; Burkill, 1985). Moreover, studies show that ethanolic and methanolic extracts exhibited a high degree of antimicrobial activity as compared to water extracts. Such findings are correlated with the medicinal preparations that use rum and liquor to extract the active plant components. In this study performed, it is deduced that the decoction procedure is not a good method in extracting phenolic compounds, flavonoids and alkaloids found in tawa-tawa, which can be responsible for its antimicrobial properties. However, the procedure is found to be effective in releasing its secondary metabolites and substances which were found to be responsible for its healing properties. This is owing to the conclusive data available on the therapeutic potential of tawa-tawa (Watt and Wijka, 1962; Perry and Metzger, 1980).

This study confirmed the safety of the traditional practice of tawa-tawa, in decoction preparations as taken orally by patients. Moreover, medicinal plants can be poisonous if wrong plant parts or wrong concentrations are used (Frohne, 1999). Some compounds of plants may be toxic in higher doses. In this case the whole plant was used and different concentrations were prepared for the decoction procedure and found to be of no antibacterial potential hence, it is safe to drink. Results imply that a decoction procedure is not sufficient to
release active compounds and substances present in the plant that are responsible for its antibacterial properties. Hence, decoction preparation is safe to be taken orally in the treatment of asthma, respiratory tract inflammation, gastrointestinal disorders, bronchial and respiratory diseases, kidney stones, diabetes and in conjunctivitis without the risk of altering the normal microflora of the gastrointestinal tract.

**Conclusion:**

This study assessed the safety of tawa-tawa (*Euphorbia hirta L.*) decoction as alternative folkloric medicine. Evaluation of antibacterial activity against several gram-positive (*Staphylococcus aureus; Bacillus subtilis*) and negative (*Escherichia coli; Pseudomonas aeruginosa*) bacterial strains *in vitro* in its decoction form proved to be negative. Results imply that a decoction procedure may not be enough or sufficient to release active compounds and substances present in the plant that are responsible for its antibacterial properties however, it was proven to be effective in releasing secondary metabolites and substances which were found to be responsible for its healing properties. Hence, this supports the safety of using tawa-tawa, decoction orally in traditional medicine without the risk of altering the normal flora of microorganisms in the gastrointestinal tract.

**Acknowledgment**

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**References**


Wong-Ting-Fook, WTH., 1980. The medicinal plants of Mauritius. ENDA publication No. 10., Dakar.