ANTI-ULCER ACTIVITY OF LEGUMINOSAE PLANTS

Noemi D. PAGUIGAN¹, Darryl Hannah B. CASTILLO² and Christine L. CHICHIKO-HERNANDEZ²

ABSTRACT - Context – Ulcer is the most common gastrointestinal disturbance resulting from an inadequate gastric mucosal defense. Several drugs are available in the market to address the disease; however, these drugs are associated with unnecessary side effects. Objectives – Previous research have confirmed the efficacy of plant extracts for possible treatment of the disease. This research aims to evaluate the anti-ulcer properties of medicinal plants. Methods – Methanol extracts from the leaves of Intsia bijuga, Cynometra ramiflora, Tamarindus indica, Cassia javanica, Cassia fistula, Bauhinia purpurea, Senna spectabilis, Senna siamea and Saraca thaipingensis were evaluated for their anti-ulcer activity using HCl-ethanol as ulcerogen. Results – All extracts showed inhibitory activity with I. bijuga, T. indica, S. spectabilis and S. thaipingensis exhibiting more than 50% inhibition. S. thaipingensis showed the highest activity at 80%. S. spectabilis and S. thaipingensis were partitioned further into hexane, ethyl acetate and aqueous fractions. The aqueous and ethyl acetate fractions of S. spectabilis showed significant increased in its activity while the hexane and ethyl acetate fractions of S. thaipingensis gave higher activity than its aqueous portions. Conclusion – We conclude that plant extracts are potential sources of new anti-ulcer agents.


INTRODUCTION

Peptic ulcer disease is the term used to describe a heterogeneous group of condition with ulcerations. It is characterized by the disruption of the mucosal integrity of the esophagus, stomach, or duodenum. As the most common gastrointestinal disturbance, it affects 10%-15% of the population at any one time. Ulcers are primarily caused by an imbalance between some endogenous aggressive and protective factors in the stomach such as acid-pepsin secretion, integrity of the mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins, and growth factors. Several factors are also associated in the occurrence of peptic ulcer including stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDS), Helicobacter pylori infections, smoking, lower socio-economic status and family history. Although ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction. Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics, antacids and proton pump inhibitors. Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are associated with unwanted side effects.

In this context, this research aims to evaluate the anti-ulcer properties of medicinal plants. Several researches have confirmed the efficacy of medicinal plants for the treatment of peptic ulcer disease. The observed activity in these plants is attributed with the presence of flavonoids, alkaloids, terpenoids, tannins, saponins, and phenolic acids. Exports of Wilbrandia ebracteata, Eruca sativa, Toona ciliata Roemer, Canna monosun, Vocaansa africana and Pedalium murex have shown anti-ulcer activity. An alkaloid from the fruit of Vocaansa africana and a protoberberine-type alkaloid from the bark of Enantia chlorantha were found to prevent ulcers. An alkaloid extract and 2-phenylnqino-line from Galipea longiflora Krause have also shown gastroprotective effects. Bauhinia purpurea, which belongs to the Leguminosae family, has been shown to inhibit aspirin-induced and ethanol-induced ulcers in mice. In this study, other species belonging to the same family were evaluated for their anti-ulcer activity.

METHODS

Plant Material

Fresh leaves of Intsia bijuga, Cynometra ramiflora, Tamarindus indica, Cassia javanica, Cassia fistula, Bauhinia purpurea, Senna spectabilis, Senna siamea and Saraca thaipingensis were collected from the Univer-
sity of the Philippines, Diliman Campus and submitted to
the Dr. Jose Vera Santos Herbarium, Institute of Biology,
University of the Philippines, Diliman for authentication.
Voucher specimen for each plant were also deposited.

**Extraction and solvent partitioning**

The plant samples were washed with running water and
air-dried. The dried samples were homogenized for overnight
soaking in methanol. The resulting extracts were filtered and
concentrated in vacuo using a rotary evaporator at 40° C. The
methanol fractions were partitioned between hexane and
water. The resulting aqueous layer was further extracted with
ethyl acetate. The hexane and ethyl acetate portions were also
concentrated in vacuo.

**Phytochemical analysis**

The phytochemical screening methods used were based
on Harborne(13) and Edeoga(8). Qualitative test for terpenoids,
saponins, tannins, flavonoids, steroids, phenolic compounds,
alkaloids and cardiac glycosides were performed.

**Bioassay**

**1) Animals**

The mice used in the assay were 6-8 weeks old, Swiss Al-
bino mice (ICR strain) purchased from the Food and Drug
Administration (FDA) Philippines, Department of Health,
Alabang, Muntinlupa City. The animals were acclimated
for at least one week in standard cages. The mice were fed
with commercial pellets with free access to purified drinking
water ad libitum, standard conditions of 12h:12h light/dark
cycle, and temperature (23°C-25°C). The protocol used for
the anti-ulcerogenic assay was approved by the College of
Science Animal Care and Use Committee (CSACUC) of the
University of the Philippines Diliman with assigned protocol
number IC 2011-06.

**2) HCl/Ethanol-induced ulcer assay**

The anti-ulcerogenic assay was adapted from the method
of Schmeda-Hirschmann(22) with slight modifications. A total
of 65 mice were randomly distributed into thirteen treat-
ment groups with 5 mice for the initial assay. Mice weighing
26± 5 g were deprived of food 24 hours prior to the experi-
ment. Group 1 was given solvent solution with 5% Tween
80, 10% DMSO and 85% distilled water. Group 2 was given
HCL/EtOH only. Group 3 was administered with Sucralfate.
Groups 4-12 were treated with the plant samples. Group 13
did not receive any treatment. The plant samples, positive
control, and solvent control were orally administered to the
mice. The plant extracts were given at a dose of 1000 mg/kg,
0.2 mL/20 g body weight; Sucralfate at a dose of 200 mg/kg,
0.2 mL/20 g body weight; and 0.3 M HCl/60% EtOH.

After an hour, the mice were given 0.2 mL/20 g b.w. of 0.3
M HCL/60% EtOH solution to induce ulceration. The mice
were sacrificed by cervical dislocation an hour after the induc-
tion of ulceration. The stomachs were excised and inflated by
injecting with 0.9% normal saline solution. The excised stom-
achs were fixed with 10% phosphate buffered solution for at
least 15 minutes, and opened along the greater curvature to
expose the gastric mucosal layer. Hemorrhagic lesions in the
mucosal membrane of the glandular region were observed
under a dissecting microscope and were manually scored.
Scoring of ulcerations was patterned after Adensawo et al.(1).
Normal gastric mucosa was scored as 0, pinpoint ulcers were
scored 0.5, one or two small hemorrhages were given 1.0 and
ulcers with diameters greater than 3 mm or characterized by
heavy bleeding were given a score 2.0.

Fifty mice were randomly distributed into ten treatment
groups for the second assay. Groups 1-3 were given similar
treatments as in the initial assay. Groups 4–9 were given plant
extracts. Group 10 did not receive any treatment. Similar
concentrations were used as in the first assay.

The ulcer index (UI) was obtained from the sum of the
scores of all lesions for each stomach, and the mean ulcer
index ($UI_{MEAN}$) was calculated for each group. Percent ulcer
inhibition of the samples was determined using the follow-
ing equation:

$$\% \text{ ulcer inhibition} = \left( \frac{UI_{MEAN} \text{ control} - UI_{MEAN} \text{ sample}}{UI_{MEAN} \text{ control}} \right) \times 100\%$$

**Data analysis**

Data were analyzed by one-way analysis of variance
(ANOVA) followed by Dunnet’s multiple comparison test
using SPSS version 16.0 to determine statistical differences
between the treated and the control group. The level of
significance was set at $P<0.05$

**RESULTS**

The methanol extracts of *I. bijuga*, *C. ramiflora*, *T. indica*,
*C. javanica*, *C. fistula*, *B. purpurea*, *S. spectabilis*, *S. siamea*
and *S. thaipingensis* were evaluated for their gastroprotective
action against HCl-EtOH-induced ulcer. Figure 1 shows the

FIGURE 1. Ulcerations caused by HCl/EtOH in mice.
ulcerations resulting from HCl-EtOH treatment. Varying gastroprotective activities of the extracts are shown in Table 1. The anti-ulcer activity of the extracts may be due to the phytochemicals they contain. The phytochemical profiles of all plant samples were determined and the results of the tests are shown in Table 2. The methanol extracts of *S. spectabilis* and *S. thaipingensis* were partitioned with hexane and ethyl acetate to further examine their high activity. The increase in gastroprotective activities of the hexane, ethyl acetate, aqueous extracts are shown in Table 3.

### TABLE 1. Gastroprotective activity of MeOH samples in HCl/EtOH-induced ulcer in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer Index (mean±SEM)</th>
<th>Ulcer Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>6.000±0.423</td>
<td></td>
</tr>
<tr>
<td>HCl/EtOH only</td>
<td>5.143±0.688</td>
<td></td>
</tr>
<tr>
<td>Sucralfate</td>
<td>1.000±0.244*</td>
<td>83.3</td>
</tr>
<tr>
<td><em>Intsia bijuga</em> (Colebr.) O.K.</td>
<td>2.500±0.775*</td>
<td>58.3</td>
</tr>
<tr>
<td><em>Cynometra ramiflora</em> L. Var. ramiflora</td>
<td>5.167±0.989</td>
<td>13.9</td>
</tr>
<tr>
<td><em>Tamarindus indicus</em> L.</td>
<td>2.417±0.800*</td>
<td>59.7</td>
</tr>
<tr>
<td><em>Cassia fistula</em> L.</td>
<td>4.417±0.889</td>
<td>26.4</td>
</tr>
<tr>
<td><em>Bauhinia purpurea</em> L.</td>
<td>3.500±1.084</td>
<td>41.7</td>
</tr>
<tr>
<td><em>Senna spectabilis</em> (DC.) Irwin &amp; Barneby</td>
<td>2.083±0.970*</td>
<td>65.3</td>
</tr>
<tr>
<td><em>Senna siamea</em> (Lam.) Irwin &amp; Barneby</td>
<td>3.917±0.908</td>
<td>34.7</td>
</tr>
<tr>
<td><em>Saraca thaipingensis</em> Cantley ex Prain</td>
<td>1.200±0.464*</td>
<td>80.0</td>
</tr>
<tr>
<td>Normal (no treatment)</td>
<td>0.000±0.000*</td>
<td></td>
</tr>
</tbody>
</table>

* Samples statistically different compared to the solvent control (P<0.05)

### TABLE 2. Phytochemical analysis of plant species in the *Leguminosae* family

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Terpenoid</th>
<th>Alkaloid</th>
<th>Cardiac Glycoside</th>
<th>Phenolics</th>
<th>Saponin</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Intsia bijuga</em> (Colebr.) O.K.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Cynometra ramiflora</em> L. Var. ramiflora</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Tamarindus indicus</em> L.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Cassia javanica</em> L. ssp. Nodosa (Buch.-Ham. Ex. Roxb)K. &amp; S.S. Larsen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Cassia fistula</em> L.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Bauhinia purpurea</em> L.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Senna spectabilis</em> (DC.) Irwin &amp; Barneby</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Senna siamea</em> (Lam.) Irwin &amp; Barneby</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Saraca thaipingensis</em> Cantley ex Prain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### TABLE 3. Gastroprotective activity of *S. spectabilis* and *S. thaipingensis* fractions in HCl/EtOH-induced ulcer in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer Index (mean±SEM)</th>
<th>Ulcer Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>6.562±0.8097</td>
<td></td>
</tr>
<tr>
<td>HCl/EtOH only</td>
<td>5.000±0.8062</td>
<td></td>
</tr>
<tr>
<td>Sucralfate</td>
<td>3.143±0.7846*</td>
<td>52.1</td>
</tr>
<tr>
<td><em>S. spectabilis</em> Aqueous extract</td>
<td>1.357±0.7377*</td>
<td>79.3</td>
</tr>
<tr>
<td><em>S. spectabilis</em> Ethyl acetate extract</td>
<td>1.857±1.0160*</td>
<td>71.7</td>
</tr>
<tr>
<td><em>S. spectabilis</em> Hexane extract</td>
<td>5.833±0.7601</td>
<td>11.1</td>
</tr>
<tr>
<td><em>S. thaipingensis</em> Aqueous extract</td>
<td>5.000±0.5323</td>
<td>23.8</td>
</tr>
<tr>
<td><em>S. thaipingensis</em> Ethyl acetate extract</td>
<td>2.429±0.7975*</td>
<td>63.0</td>
</tr>
<tr>
<td><em>S. thaipingensis</em> Hexane extract</td>
<td>2.429±0.8690*</td>
<td>63.0</td>
</tr>
<tr>
<td>Normal (no treatment)</td>
<td>0.429±0.2974*</td>
<td></td>
</tr>
</tbody>
</table>

* Samples statistically different compared to the solvent control (P<0.05)
DISCUSSION

All extracts were active and showed varying degrees of gastroprotection. It is possible that plants belonging to the Leguminosae family are able to inhibit ulcers. *I. bijuga*, *T. indica*, *S. spectabilis* and *S. thaipingensis* showed higher than 50% inhibition. *S. thaipingensis* showed the highest activity at 80% which is comparable with the activity of the positive control sucralfate at 83% inhibition. These plants showed significant anti-ulcer action against HCl-EtOH ulcerogen. Alcohol consumption is a contributor to gastric ulceration (11) and excessive consumption increases the risk for gastric mucosal damage. Ethanol causes gastric ulcers by lowering protective factors in the gastric mucosa (5). Ethanol-induced ulcers in mice are characterized by heavy bleeding since it can cause immediate stasis in the blood flow (20). It is possible that the extracts contain compounds that can enhance protective factors and restore gastric blood circulation.

Phytochemicals refer to a wide-variety of compounds produced by plants with no nutritive value. They are promoted for their protective and disease-preventive properties according to the American Cancer Society website. Stilbenes and flavonoids were isolated from the heartwood of *I. bijuga* (14). *T. indica* showed 59.6% ulcer inhibition. The results supported its used for gastrointestinal disorders in India (7, 25). *S. spectabilis* activity showed significant increased inhibition for its ethyl acetate and aqueous fractions at 71.7% and 79.3%, respectively. Result showed that hexane and ethyl acetate extracts of *S. thaipingensis* exhibited significant bioactivity, both at 63 %. No significant activity was observed for the aqueous extract. The flowers of *S. spectabilis* previously yielded three new bioactive piperidine alkaloids (31). Its leaves, roots and stems also gave different alkaloids (24, 32). Phytochemical screening of the methanolic extract of *S. thaipingensis* showed the presence of flavonoids, terpenoids, tannins, saponins, and phenolic acids which are known to have anti-ulcer activities (4, 19, 21). The bioactivity of the extracts could be attributed to these secondary metabolites.

CONCLUSION

The different plant extracts gave varying degrees of anti-ulcer activity and could be a potential source of new anti-ulcer agents. Further studies are underway to identify these compounds.

ACKNOWLEDGEMENT

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REFERENCES


