ANTIDIARRHOEAL ACTIVITY OF VARIOUS ROOT EXTRACTS OF
GMELINA ARBOREA ROXB. IN EXPERIMENTALLY INDUCED
DIARRHOEA IN MICE

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ABSTRACT
Diarrhoea has long been recognized as one of the most important health problems in the developing countries. Consumption of medicinal herbs is tremendously increasing over the past decade as alternative approach to improve the quality of life and maintain good health. Gmelina arborea root has been used traditionally for the treatment of anthelmintic, laxative, fever, piles, diarrhoea and urinary discharges. Therefore, the present study was undertaken to evaluate the antidiarrhoeal potential of G.arborea root extract in castor oil induced diarrhea model. For this Swiss albino mice of either sex were used. all the crude extracts of G.arborea root such as ethanol, ethyl acetate, n-butanol and petroleum ether were tested for antidiarrhoecal activity at 200 and 400 mg/kg body weight. Where as loperamide (3 mg/kg) were used as standard drugs and given orally. The ethanol & n-butanol extracts of root of G.arborea was found to be effective in a dose dependent manner against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight.

KEYWORDS: Gmelina arborea root, antidiarrhoeal potential, laxative, fever, piles, diarrhoea and urinary discharges.
INTRODUCTION

Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries effecting mainly the infants and children’s.\(^1\) The major causative agents of diarrhoea in humans include: *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*.\(^2\) WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.\(^3\) In developing countries like India, a majority of people who live in the rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea.\(^4\) It is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. *Gmelina arborea* Roxb. belonging to family Verbenaceae locally named as Gambhari(Oriya), Gambhar (Hindi), Gambhar (Bengali), Sriparni (Sanskrit) and Gummadi (Telgu).\(^5\) Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June. Flowers occur in narrow branching clusters at the end of branches. The yellow flower, tinged with brown, is trumpet shaped, 3-4 cm long. The trumpets flare open into a gaping mouth with 5 distinct lobes.\(^6\) The root of this plant has been used in traditional Indian systems of medicines as a demulcent, stomachic, bitter tonic, refrigerant, laxative, and galactagogue. The tender leaves are used as demulscent, in headache, fevers, gonorrhea, cough etc. The whole plant is used in snake bite and scorpion sting throughout India.\(^7\) As per the folklore medicine the root decoction is used in folk remedies for, demulcent, stomachic, and tonic, diarrhea, dropsy, dyspepsia, epilepsy, fever, gout, headache, hemorrhage,rheumatism, smallpox, snakebite, sores, sore throat, stomachic and urticaria. Ayurvedics. prescribe them for alopecia, anemia, consumption, leprosy, thirst, and vaginal discharges; the flowers for blood disorders and leprosy; the root, deemed anthelmintic, laxative and stomachic, for abdominal pains, burning sensations, fever, hallucinations, piles and urinary discharges.\(^8,9\) According to scientific studies, the root decoction is used as a folk remedy for abdominal tumors. The roots are useful in hallucination, piles, abdominal pains, fevers, ‘tridosha’ and urinary discharge.\(^10,11\) Traditional people are using to get relieve from Post delivery weakness. They are using half glass of boiled root extract. The extract is prepared by boiling roots with one glass of water till it gets reduced to half aglass. The plant has also been
reported to have anti-inflammatory activity, hypoglycaemic and anti-viral activities against Ranikhet disease virus.\cite{12}

\section*{MATERIAL AND METHODS}

\subsection*{Drugs and chemicals}
Loperamide was procured as gift sample from Provizer Pharma, Surat, Gujarat, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C, petroleum ether AR 40-60°C, Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

\subsection*{Collection of Plant Material}
The root of \textit{Gmelina arborea} were collected from the tribal belts of the local area of Baipariguda of Koraput district, (India) in the month of November 2011. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-198/11, dated 16.12.2011). After authentication, roots were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder and stored in a closed air tight container for further use.

\subsection*{Preparation of Extracts}
The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.\cite{13,14,15}

\subsection*{Evaluation of Antidiarrhoea Activity}

\subsection*{Animals}
Healthy albino mice of Swiss strain of either sex were used. They were housed in standard conditions of temperature (25±2 °C), 12 hours light per day cycle, relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition.
Drugs
Loperamide (3 mg/kg) and a dose of 200 & 400 mg/kg of different *G. arborea* root extracts used for activity study and the route of administration for both standard and test drug was orally.

Experimental protocol
Animals were selected, weighed (25-30 g) and divided into ten groups (n=3), namely control group, standard group and eight groups belonging to four different root extracts of *G. arborea*. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar, Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies
The acute toxicity was performed according to OECD 423, 2001. The selected female albino rats were used to determine the dose. The animals were divided into twelve groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the different root extracts of *Gmelina arborea* and administered orally as following doses of 100, 300, 600, 1000 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively. The acute toxicity study in mice showed that all the extracts were not toxic (LD50 > 1000 mg/kg).[16]

Castor oil induced diarrhea
The method described by Shoba and Thomas was followed for this study with slight modification. The animals were all screened initially by giving 0.5 ml of castor oil one week before the actual experiment. Only those showing diarrhoea were selected for the final experiment. Thirty mice fasted for 24 h were randomly allotted to five groups of five animals each. Group I (received 1% tween 80 at a dose of 10 ml/kg) served as control group, Group II received the standard drug loperamide 3 mg/kg, p.o. Group III to X received the different root extracts of *G. arborea* at the doses of 200 and 400 mg/kg p.o., respectively. One hour after administration, all animals received 0.5 ml of castor oil and then they were individually placed in cages the floor of which was lined with transparent paper. During an observation period of
4 h, the time of onset of diarrhoea, the total number of faecal output (frequency of defecation) and weight of faeces excreted by the animals were recorded.\cite{17}

**Statistical analysis**

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where \( P < 0.05 \) was considered statistically significant.\cite{18}

**Table no. 1. Effect of *Gmelina arborea* root extract on castor oil induced diarrhea in mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (mg/Kg)</th>
<th>Time of onset of diarrhoea(min.)</th>
<th>Total number of faeces in 4h(frequency of defecation in 4 h)</th>
<th>% Inhibition of defecation</th>
<th>Weight of stool(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---------------</td>
<td>93 ± 14.2</td>
<td>7.9 ± 1.3</td>
<td>0.82 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Loperamide (standard)</td>
<td>3</td>
<td>233.8 ± 7.0</td>
<td>1±0.2</td>
<td>83.77</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>200</td>
<td>182 ± 17</td>
<td>3.6 ± 0.6</td>
<td>52.78</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>217 ± 12.3</td>
<td>1.3 ± 0.3</td>
<td>77.83</td>
<td>0.02 ± 0.07</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>200</td>
<td>157.7±22.6</td>
<td>4.5±0.7</td>
<td>52.33</td>
<td>0.23±0.018</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>177.9±14.7</td>
<td>9±0.4</td>
<td>62.47</td>
<td>0.41±0.022</td>
</tr>
<tr>
<td>n-butanol extract</td>
<td>200</td>
<td>171.3±18</td>
<td>3.7±0.6</td>
<td>53.62</td>
<td>0.26±0.033</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>203 ±14</td>
<td>1.9±0.6</td>
<td>73.38</td>
<td>0.33±0.024</td>
</tr>
<tr>
<td>Pet. ether extract</td>
<td>200</td>
<td>152.3±9</td>
<td>4±0.9</td>
<td>42.30</td>
<td>0.28±0.023</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>190±11.4</td>
<td>6±0.2</td>
<td>31.27</td>
<td>0.18±0.016</td>
</tr>
</tbody>
</table>

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where \( P < 0.05 \)

**RESULT AND DISCUSSION**

The ethanol & n-butanol extracts of root of *G.arborea* was found to be effective in a dose dependent manner against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight, The extract produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in albino mice. At the same dose, the extract showed significant antidiarrhoeal activity showing 77.83
% &73.38 % (ethanol & n-butanol ) extracts reduction in diarrhoea comparable to that of the standard drug loperamide that showed 83.77% reduction in diarrhea. Which showed in (Table 1). Anti-diarrhoea activity was found in plants possessing tannins, alkaloids, saponins, flavonoids, steroids and terpenoids.\textsuperscript{[19,20]} Anti-diarrhoea activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions.\textsuperscript{[21]} Tannins present in anti-diarrhoea plants denature proteins in the intestinal mucosa by forming protein tannates which may reduce secretion. Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca\textsuperscript{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation).\textsuperscript{[22]} The preliminary phytochemical screening of \textit{G.arborea} root extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and saponins. These constituents may be responsible for the \textit{in vivo} anti-diarrhoea activity of \textit{G.arborea} root.

CONCLUSION
Among all the extracts ethanol & n-butanol extracts showed dose dependant &significant anti-diarrhoea activity as compared to reference drug loperamide. The folklore claim of root of \textit{G.arborea} used as an anti-diarrhoeal have been confirmed. Further studies to isolate and reveal the active compound present in the crude extract of \textit{G.arborea} root and to establish the MOA of anti-diarrhoeal activity.

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REFERENCES


