Evaluation of Anti-Diarrheal Potential of *Clerodendrum Wallichii* (Merr.) Leaves

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**Abstract**

*Clerodendrum wallichii* Merr. (Family: Lamiaceae) commonly known as Sampul is native to southern Asia from the Himalayas to Southern China. In India, it is found in Sikkim, Tripura, Mizoram, Meghalaya, Assam, Maharashtra and Uttarakhand. Traditionally, it is used against diarrhea, skin infection, inflammation and fever. The plant has never been evaluated for anti-diarrheal activity. Thus, it was considered worthwhile to evaluate anti-diarrheal effect of *C. wallichii* leaves in rats. Hydro-alcohol extract was prepared and fractionated using n-hexane and chloroform. Diarrhea was induced with castor oil and magnesium sulphate. Colorimetric method was employed for the determination of total phenolic and flavonoid content. Hydro-alcohol extract showed significant (*p* < 0.05) anti-diarrheal activity. Furthermore, amongst various fractions of hydro-alcohol extract, only chloroform fraction exhibited potent anti-diarrheal activity (*p* < 0.05). The findings of current investigation suggest that *C. wallichii* possesses anti-diarrheal activity and could serve as a potential source for the treatment of diarrhea.

**Keywords:** *Clerodendrum wallichii* (Merr.); Anti-diarrheal potential; Castor oil-induced diarrhea

**Introduction**

Diarrhea is the second leading cause of deaths among the youngest generation and responsible for about 15% of all under-five deaths [1,2]. In developing countries like African and South-East Asian regions about four fifths (nearly 78%) of all under-five mortality occurred [3,4].

As per the World Health Organization (WHO) and UNICEF report, globally about two billion people are suffering from diarrheal disease annually [5,6]. However, in spite of various synthetic drugs available, people believe and utilizing natural product for the management of diarrhea particularly in developing countries. Moreover, WHO encourages traditional medical practices and utilization of natural remedies for the treatment and management of diarrhea [7]. Therefore, medicinal plants represent a promising source for the discovery of new anti-diarrheal agents [8].

*Clerodendrum wallichii* Merr. (Family: Lamiaceae) commonly known as Sampul is native to southern Asia from the Himalayas to Southern China [9,10]. In India, it is found in Sikkim, Tripura, Mizoram, Meghalaya, Assam, Maharashtra and Uttarakhand [11-13]. Ethnopharmacological reports on *Clerodendrum wallichii* revealed that leaf extract of this plant is taken to treat diarrhea and dysentery. Leaves pounded with slaked lime were used for the treatment of inflammation and skin infection by Mao Naga tribe in India. Ethanol extract of aerial parts is used as diuretic. Root juice is given for the treatment of high fever (Marma tribe). It is uses as vegetable...
and in folkloric medicine among Khasi and Jentia tribes in Meghalaya in India [10-12]. The plant possesses phenolic and flavanoid compounds and other chemical groups like steroids, alkaloids etc [14]. Aerial parts of the plant are reported clerodolone, clerosterol, β-sitosterol, stigmasterol and 24(S)ethyl-cholesta-5,22,25-trien-3β-ol [15].

Amongst various species of Clerodendrum genus, C. wallichii species has never been subjected to evaluation of anti-diarrheal activity. Moreover, a vast literature survey on phytochemical and pharmacological activities of C. wallichii revealed that sporadic reports are available on this plant. Thus, it was considered worthwhile to evaluate C. wallichii for anti-diarrheal activity.

**Material and Methods**

**Plant Material**

The plant C. wallichii was collected from Forest Research Institute, Dehradun, Uttarakhand, India. The plant was identified and authenticated from Department of Botany, Botanical Survey of India, Dehradun, Uttarakhand, India vide ref. no. 118175. The voucher specimen was maintained in Botanical Survey of India laboratory for the further reference.

**Reagents and Chemicals**

Petroleum ether (CDH, New Delhi, INDIA), chloroform, ethanol (Himedia), all other chemicals (Loba chemie, Himedia, Mumbai, India), all of LR grade were employed for extraction of the plant material. Standard drug - Loperamide and magnesium sulphate (CDH, New Delhi, INDIA) were used in the present study.

**Extraction Process**

The dried leaves of the plant were collected and crushed by a mixer grinder and pass through a sieve. The powdered material was dried. The plant material was defatted with petroleum ether and then macerated with hydro-alcohol (30:70). After that the extract was concentrated, weighed and subjected to fractionation with each of n-hexane and chloroform.

**Animal**

Wistar albino rats of either sex 150-255 g were used in the study. Rats were fed with standard rodent diet and tap water *ad libitum*. Animals were housed with exposure of natural photoperiod. The protocol of experimental was approved by Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research, Animal Ethics Committee and care of the animals (CPSEA/IAEC/SBS/2017-18/013) was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India.

**Toxicity Study**

The acute toxicity was carried out as per Organization for Economic Cooperation and Development (OECD) 423 Guidelines [16]. The study was carried out in Wistar albino rat. The acute toxicity explored the non toxic nature of all the extracts even at highest starting dose of 2000 mg/kg body weight of animal for oral route of administration.

**Standardization of Plant Extract and its Fraction**

**Determination of total phenolic content:** The plant extract (1 mg/ml) was dissolved in 100 ml of 50% methanol (100 mg/ml) and then further diluted to 1, 2, 4, 8, and 16 mg/ml. Now, 1 ml of each aliquot of each dilution was diluted with 10 ml distilled water. Then, Folin Ciocalteu reagent (1.5 ml) was added to each sample and incubate at room temperature for 5 min. Sodium carbonate solution (20%) was prepared and 4 ml of it was added in each test tube and then further adjust the volume with distilled water up to 25 ml mark. Shaken well and left to stand for 30 min at room temperature. Absorbance of standard was measured at 765 nm using spectrophotometer against blank (distilled water). Total phenolic content was expressed in milligrams of gallic acid equivalents per gram of extract/fraction [17].

\[
\text{Total phenolic content (mg/g)} = \frac{\text{GAE} \times V \times D \times 10^{-6} \times 100}{W}
\]

GAE - Gallic acid equivalent (μg/ml); V - Total volume of sample (ml); D - Dilution factor; W - Sample weight (g)

**Determination of total flavanoid content:** 1 mg/ml of plant extract was dissolved in 100 ml of 80% methanol (100 mg/ml) and further diluted to 7.5, 15, 30, 60 and 120 mg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol (95%), 0.1 ml of aluminium chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. Absorbance at 415 nm was recorded after 30 minutes of incubation against blank (distilled water). A standard calibration plot was generated at 415 nm using known concentration of quercetin. Total flavanoid content was expressed in milligrams of rutin equivalents per gram of extract [17].

\[
\text{Flavonoid content (mg/g)} = \frac{\text{RE} \times V \times D \times 10^{-6} \times 100}{W}
\]

RE - Rutin equivalent (μg/ml); V - Total volume of sample (ml); D - Dilution factor; W - Sample weight (g)
Evaluation of Anti-Diarrheal Activity

Castor oil-induced diarrhea test: Castor oil-induced diarrhea was employed following the Awouters, et al. [18] method. Animals of either sex were divided into eight groups of six rats each. Animals were fasted for 18 h prior to the experiment. Group I served as control and treated with normal saline; Group II received standard drug loperamide (5 mg/kg). Groups III - IV received orally 100 or 200 mg/kg of hydro-alcohol extract while groups V-VIII treated with 50 & 100 mg/kg each n-hexane and chloroform fraction. 1 h later, castor oil (1 ml) was given orally to all the groups of animals. After that animals were placed in cages lined with adsorbent papers and observed for 4 h for the presence of diarrhea defined as watery (wet) or unformed stool. 100% result was considered for control group. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated as per the following formulae:

\[
\% \text{ Inhibition of defecation} = \left[ (A-B) / A \right] \times 100
\]

Where A- indicates mean number of defecation caused by castor oil; B- indicates mean number of defecation caused by extract/fraction.

Magnesium Sulphate-Induced Diarrhea: In this model similar procedure as for castor oil-induced diarrhea was employed. Diarrhea was induced by magnesium sulphate at a dose of 2 g/kg, orally to the animals 30 min after pre-treatment with vehicle (10 ml/kg, p.o.) to the control group; loperamide (3 mg/kg) to the positive control group. Groups III - IV received orally 100 or 200 mg/kg of hydro-alcohol extract while groups V-VIII treated with 50 & 100 mg/kg each n-hexane and chloroform fraction [18,19].

Statistical Analysis

The present findings were analyzed using one way analysis of variance followed by Tukey’s multiple range comparison tests. Data expressed as Mean ± SEM, n = 6. P < 0.05 was considered as statistically significant.

Results

Yield of Extract/ Fractions of C. wallichii Leaves

The yields of hydro-alcohol extract and its fractions of C. wallichii are presented in table 1.

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>Yield (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-alcohol extract</td>
<td>13</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>10</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1: Yield of different fractions and extract of C. wallichii leaves.

Toxicity Study

The acute toxicity was carried out as per OECD 423 Guidelines, 2001. Swiss albino rats were employed in the study. The acute toxicity explored the non-toxic nature of all the extracts even at highest starting dose of 2000 mg/kg body weight of animal for oral route of administration.

Estimation of Total Phenolic and Flavonoid Content

The total phenolic and flavonoid content was estimated to be 56.2 ± 0.51 mg gallic acid equivalents/g of the extract, and 25.2 ± 0.44 mg rutin equivalents/g of hydro-alcohol extract respectively. Where as in chloroform fraction high amount i.e. 110 ± 0.65 mg gallic acid equivalents/g and 49.9 ± 0.39 rutin equivalents/g of chloroform fraction was estimated respectively (Table 2).

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>Total phenolic content (mg gallic acid equivalent/gm of dried extract)</th>
<th>Total flavonoid content(mg rutin equivalent/gm of dried extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-alcohol extract</td>
<td>56.2 ± 0.51</td>
<td>25.2 ± 0.44</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>110 ± 0.65</td>
<td>49.9 ± 0.39</td>
</tr>
</tbody>
</table>

Table 2: Total phenolic and flavonoid content of C. wallichii leaves.

Screening of Anti-Diarrheal Activity

Castor oil- induced diarrhea: In the castor oil-induced diarrhea model, hydro-alcohol extract of C. wallichii and its fractions showed anti-diarrheal effect in rats. The standard anti-diarrheal drug loperamide exhibited superior activity in reducing the number of diarrheal feces by 71.53%, while amongst all tested extract/fractions; chloroform fraction (100 mg/kg) was found to be the most effective, reducing diarrheal droppings by 62.50%. Hydro-alcohol extract (200 mg/kg) also exhibited significant anti-diarrheal activity however, n-hexane fraction was found devoid of anti-diarrheal activity. The results are shown in Table 3.

**Discussion**

Diarrhea is frequently considered a consequence of altered motility and fluid accumulation within the intestinal tract. In some of the cases of diarrhea, the secretory component predominates, while in others diarrhea is characterized by hypermotility [20]. Peristaltic activity is inhibited and tone is reduced by activation of sympathetic innervations of the intestines. Adrenergic α2 receptor on the parasympathetic terminals may also play role in inhibition of sympathetic nerve resulting in stimulation of gastrointestinal motility by inhibiting release of acetylcholine. Activation of the mucosal α2-adrenergic receptor also controls the balance of absorption and secretion in the ileum. Stimulation of these α2-adrenergic receptor in ileum results in a decline of ion fluxes, which is consistent to the of α2-adrenergic receptor agonist to inhibit intestinal fluid secretion [21].

High content of the hydroxylated unsaturated fatty acid ricinoleic acid is present in Castor oil. More than 90% of ricinoleic acid present in castor oil is responsible for production of diarrhea [22] and causes irritation and inflammation of gastric mucosa resulting in release of prostaglandins causing stimulation of secretion [23,24]. In addition, ricinoleic acid sensitizes intramural neurons of the gut. Other alternative mechanisms of castor oil-induced diarrhea: In this diarrhea test model, again hydro-alcohol extract of *C. wallichii* and its fractions exhibited anti-diarrheal effect in animals. The standard anti-diarrheal drug loperamide again exhibited superior activity in reducing the number of diarrheal feces by 68.16%, while amongst all tested extract/fractions; chloroform fraction (100 mg/kg) was found to be the most effective, reducing diarrheal droppings by 60.08%. Hydro-alcohol extract (200 mg/kg) was also exhibited significant anti-diarrheal activity however, n-hexane fraction was found devoid of anti-diarrheal activity. The results are reported in Table 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean of total number of feces</th>
<th>Mean of total number of diarrheal feces</th>
<th>% Inhibition of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>19.25 ± 0.79</td>
<td>11.15 ± 0.82</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Loperamide (3 mg/kg)</td>
<td>7.11 ± 0.45**</td>
<td>3.55 ± 0.40**</td>
<td>68.16</td>
</tr>
<tr>
<td>III</td>
<td>HE (100 mg/kg)</td>
<td>11.35 ± 0.74*</td>
<td>6.62 ± 0.72*</td>
<td>40.62</td>
</tr>
<tr>
<td>IV</td>
<td>HE (200 mg/kg)</td>
<td>9.57 ± 0.64*</td>
<td>5.51 ± 0.50*</td>
<td>50.58</td>
</tr>
<tr>
<td>V</td>
<td>nHF (50 mg/kg)</td>
<td>16.37 ± 0.87</td>
<td>9.98 ± 0.47</td>
<td>40.49</td>
</tr>
<tr>
<td>VI</td>
<td>nHF (100 mg/kg)</td>
<td>15.78 ± 0.77</td>
<td>9.79 ± 0.85</td>
<td>12.19</td>
</tr>
<tr>
<td>VII</td>
<td>CF (50 mg/kg)</td>
<td>9.77 ± 0.66*</td>
<td>5.60 ± 0.70*</td>
<td>49.77</td>
</tr>
<tr>
<td>VIII</td>
<td>CF (100 mg/kg)</td>
<td>7.98 ± 0.48*</td>
<td>4.45 ± 0.50**</td>
<td>60.08</td>
</tr>
</tbody>
</table>

Table 4: Effect of hydro-alcohol extract and its fractions of *C. wallichii* on magnesium sulphate-induced diarrhea in rats. All values are expressed as mean ± SEM, n = 6. * denotes p < 0.05 in comparison to control and ** denotes p < 0.01 in comparison to control.
diarrhea are adenylyl cyclase activation, cyclic adenosine mononucleotide phosphate mediated active secretion and inhibition of Na⁺, K⁺ ATPase activity [25,26]. The current study was designed to assess the anti-diarrheal activity of C. wallichii. The study showed that hydro-alcohol extract of C. wallichii and its chloroform fraction significantly inhibited castor oil-induced diarrhea in rats, as shown by the significant reduction of the number of diarrhea and total faces (table 3).

In the magnesium sulphate-induced model of diarrhea, magnesium sulphate-induced diarrhea by enhancing the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and causes prevention of reabsorption of water and sodium chloride consequently increase intestinal content volume [27]. Furthermore, fluid in the intestinal lumen increased and flow from the proximal to distal intestine enhanced by magnesium sulphate [28]. In the present study hydro-alcohol extract of C. wallichii and its chloroform fraction significantly inhibited magnesium sulphate-induced diarrhea in rats (Table 4). This improvement is expected due to increase in water and electrolyte reabsorption from the gastrointestinal tract.

Anti-diarrheal activity has been exhibited by the various groups of phytoconstituents like flavonoids, alkaloids, tannin, saponins and steroids [29]. The leaves of C. wallichii comprise numerous phenolics and flavonoids content which may be responsible for its consequence. Phytochemical screening of hydro-alcohol extract of C. wallichii and its fractions showed the presence of mainly steroids, carbohydrates, phenolic and flavonoid compounds. Flavonoids consist of a large group of polyphenolic compounds and are ubiquitously found in plants which inhibit autacoids release and prostaglandins, causing motility reduction and secretion-induced by castor oil [30,31]. It has been reported that methanol extract prepared from Hymenaea stigonocarpa showed anti-diarrheal effect due to presence of flavonoids and tannins [32]. These properties of such phytoconstituents may justify the reason for the effective use of the plant as anti-diarrheal agent. Therefore, it is proposed that flavonoids content of hydro-alcohol extract of C. wallichii and its chloroform fraction among others would be responsible for the anti-diarrheal action. High quantities of phenolic and flavonoid contents were estimated in this study (Table 2). In this study, hydro-alcohol extract of C. wallichii and its chloroform fraction, standard drug loperamide reduced diarrhea could be due to reduction in gastrointestinal motility or by inhibiting induced intestinal accumulation of fluid or by enhancing reabsorption of electrolytes and water.

Conflicts of Interest

Authors have no conflict of interest.

Acknowledgment

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