Phytochemical screening, acute toxicity, antinociceptive and antidiarrheal activity of *Gendarussa vulgaris* leaves extract


Abstract

*Gendarussa vulgaris* Nees (Acanthaceae) is used in folk medicine for the treatment of asthma, rheumatism, colic’s, arthritis, jaundice, inflammation, cephalgia, eczema, diarrhea, wounds, dyspepsia, pain and fever. The present study was designed to qualitatively evaluate the profile of phytochemical constituents as well as acute toxicity, antinociceptive and antidiarrheal activities of ethyl acetate extract of *Gendarussa vulgaris* leaves (EAGVL). Phytochemical constituents, acute toxicity, antinociceptive and antidiarrheal activities were determined and assessed by various tests such as Molisch’s test, Fehling test, Mayer’s test, frothing test, FeCl₃ test, alkali test, Salkowski’s test, Keller-killiani test and CuSO₄ test, OECD guidelines, formalin-induced paw licking, acetic acid-induced writhing, castor oil and MgSO₄ induced diarrheal test. This extract figured the presence of carbohydrates, alkaloids, flavonoids, tannins, glycosides, triterpenoids, fat and fixed oils. Mortality, behavioral changes or sign of any toxicity were not observed up to the dose as high as 4000 mg/kg. The crude extract was found to have significant (*P* < 0.05, vs. control) analgesic activity at the oral dose of 200 mg/kg and 400 mg/kg (b. wt.) in the tested animals. Moreover, both doses of (200 mg/kg and 400 mg/kg) ethyl acetate extract significantly (*P* < 0.05, vs. control) reduced the gastrointestinal motility and inhibit the percentage of diarrhea in antidiarrheal models. But 400 mg/kg dose showed better antinociceptive and antidiarrheal activity than 200 mg/kg dose compared to control. The results indicate that *Gendarussa vulgaris* leaves may provide a potential source of antinociceptive and antidiarrheal activities.

Keywords: Phytochemical screening, *Gendarussa vulgaris*, acute toxicity, antinociceptive, antidiarrheal

1. Introduction

*Gendarussa vulgaris* (Family: Acanthaceae, commonly known as willow-leaved justicia, Nili nargandi, bakas, kala adulasa, kasanah, Gandharasa, vaidyasinha) is a small erect, fast-growing, branched shrub with attractive, lanceolate (shaped like a lance-head), ascending to spreading variegated leaves in shades of green, white and grey, and produces dainty white flowers. It has been described as rare and endemic to India, though those claims are at least confusing, in the context of statements that the plant is widely used in various forms for many of its medicinal and insecticidal properties [1] and that it is a quick-growing, evergreen forest shrub considered to be a native of China and distributed in Bangladesh, Sri Lanka, India, and Malaysia [2].

The plant is shrubby, about 2–4 ft. high. Leaves are simple, entire, opposite, lanceolate, variegated in shades of white, green and grey, 7 to 14 cm long and 1 to 2.5 cm wide, glabrous on both sides, apex acute-acuminate. The rather small flowers are borne in 4–12 cm long spikes, at the end of branches or in leaf axils. The teeth of the sepals cup are smooth, linear, and about 3 mm long. The flowers are about 1.5 cm long, white or pink, with purple spots. The capsule is club-shaped, about 1.2 cm long, and smooth. Capsule 1-2 inch. long, clavate, glabrous; seeds unknown [3].

The chemical constituents of the leaves include O-disubstituted aromatic amines, 2-aminobenzyl alcohol and their respective O-methyl ethers, friedelin, lupeol and β sitosterol [4] which are revealed from the present study.

Generally, the plant is considered as an emetic, emmanagogue, febrifuge, diaphoretic and leaves are traditionally used in the treatment of respiratory disorders like cough, cold, bronchitis, throat infections, pulmonary infections, arthritis, jaundice, cephalgia, hemiplegia, eczema, and allergic disorders like bronchial asthma etc [5].

Therefore, the present study was designed to justify the antinociceptive and antidiarrheal activities of *Gendarussa vulgaris* leaves, and evaluate the traditional usage scientifically.
2. Materials and Methods

2.1. Collection and Identification of the Plant
For performed this study, green and freshness leaves of *Gendarussa vulgaris* plant was collected from Jessore University of Science & Technology, Jessore, Bangladesh, in January, 2018. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

2.2. Extraction
300 gm of powdered leaves were taken for ethyl acetate extraction. First, the leaves of *Gendarussa vulgaris* were separated from the plant and thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature (25±2 °C) for two weeks. The materials were ground into coarse powder and cold extraction method was used to extract the active components. The ground leaves (300 gm) were soaked in sufficient amount (approximately 2 L) of ethyl acetate for 14 days at room temperature with periodical shaking and stirring. The whole mixture was primarily filtered through cotton and then through Whatman No.1 filters. The solvent was evaporated with a rotary evaporator under reduced pressure at 40°C temperature to yield semisolud crude extract. The percentage yield of the extract was 2.97% (w/w). The extract was then preserved in a refrigerator till further use.

2.3. Experimental Animals
To run the experiment of antinociceptive and anti diarrheal activity, one hundred and fifteen Swiss albino mice of either sex, aged 4-5 weeks, weighing about 25-30 gm were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Before initiating the experiment, the animals were exposed to alternative 12:12 hours light and dark cycle at an ambient temperature of 26±2°C. It was ensured the proper supplies of foods and water ad libitum. All protocols for the animal experiment were approved by the Institutional Animal Ethical Committee of Jessore University of Science & Technology, Jessore, Bangladesh. Prior to the study, mice were aclimatized for 7 days in the laboratory environment and maintained the constant environmental and adequate nutritional conditions throughout the period of the experiment.

2.4. Phytochemical Screening
Freshly prepared *Gendarussa vulgaris* leaves extract were subjected to different qualitative tests.

2.4.1. Molisch’s test for carbohydrates
About 500 mg of crude extract was dissolved in 5 mL of distilled water and later filtered. A few drops of Molisch’s reagent (α-naphthol 10% (w/v) in 90% ethanol) were added to the filtrate. Then 1 mL of concentrated H$_2$SO$_4$ was poured carefully along the side of the test tube. Two minutes later, 5 mL of distilled water was added. A positive test, indicating the presence of carbohydrates, was confirmed with the formation of dull violet or red color at the interphase of the two layers [6].

2.4.2. Fehling’s test for reducing sugars
2 mg plant extract was dissolved in 1 mL of distilled water and filtered. Then, 1 mL mixture of Fehling’s solutions A and B (a ratio of 1:1) was added to the filtrate, which was heated in a water bath for a few minutes. Formation of brick-red precipitate confirmed the presence of reducing sugars [7].

2.4.3. Mayer’s test for alkaloids
In Mayer’s test, one or two drops of 0.35 mol/L Mayer’s reagent (potassium- mercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 mL distilled H$_2$O$_2$) was added to 2 mL (50 mg extract dissolved in 5 mL of 1% aqueous HCl) filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a white creamy precipitate [8].

2.4.4. Frothing test for saponins
100 mg plant extract was dissolved in 10 mL of methanol for making stock solutions. These stock solutions were diluted to 0.5 mg/mL by the additions of 20 mL of distilled water. The test tube containing the dilution was then shaken for 15 min. Formation of foam on the top of the test tubes indicated the presence of saponins [7].

2.4.5. FeCl$_3$ test for tannins
Approximately 50 mg plant extract was dissolved in 5 mL distilled water, followed by the addition of a few drops of 5% FeCl$_3$. Tannin was confirmed by the development of a bluish-black color [9].

2.4.6. Alkali test for flavonoids
For this test, a few drops of 5% NaOH solution were added to 1 mL of filtered stock solution (100 mg of extract dissolved in 10 mL of methanol), which produced a deep-yellow color. The color was lost in the presence of dilute HCl and confirmed flavonoids [9].

2.4.7. Salkowski’s test for triterpenoids
2 mg plant extract was shaken in 1 mL of CHCl$_3$. Then, a few drops of concentrated H$_2$SO$_4$ were added to the solution along the side of the test tube. Development of a red-brown color at the interface indicated the presence of triterpenoids [7].

2.4.8. Keller-killiani test for glycosides
For this screening, 1ml of extract, 1ml of Glacial acetic acid and few drops of 2% FeCl$_3$ were added and then 1ml of con. H$_2$SO$_4$ is also added to the mixture. The appearance of Brown ring shows the presence of glycosides [10].

2.4.9. CuSO$_4$ test for fat and fixed oils
In this test, 5 drops of extract solution (0.25g extract dissolved in 25 mL mother solvent) mixed with 1 mL of 1% CuSO$_4$ and then few drops of 10% NaOH was added. The appearance of the clean blue solution shows presence of fat and fixed oils.

2.5. Acute Oral Toxicity Study
Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. According to Organization of Economic Cooperation and Development (OECD) guidelines, the acute toxicity study of *Gendarussa vulgaris* leaves was designed to estimate the half lethal dose (LD50) of the experimental samples [11]. Fifteen mice were divided into two groups: control group and test group (EAGVL), with five animals per group. The experimental sample (EAGVL) was administered orally at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After that the animals were observed every 1 h for the next 5–6 h for mortality, behavioral pattern changes such as salivation, weakness, aggressiveness, food or water refusal, diarrhea, discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any sign

~ 578 ~

*Journal of Pharmacognosy and Phytochemistry*
of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted.\textsuperscript{[11]}

### 2.6. Antinociceptive Study

#### 2.6.1. Formalin-Induced Paw Licking Test

The slightly modified method of Hunskaar and Hole\textsuperscript{[12]} was followed for the formalin-induced paw licking test. Twenty Swiss albino mice were selected for this test and divided into four groups containing five mice in each group, and they have fasting for 16h with water ad libitum. Control group, standard group, and test groups were treated with distilled water (10mL/kg), diclofenac sodium (DS, 100 mg/kg), EAGVL at 200 and 400mg/kg, respectively. All of the treatments were administered orally [per oral (p.o.) route]. After 1h of treatment, each mouse was injected with 20μL of 2.7% (v/v) formalin solution into the dorsal surface of the left hind paw. Mice were observed for 5min after injection and the time spent in licking, biting, and shaking behaviors was measured in seconds, which was considered as the acute phase (0-5 min). Again, they were monitored for 5min after 40min of injection which was defined as late phase (20-25 min).

The percentage of inhibition of licking was calculated by the following formula:

\[
\text{Inhibition} \% = \left[ 1 - \frac{\text{No.of licking (standard or extracts)}}{\text{Licking time (normal control)}} \right] \times 100
\]

#### 2.6.2. Acetic Acid-Induced Writhing Test

The method of Koster et al.\textsuperscript{[13]} was used for acetic acid induced writhing test. Mice were kept unfed for 16h with water ad libitum prior to the experiment and pretreated with extracts as mentioned before. DS (100mg/kg) was used as the standard or positive control and distilled water as the normal control. After 4h of respective treatment, each mouse was injected intraperitoneally with 0.7% (v/v) acetic acid at a dose of 10mL/kg body weight. The number of writhing responses of each mouse was counted for 5 min period, which began 15 min late of acetic acid administration.

To calculate the percentage of inhibition of writhing, the following formula was used.

\[
\text{Inhibition} \% = \left[ 1 - \frac{\text{No.of writhing (standard or extracts)}}{\text{No.of writhing (normal control)}} \right] \times 100
\]

### 2.7. Antidiarrheal Study

#### 2.7.1. Castor Oil Induced Antidiarrheal Test

A slightly modified method of Shoba and Thomas\textsuperscript{[14]} was used in castor oil induced antidiarrheal test in mice. By administering 0.5mL of castor oil orally the preliminary screening of animals was performed, and those animals that started diarrhea were selected finally for the test. Twenty diarrheal screened mice were divided into control group (distilled water), positive control or standard group (Loperamide HCl, 3 mg/kg b.w.), and test groups (EAGVL 200 mg/kg and 400 mg/kg b.w.), containing five mice in each group. Experimented animals were fasted for around 16h with water ad libitum. Mice in the control group, standard group, and test groups orally received one dose of distilled water, Loperamide HCl, EAGVL 200 mg/kg and 400 mg/kg respectively. Then, each animal received 0.5mL of castor oil orally for initiating diarrhea after 30min of the above treatments. Observation for defecation continued up to 4h on blotting paper lined individual cage was used for placing every animal. Blotting papers were replaced every hour. The number of diarrheal feces was count and recorded for a period of 4h and the percentage of inhibition of defecation was calculated for every group of animals.

#### 2.7.2. MgSO\textsubscript{4} Induced Antidiarrheal Test

MgSO\textsubscript{4} induced antidiarrheal test was performed according to the method described by Doherty\textsuperscript{[15]} with slight modification. Here, a similar procedure as for castor oil induced diarrhea test was maintained for magnesium sulphate induced diarrheal model. The animals all were screened for diarrhea was done by administering magnesium sulphate at a dose of 2g/kg orally. Experimented animals have fasted for 16h with water ad libitum. Then, mice were grouped and treated as described before. Then, each animal received 2g/kg of magnesium sulphate orally for initiating diarrhea after 30min of the above treatments. Observation for defecation is same as for castor oil induced diarrhea test, and the antidiarrheal activity was expressed by comparing the percent of inhibition of defecation of different groups with the control group.

### 2.8. Statistical Analysis

The experimental results were expressed as mean ± SEM (Standard Error of mean). Statistical analyses for antinociceptive and antidiarrheal studies were evaluated by one-way ANOVA following Dunnett’s test through the SPSS software (version 16; IBM Corporation, New York, USA). The obtained results were compared with the vehicle control group. The $P<0.05$ was considered to be statistically significant.

### 3. Results

#### 3.1. Phytochemical Screening

It is important to depict the chemical nature of plant materials after evaluation of the pharmacological activities of plant extract. Phytochemical screening of the \textit{Gendarussa vulgaris} leaf showed the presence of several primary and secondary metabolites, or phytoconstituents, which are summarized in table-1. In the phytochemical screening, EAGVL showed the presence of almost all of the phytoconstituents like alkaloids, carbohydrates, flavonoids, tannins, phenols, glycosides, triterpenoids, fat and fixed oils that were tested here. However, some tests did not show consistent results such as carbohydrate content in EAGVL was indicated by Molisch’s test, but not by Fehling’s test.

#### 3.2. Acute Oral Toxicity Study

No mortality was viewed up to the dose as high as 4000 mg/kg for EAGVL or control group in acute oral toxicity study. Any signs of toxicity or behavioral changes were not observed up to the dose as high as 4000 mg/kg for EAGVL (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently indicated that the test group does not show acute oral toxicity.

### 3.3. Antinociceptive Study

#### 3.3.1. Formalin-Induced Paw Licking Test

Of the ethyl acetate extract of \textit{Gendarussa vulgaris} leaves, both doses (200 mg/kg and 400 mg/kg) were showed a significant reduction in the duration of paw-licking as compared to the control group in the early phase. Mean paw licking time for 200 mg/kg and 400 mg/kg at the early phase were 62.50±7.39 and 48.50±3.70 sec respectively and percent inhibition were 50.74% and 61.78% respectively. In the late phase, the 200 mg/kg and 400 mg/kg dose were reduced paw licking time by 88.37% (5.86±0.97 sec) and 93.23% (3.38±0.31 sec) which were very significant compared to control. Increasing the dose also increasing the analgesic...
activity of that extract. Results are showed in table-2 and illustrated in figure 1.

3.3.2. Acetic Acid-Induced Writhing Test
In this test, both doses of extract (200 mg/kg and 400 mg/kg) were highly inhibited the numbers of writhing. The extract was more effective at high dose (400 mg/kg) with a mean value of 13.60±1.57 than the low dose (200 mg/kg) with a mean value of 17.60±1.33. But both of their results were very significant (P= 0.000, 0.006 in respectively). Percent protection offered by 200 and 400 mg/kg was 33.83% and 48.87% in respectively. Where the mean number of writhing’s of the standard drug was 12.00±1.22, which was very highly significant (p = 0.000) compared to that of the control (26.60±2.98). Both doses of extract (200 mg/kg and 400 mg/kg) are dose dependent in manner. The effects are displayed in table-3 and illustrated in figure 2.

3.4. Antidiarrheal Study
3.4.1. Castor Oil Induced Antidiarrheal Test
In the castor oil induced diarrheal mice, Loperamide HCl (3 mg/kg) and ethyl acetate extract of Gendarussa vulgaris leaves at the doses of 200 mg/kg and 400 mg/kg significantly (P<0.05, vs. control) reduced the total number diarrheal feces. Here, the decrease of the total number of diarrheal feces is dose dependent in manner. Highest and most significant (P<0.05, versus control) percentage of inhibition of diarrhea (52.63%) was revealed by EAGVL 400 mg/kg. Castor oil induced antidiarrheal results are showed in table-4 and illustrated in figure 3.

3.4.2. MgSO4 Induced Antidiarrheal Test
In the magnesium sulphate induced diarrheal mice, Loperamide HCl (3 mg/kg) and ethyl acetate extract of Gendarussa vulgaris leaves at the doses of 200 mg/kg and 400 mg/kg significantly (P<0.05, vs. control) reduced the total number diarrheal feces. Here, the decrease of the total number of diarrheal feces is dose dependent in manner. Highest and significant (P<0.05, versus control) percentage of inhibition of diarrhea (50.00%) was revealed by EAGVL 400 mg/kg. Magnesium sulphate induced antidiarrheal results are showed in table-5 and illustrated in figure 4.

4. Discussion
From the phytochemical analysis of the leaves of Gendarussa vulgaris, it revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, glycosides, and triterpenoids which are act as bioactive compounds of plant extracts and may be responsible for the diverse activities when herbs are used medicinally [16]. The acute oral toxicity study is a vital factor in the investigation of therapeutic index of drugs and xenobiotics [17]. As no mortality was observed up to the dose as high as 4000 mg/kg, LD₅₀ of Gendarussa vulgaris leaves extract could not be obtained. For this, the extract was found to be safe with a broad therapeutic range and two comparatively high doses (200 mg/kg and 400 mg/kg) of EAGVL were used for in-vivo doses.

In the antinociceptive study, both peripheral and central activities of nociception are revealed in formalin-induced paw licking test. The response time of the animals spends in licking the injected paw were measured in both acute and late phase during this test. Two different periods of licking activity, an early response (0-5 min after the formalin injection) and a late response (20-25 min after the formalin injection). The direct effect of formalin on nociceptors and prostaglandins has occurred in early phase which was not significantly in the early phase (no inflammatory pain) where the late phase reflects pain from formalin-induced inflammation which can be inhibited by anti-inflammatory drugs. The late response is inhibited by peripheral analgesic only where both phases response are inhibited by the narcotic analgesic [18]. Ethyl acetate extract of Gendarussa vulgaris leaves inhibited the percentage inhibition of licking at both phases.

In the acetic acid-induced writhing study, the pain sensation is arising through the activation of the localized inflammatory response by acetic acid. It is a reliable and simple model to evaluate the peripheral type of analgesic action of crude and other drugs. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally active analgesic [19]. Here endogenous substances such as serotonin, bradykinin, histamine, and prostaglandin are involved in pain generation [20, 21]. Therefore, plant extract might be inhibiting the synthesis and/or release of these endogenous substances and thus reduce pain.

The castor oil induced diarrhoea demonstrates secretory diarrhoea, since recinolic acid the active ingredient of castor oil, induces diarrhoea by hypersecretory response [22] and increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water, which is associated with prostaglandin release [23]. As a result, absorption of sodium and potassium ions are reduced, which sequentially lessens the function of Na+, K+-ATPase in colon plus small intestine [24]. In the study, both doses of ethyl acetate extract (200 mg/kg and 400 mg/kg) of Gendarussa vulgaris leaves showed a significant inhibition (P<0.05, versus control) of castor oil induced diarrhea in mice and may be due to the inhibition of electrolyte permeability of the intestine and prostaglandin release and it can be assumed that the antidiarrheal action of plant extract was exerted by antisecretory mechanism.

On the other hand, magnesium sulphate induces diarrhea by promoting cholecystokinin release from the duodenal mucosa preventing the reabsorption of sodium chloride and water from the lumen. Discharge of cholecystokinin and nitric oxide from duodenal mucosa occurs after its oral administration. Then two recurrently results come about and one is the rise of secretion and motility of small intestine. Another is the inhibition of reabsorption of NaCl and water that occurs from the previous case [25]. Ethyl acetate extract of Gendarussa vulgaris leaves extract (200 mg/kg and 400 mg/kg) was effective in reducing diarrhea and that was expected due to increase in electrolyte and water reabsorption from the gastrointestinal tract and thus reduce diarrhea.

All activities seem to be due to the presence of tannins and flavonoids in the ethyl acetate extract of Gendarussa vulgaris leaves. In fact, tannins are responsible for the denaturation of proteins and form protein tannate, which reduces the intestinal mucosa permeability [26]. The ethyl acetate extract of Gendarussa vulgaris leaves was administered at the dose of 200 mg/kg and 400 mg/kg showed 31.58% and 52.63% reduction of diarrhea in castor-oil induced diarrheal test and 34.38% and 50.00% reduction of diarrhea in MgSO₄ induced diarrheal test respectively.

So we can conclude that the present study seems to support the claims of a traditional medicine practitioner about the use of Gendarussa vulgaris in pain and diarrhea.
Table 1: Phytochemical screening of ethyl acetate extract of *Gendarussa vulgaris* leaves.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Test name</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkali test</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Salkowsky’s test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller-killiani test</td>
<td>+</td>
</tr>
<tr>
<td>Fat and Fixed oils</td>
<td>CuSO₄ test</td>
<td>+</td>
</tr>
</tbody>
</table>

*+* mean presence of specific phytoconstituents and *-* mean absence of specific phytoconstituents.

Table 2: Effects of ethyl acetate extracts of *Gendarussa vulgaris* leaves on formalin-induced paw licking test

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose</th>
<th>Licking time (s)</th>
<th>% inhibition</th>
<th>Licking time (s)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>126.90±5.41</td>
<td>-</td>
<td>50.39±5.44</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100 mg/kg</td>
<td>60.28±7.28</td>
<td>52.49</td>
<td>2.49±0.36</td>
<td>95.06</td>
</tr>
<tr>
<td>EAGVL</td>
<td>200 mg/kg</td>
<td>62.50±7.39</td>
<td>50.74</td>
<td>5.86±0.97</td>
<td>88.37</td>
</tr>
<tr>
<td>EAGVL</td>
<td>400 mg/kg</td>
<td>48.50±3.70</td>
<td>61.78</td>
<td>3.38±0.31</td>
<td>93.23</td>
</tr>
</tbody>
</table>

Numbers of licking time inhibition are presented as (mean ± standard error of mean). *P*<0.05, vs. control; (Dennett’s *t* test)

Table 3: Effects of ethyl acetate extracts of *Gendarussa vulgaris* leaves on acetic acid-induced writhing test

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg</th>
<th>No of writhing</th>
<th>% of inhibition</th>
<th><em>p</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>26.60±2.98</td>
<td>-</td>
<td>.000</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100 mg/kg</td>
<td>12.00±1.22</td>
<td>54.89</td>
<td>.000</td>
</tr>
<tr>
<td>EAGVL</td>
<td>200 mg/kg</td>
<td>17.60±1.33</td>
<td>33.83</td>
<td>.006</td>
</tr>
<tr>
<td>EAGVL</td>
<td>400 mg/kg</td>
<td>13.60±1.57</td>
<td>48.87</td>
<td>.000</td>
</tr>
</tbody>
</table>

Numbers of writhing are presented as (mean ± standard error of mean). *P*<0.05, vs. control; (Dennett’s *t* test)

Table 4: Effects of ethyl acetate extracts of *Gendarussa vulgaris* leaves on castor oil-induced diarrhea test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Number of diarrheal feces</th>
<th>% of inhibition of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>7.60±0.81</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide HCL</td>
<td>3 mg/kg</td>
<td>1.80±0.37</td>
<td>76.32</td>
</tr>
<tr>
<td>EAGVL</td>
<td>200 mg/kg</td>
<td>5.20±0.37</td>
<td>31.58</td>
</tr>
<tr>
<td>EAGVL</td>
<td>400 mg/kg</td>
<td>3.60±0.24</td>
<td>52.63</td>
</tr>
</tbody>
</table>

Numbers of feces are presented as mean ± SEM (standard error of mean). *P*<0.05, vs. control (Dennett’s *t* test)

Table 5: Effects of ethyl acetate extracts of *Gendarussa vulgaris* leaves on MgSO₄ induced diarrhea test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Number of diarrheal feces</th>
<th>% of inhibition of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>6.40±0.75</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide HCL</td>
<td>3 mg/kg</td>
<td>1.40±0.40</td>
<td>78.13</td>
</tr>
<tr>
<td>EAGVL</td>
<td>200 mg/kg</td>
<td>4.20±0.58</td>
<td>34.38</td>
</tr>
<tr>
<td>EAGVL</td>
<td>400 mg/kg</td>
<td>3.20±0.58</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Numbers of feces are presented as mean ± SEM (standard error of mean). *P*<0.05, vs. control (Dennett’s *t* test)

Fig 1: Effect of ethyl acetate extract of *Gendarussa vulgaris* leaves in formalin-induced paw licking test X-axis – group of experimented animal Y-axis – number of paw licking time Level of Significance = *P*<0.05 compared to control (Dennett’s *t* test).
Fig 2: Effect of ethyl acetate extract of *Gendarussa vulgaris* leaves in acetic acid-induced writhing test X-axis – group of experimented animal Y-axis – number of writhing Level of Significance = $P<0.05$ compared to control (Dennett’s t test).

Fig 3: Effect of ethyl acetate extract of *Gendarussa vulgaris* leaves in castor oil-induced diarrheal test X-axis – group of experimented animal Y-axis – number of diarrheal feces Level of Significance = $P<0.05$ compared to control (Dennett’s t test).
5. Conclusion
From the above study, it could be suggested that Ethyl acetate extract of Gendarussa vulgaris leaves might possess analgesic and antidiarrhoeal activities. Data obtained in this study showed that all activities were dose-dependent and statistically significant. The presence of flavonoids, alkaloids, sitosterol, tannin and phenolic compounds might be responsible for these activities and which are probably mediated via inhibition of various autacoids formation and release. We hope that the further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities. The genotoxicity study of this extract may be a promising area for novel 'lead' discovery for analgesic and antidiarrhoeal drug development.

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8. References


