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Original Article

Thrombolytic, Membrane stabilizing, Antidiarrhoeal, and Antimicrobial Properties of Bioactive Compounds Isolated from leaves of *Sesbania grandiflora* Naturally Growing in Bangladesh

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Abstract

Sesbania grandiflora leaves are extensively used locally since ancient time as in traditional systems. Based on mainly folkloric use, we investigated to know the potential of *Sesbania grandiflora* leaves extracts through biological works *in vivo* and *in vitro*. The ethanolic extract of *S. grandiflora* leaves was partitioned into ethyl acetate soluble fraction (ESF), petroleum ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF), and aqueous soluble fraction (AQSF). The extracts were evaluated for their thrombolytic, membrane stabilizing, antimicrobial and antidiarrhoeal activities and the results were compared with standard drugs; streptokinase for thrombolytic, acetyl salicylic acid for anti-inflammatory, kanamycin for antimicrobial activities and loperamide against diarrhoea. In thrombolytic investigation, among all partitionates, the ESF showed highest % of clot lysis (59.57%) as compared to (69.23%) and (3.07%) exhibited by the standard streptokinase and water. In case of membrane stabilizing study, ESF also significantly inhibited the haemolysis of human erythrocyte membrane both induced by hypotonic solution (64.30 ± 0.64%) and by heat (57.21 ± 0.69%), respectively as compared to (70.12 ± 26%) and (73.90 ± 0.29%) demonstrated by acetyl salicylic acid. In the antidiarrhoeal assay, the ethanolic extract (EE) inhibited the mean number of defecation by 24.97% and 41.05% at 200 mg/kg and 400 mg/kg body weight, respectively. In antibacterial activity, it was exhibited by the extracts being comparatively more prominent on the gram negative bacteria than the gram positive bacteria. Our study revealed that satisfactory amount of flavonoid and tannin presence showed a significant and positive correlation between total phenolic compound contents with pharmacological activities of *S. grandiflora.*

Keywords: *antimicrobial and antidiarrhoeal activities, membrane stabilizing, Sesbania grandiflora*, thrombolytic.

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1. Introduction

Medicinal plants are one of the important contributors to most of the medicinal preparations as raw plant materials, refined crude extracts, mixtures and isolated pure compound. Numerous plants species have been known to possess medicinal values and are used to treat variety of ailments in various cultures worldwide [1]. Despite the great advancement observed in modern medicine in recent decades, plants still play an important role in health care [2] and according to WHO more than 75% of the world population relies on medicinal plants or natural products to treat common ailments. Despite the toxicity profile of most medicinal plants, which are yet to be thoroughly evaluated, it is well accepted that medicines derived from plant products are safer, more low cost, and more easily available than their synthetic counterparts [3]. *Sesbania grandiflora* (L) Pers. belongs to the family: Fabaceae and in Bangladesh, it is locally known as Bagful due to its medicinal values. It is a species of tropical climate, short lived, quick growing and soft wooded tree. It grows up to 6-9 meters high and is available in several parts of Bangladesh. Its flowers are fleshy with large snowy white, pink or crimson petals which are 7.5 to 10 cm long with short axillary racemes. Leaves are long, abruptly pinnate; leaflets 41-61 linear, oblong, and glabrous. Tender leaves, flowers and pods are used as vegetable and considered excellent source of vitamin C and calcium. Seeds are rich in protein (4). Almost every single part of *S. grandiflora* is used as folkloric or traditional medicine to treat an array of diseases such as dysentery, stomatitis, fever, small pox, sore throat, headache, etc. This plant is also used in Indian traditional system of medicine, Sidha and Ayurveda, for the treatment of various acute and chronic disorders. The dried leaves are often used to make tea and are considered to have good antibacterial, anthelminthic, antitumor and contraceptive properties [5]. A poultice made from the leaf juice is used in folkloric system as an effective treatment for bruises [6]. The leaf is widely used in detoxification process of Ayurvedic metallo mineral drug, Manacle [7]. *S. grandiflora* leaves have been shown to contain protein-8.4 gms, fat-1.4 gms, minerals-3.1 gms, crude fibers 2.2gms, carbohydrates-11.8 mg, energy-93 mg, calcium-1130 mg, phosphorus-80 mg, iron-3.9 mg making it highly nutritious [8].Young leaves of *S. grandiflora* are edible and are quite often used to supplement meals. It has also been reported to be a potent antidote for tobacco and smoking-related diseases [9]. Numerous literatures mentioned that *S.grandiflora* leaves, flowers, and aerial parts of

this plant successfully isolated sterols, saponins and tannins. These bioactive constituents are useful as potential health benefits and have been claimed to possess important biological activities such as antibacterial and antifungal [10], antioxidant [11, 12, 13], antiurolithiatic [14], anticonvulsant and anxiolytic [15], and hepato protective properties [16]. In Bangladesh, Tangail (district) especially in rural area, the villagers use *S. grandiflora* leaves as concentrated leaf juice with sugar which is a remedy for diarrhoea since ancient times. Its potential medicinal value is approved by local users as they are greatly benefited by the plant. On the basis of their traditiontional use of the leave and presence of bioactive compound in the *S.grandiflora* leaves in significant amount which, has extended to find out the potentiality of the leaf extract as an antidiarrhoeal. Use of crude ethanolic extracts of *Sesbania grandiflora* moderately reduces the defecation number and prolongation of frequency of defecations. Although we did not find out scientific investigations for diarrhoea using this plant but we have undertaken to validate the local use of this plant as remedy of diarrhoea by scientific investigation *in vitro*. Because of its copious, widespread availability and folkloric use, the present research was undertaken also to enquire into the potentiality of *S.grandiflora* extract as membrane stabilizing, thrombolytic and antibacterial activity against important human pathogens.

2. Materials and Methods

2.1. Plant Materials

The leaves of *S. grandiflora* were collected from Tangail district near Dhaka, Bangladesh in February 2015 and were identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. The Voucher specimens (DACB accession no. 42103) for this plant were also preserved in Bangladesh National Herbarium office.

2.2. Preparation of Extract

To avoid the loss of essential plant components, the collected plant leaves were shade dried for several days and then oven dried for 12 hours at 37°C to facilitate grinding and to obtain powder of uniform particle size that can pass through sieve # 40. The powdered leaves were then preserved in an air- tight container. The dried powder material (410 gm) was soaked in 1500 ml of 90% ethanol for ten days and was shaken occasionally. The whole mixture was filtered by a piece of clean, white cotton followed by Whatman filter paper. The filtrate was dried using a vacuum rotary evaporator at optimum temperature of 40 °C to prevent loss of important plant constituents and to obtain the crude ethanolic extract of *S. grandiflora* (yield-9 gm, Table 1). The concentrated aqueous ethanol extract was partitioned by the Kupchan method [17] and the resultant partitionates, i.e. ethyl acetate (ESF), petroleum ether (PSF), carbon tetrachloride (CTSF), chloroform (CSF), and

Table 1. Fractions of 9 gm ethanolic crude extract (EE) of *S. grandiflora*.

Plant part	**L eaves**				
Extract fraction	PSF	CTSF	CSF	ESF	ASF
	2.3	0.7	1.6	2	2.4
Fraction amount (%)	25.5	7.7	17.7	22.2	26.6

aqueous soluble (ASF) extracts were used for the current investigation.

2.3. Phytochemical Screening

The freshly prepared organic extracts were qualitatively tested for the presence of various categories of phytochemicals. These were identified by characteristic color changes using standard procedures, previously described by Software [18].

2.4. Determination of Total Flavonoid Content

The total flavonoid content was estimated using a method previously described by Kumaran and Karunakaran [19] using quercetin as a reference compound. To determine total flavonoids content in this study, 1 ml of leaves extract in ethanol (250 µg/ml) was mixed with 1 ml aluminum chloride in ethanol (20 mg/ml) and a drop of acetic acid. The mixture was then diluted with ethanol to 25 ml. The absorption was read after 40 min at 415 nm. A blank sample was prepared in similar way without the extract. The absorption of various concentrations of standard quercetin solution in ethanol was measured under the same conditions to plot a calibration curve.

2.5. Determination of Total Tannin Content

The total tannin content of *S. grandiflora* was determined by Folin - Coicalteu method [20]. Briefly, 0.3 ml (300 µl) of the ethanolic extract and its different fractionates were added to a volumetric flask (10 ml) containing 2.7 ml of Folin-Coicalteu (1:10) phenol reagent. After 5 min, 2 ml of 7.5 % sodium carbonate solution was added to each tube, the mixture were shaken, followed by heating at 45 ^{0}C for 15 minutes and was kept at room temperature for 30 min in dark place. A set of reference standard solutions of tannic acid (50 to 300 µg/ml) were prepared similarly without extract. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The total tannin content was determined from extrapolation of tannic acid calibration curve.

2.6. *In Vitro* Thrombolytic Activity

The crude extracts were individually suspended in 1 ml of sterile distilled water and shaken vigorously on a sonicator to obtain a uniform liquid. The suspension of the extract was kept overnight and decanted later to remove soluble supernatant, which was filtered through a filter paper to obtain residue less solution. The

resulting solution used as such for *in vitro* evaluation of clot lysis activity**.**

The thrombolytic activities of prepared extracts were evaluated by the method described by Daginawala [21]. By sterile hypodermic syringe 5 ml sterile distilled water were added in freeze- drying streptokinase (SK) vial (15, 00,000 IU) and mixed properly. This suspension used as a stock from which 100 µl (30,000 I.U) was used for *in* vitro thrombolytic activity. Venous blood (6 ml) was provided by healthy and willing volunteers, noting that they all did not have any history of oral contraceptive or anticoagulant therapy. The blood was taken maintaining all the septic conditions and precautions. In six different pre-weighed sterile vials (1 ml/vial), 1 ml of withdrawn blood was transferred and incubated at room temperature without being disturbed for 45 minutes to form clot and serum. The serum was completely removed without disturbing the formed clot and each vial having clot, were again weighed to determine the clot weight. The vials containing pre-weighed clot were separately labeled. An aliquot of 100 µl, crude extract and aqueous solutions of various fractionates were added to the different vials one by one. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of isotonic solution were separately added to the clot containing respectively. To observe clot lysis, all the vials were incubated at room temperature for 90 minutes then the released fluid was removed and vials were again weighed to observe and note down the difference in weight after clot disruption. Differences obtained in weight were taken before and after clot lysis were expressed as percentage of clot lysis as shown in the equation:

% of clot lysis = (wt. of released clot /clot wt.) × 100.

2.7. In Vitro Membrane Stabilizing Assay

The membrane stabilizing activity of the extracts was assessed by evaluating their ability to inhibit hypotonic solution and heat-induced haemolysis of human erythrocytes following the method of Omale [22]. Hypotonic solution induced hemolysis: The test sample contained stock erythrocyte (RBC) suspension (0.5 ml) with 5 ml hypotonic solution with (50 mM NaCl in 10 mM sodium phosphate buffered saline, pH 7.4) containing different fractions of ethanolic extract (2 mg/ml) and acetyl salicylic acid (0.1 mg/ml). The acetyl salicylic acid was used as the reference standard. The mixtures were centrifuged for 10 min at 3000 rpm, and incubated for 10 min at a room temperature of 25 °C.The absorbance of supernatant content haemoglobin was measured at 540 nm using UV spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was determined using the following equation:

% inhibition of haemolysis $=100\times \{(OD_1-OD_2)/OD_1\}$

where, OD_1= Optical Density of hypotonic buffered saline solution alone (control) and

OD_2= Optical density of the test sample in hypotonic solution.

Heat induced haemolysis, Isotonic buffer solution containing 2 mg/ml of different partitions of *S.grandiflora* were placed into two centrifuging tubes [23]. These two sets of control tubes contained 5 ml of sterile vehicle and 5 ml of acetyl salicylic acid (0.1 mg/ml) respectively and Erythrocyte suspension (30 ul) was added to each tube separately and mixed gently by inversion. One pair of tubes was incubated at 54 °C for 20 min in a water bath. The other pair was maintained at 0-5 °C in an ice bath. The reaction mixture was centrifuged for 10 min at 3000 rpm and the absorbance of the haemoglobin content in supernatant was measured at 540 nm, and the results obtained were recorded.

2.8. Statistical Analysis

Three replicates of each sample were used for each test to facilitate statistical analysis and the data were presented as mean ± standard deviation (SD)**.**

2.9. Test for Antidiarrhoeal Assay

White healthy albino mice (Swiss-Wistar strain, body weight: 25-30 gm) of aged 8 weeks were selected for *in vivo* antidiarrhoeal activity. They were kept in clean cage for 48 hours to adjust standard environmental conditions at animal house of Pharmacology Laboratory, World University of Bangladesh, Dhaka. Ethics committee of pharmacology and toxicology section of World University of Bangladesh have given approval. The approval number PTWUB # WE3091, was preserved in their official system for future reference. All experimental procedures were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines (1985).We also reviewed literature of Zimmermann's' Ethical guidelines for investigations of experimental pain in conscious animals [24]. Loperamide (Square Pharmaceuticals Ltd., Bangladesh) was used as standard drug for this study.

2.10. Castor oil-induced Diarrhoea

Antidiarrhoeal activity of leaves' extract of *S. grandiflora* was tested by using castor oil induced method on mice [25, 26]. Twenty Swiss albino mice were randomly divided into four groups (n = 5). The control group received only distilled water of 1 ml/mice, the positive control group received loperamide 50 mg/kg body weight (1.25 mg loperamide for 25 gm mice) as standard and the test groups received the extract at a concentration of 1.2 mg/0.2 ml for the dose of 200 mg/kg and 2.4 mg/0.2 ml for the dose of 400 mg/kg body weight respectively. Mice were housed in separate properly labeled cages having paper placed below for collection of faecal matters. Diarrhoea was induced after oral administration of castor oil 1ml (mixed with few drops of 1% Tween 80 in water, 34 mL/kg body

weight)). Extracts and drugs were given orally 1 hour before the administration of castor oil. Examined and recorded for the presence of diarrhea every 60 minutes for 4 h study after castor oil administration. The time for first excretion of feces and the total number of faecal output by the animals were recorded. Inhibition of defecation in mice was calculated by using the following equation:

% inhibition = [(Mo–M) / Mo) ×100

Where, Mo = Mean defecation of control and M = Mean defecation of test sample.

2.11. Acute Toxicity Test

The acute toxicity of *S. grandiflora* ethanolic extract was determined in mice according to the method of Hilaly et al., [27] with slight modifications. The acute toxicity studies precisely aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the mice. Mice fasted for 16 h were simply divided into groups of five mice per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o) were separately administered to the mice in each of the groups by means of bulbed steel needle intraperitoneal. All the animals were then allowed to normal food and the animal behavioral-activities were closely monitored for the first 3 h then at an interval of every 4 h during the next 48 h. The extract did not cause mortality in mice during 48 h observation but insignificant behavioral changes such as locomotors ataxia, diarrhea, and weight loss were observed. In the present study, it was found to be safe at all doses of *S. grandiflora* ethanolic extract.

2.12. Antimicrobial Activity

The samples were examined for antimicrobial activity by the standardized disc diffusion method. A total of nine bacterial strains were used in the present study. Five Gram negative strains namely *Escherichia coli, Proteus mirabilis, Pseudomonas fluroescens*, *Pseudomonas aeruginosa, Salmonella typhi.* Four Gram positive bacteria species viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Sarcina lutea* and *Bacillus cereus* were used to investigate the antibacterial potential of the leaves' extracts. These pathogenic strains were obtained from the Department of Microbiology, University of Dhaka. The bacterial strains were maintained on nutrient agar slants tubes at 4 °C at all times.

2.13. Determination of MICs

The minimum inhibitory concentrations (MICs) of the roots extract was carried out by using seven test organisms; *Escherichia coli, Shigella dysentria, Staphylococcus aureus*, *Bacillus subtilis, Salmonella typhi. Bacillus cereus* and *Sarcina lutea* using the procedure with slight modifications [28, 29]. An aliquot of 500 µl of extracts were diluted serially to 0.5-2000 µg/ml, a sample of test organism previously standardized to 0.5 as McFarland turbidity standard (1.5×10^8 CFU/ml; are used

as a reference to adjust the turbidity of bacterial suspensions) was introduced into the tubes containing 2 ml of sterile nutrient broth followed by tubes were then incubated at 37 °C for 24 h. Bacterial growth was examined and recorded by observing the rate of turbidity. The lowest concentration of extract (maximum dilution) exhibited no bacterial growth. The procedure was repeated in triplicates and compared with the extract free standard antibiotics kanamycin.

2.14. Antibacterial Activity

The prepared extracts were screened for their antibacterial activity in comparison with standard kanamycin (30 µg/disc) *in vitro* by disc diffusion technique using various bacterial strains [30]. Nutrient agar was sterilized in a flask to avoid sepsis and cooled to 45-50 °C and then taken in sterilized, autoclaved petridishes with a diameter of 120 mm. The paper discs (6 mm diameter, Whatman No. 1 filter paper) contained 100 µg/ml plant extract. Each extract was dried on to separate paper disc and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface which is previously inoculated agar plates with the test microorganisms [31].The petridishes were kept at 4 °C for 12 h followed by the incubation at 37 °C for 16 h for the growth of the microorganisms. The inhibition zone around each disc was measured in nearest millimeter and the assay was performed in triplicate for each extract for accuracy. The results were recorded by measuring the zone of growth inhibition of microbes surrounding the disc.

3. Results and Discussion

The results of qualitative phytochemical screening of various extracts of *S. grandiflora* leaves are presented in (Table 2) which showed presence of important classes of plant secondary metabolites. All the prepared extracts showed the presence of alkaloids, carbohydrates, tannins, and flavonoids while steroids were detected only in Petroleum soluble fractions

Total flavonoid and tannin content, Since, all

Table 2. Results of phytochemical constituents of EE and different partitionates of *S. grandiflora* leaves.

Test for	PSF	CSF	ESF	EE	CTSF
Carbohydrates	-	+	+	+	+
Reducing sugar	-	+	+	-	-
Steroid	+	-	-	-	-
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+

+ indicate present, -indicate absent

the partitionates demonstrated positive results for flavonoid and tannin in the preliminary phytochemical screening; the same is quantified by the analysis of total tannin and flavonoid content. Aluminum chloride colorimetric method was used to determine the total flavonoid content in the plant extract of different fractionates of the *S. grandiflora*. This method is based on the quantitative determination of the flavonoid-aluminum complex between flavonoid of the crude extract and aluminum chloride [32].The total flavonoids contents of leaves of *S. grandiflora* were calculated using the linear equation obtained from the standard curve of quercetin ($y = 0.0098x - 0.0364$; $R^2 = 0.9724$) and expressed as quercetin equivalents (QAE) per gram of the plant extract. In this investigations, ESF revealed the highest flavonoid content (57.37 ± 0.15 mg/gm) while PSF was found with the lowest flavonoid content (44.44 ± 0.37 mg/g). The tannin content was examined in extracts using the Folin-Coicalteu reagent and is expressed in terms of tannic acid as mg of TAE/gm equivalent (the standard equation $Y=0.0999x-0.0161$.$R^2=0.9996$). The total tannin content was also the most significant in ethyl acetate soluble fraction (47.08 ± 0.22 mg /gm) whereas ethanolic extract was (20.98 ± 0.59mg/gm) lowest in amount (Table 3).

Table 3. Total flavonoid and tannin content of EE and various extracts of *S. grandiflora.*

Extracts	Total flavonoid content (mg QAE/gm) of dry extract	Total tannin content(mg of TAE/gm of dry extract
ESF	57.37 ± 0.25	47.08 ± 0.22
CSF	51.76 ± 1.80	30.09 ± 0.11
PSF	44.44 ± 0.47	39.47 ± 0.03
EE	-	20.98 ± 0.59

Results are expressed as mean ± SD (n = 3)

The results expressed as mean ± SD (n = 3), are statistically significant ($P < 0.05$). In the study of thrombolytic activity, the results of *in vitro* thrombolytic activity revealed that addition of a positive control (a fibrinolytic drug) to the clots showed 69.23% lysis of clot. On the other hand sterile distilled water a, negative control exhibited a negligible percentage of lysis of clot 3.07%. The % clot lysis by various extracts were observed in the following order, ESF (59.57%) <PSF (57.40%) <CSF (51.5%) <EE (33.18) <AQSF (23.80%). In our thrombolytic assay, negative control clearly demonstrated negligible percentage of lysis of clot (3.07%) when water was added to the clot whereas a positive control (a fibrinolytic drug) showed 69.23% lysis of clot. So, it is clear that significant (p value < 0.001) percentage of thrombolytic activity was exhibited by all extract of S.grandiflora presented in the (Table 4). The possible mechanism of clot lysis is; briefly**,** Streptokinase (SK) is an enzyme secreted by several species of

streptococci that can bind and activate human plasminogen. Standard SK is used as an effective and inexpensive thrombolysis medication in some cases of myocardial infarction (heart attack) [33] and pulmonary embolism [34]. It is a medication well known as fibrinolytic and bind with human plasminogen. Complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin [35]. Plasmin is an important enzyme (EC 3.4.21.7) present in blood that degrades many blood plasma proteins, including fibrin clots. The degradation of fibrin is termed fibrinolysis. It is evident that plants which have plasminogen receptors that bind plasminogen and easily activate it could lead to fibrinolysis [36]. The results from the study clear that S. grandiflora extract potentially lysis clot.

In the study of membrane stabilizing activity, the *in vitro* membrane stabilizing activities of different partitionates of crude extracts of *S. grandiflora* significantly protected the haemolysis of HRBC membrane induced by hypnotic solution and heat as compared to the standard ASA. The membrane stabilizing activity against hypnotic solution was observed in the following order ASA (73.90 ± 0.29%), ESF (64.30% ± 0.64), PSF (51.30 ± 0.64%), CSF (48.65 ± 0.77) and AQSF (31.30% ± 0.51), respectively. In heat induced method, the values of ASA (70.12 ± 0.26), CSF (44.23 ± 0.57 %) and ESF (57.21 ± 0.69%) were decreased

Table 4. Percentage (%) inhibition of heat and hypotonic solution induced haemolysis of erythrocyte membrane and thrombolytic activity (in terms of % of clot lysis) of different fractions of *S. grandiflora.*

Samples	**% Inhibition of haemolysis**		**% of clot lysis**
	Heat induced	**Hypotonic solution induced**	
ESF	57.21 ± 0.69*	64.30 ± 0.64**	59.57**
CSF	44.23 ± 0.57	48.65 ± 0.77*	51.5**
PSF	52.30 ± 0.61	51.30 ± 0.64*	57.40**
AQSF	31.34 ± 0.42	31.30 ± 0.51*	23.80*
ASA	70.12 ± 0.26*	73.90 ± 0.29*	-
SK	-	-	69.23**
EE	-	-	33.18*
water	-	-	3.07*

Values are expressed as mean ± SD (standard deviation);*P < 0.005, **P < 0.001; statistically significant as compared to positive control and negative control.

slightly but for AQSF (31.34% ± 0.42) and PSF (52.30 ± 0.61) a little increase in membrane stabilizing activities was noted (Table 4). The exact possible mechanism for the membrane stabilizing effect of *S.grandiflora* leaves was not known. However, a number of studies have shown that flavonoids and other phenolic compounds exhibited analgesic and anti-inflammatory effects [37].Presence of significant amount of phenolic compound in the extract of *S. grandiflora* indicated that *S. grandiflora* has potential to inhibit hemolysis induced by hypotonic solution as well as by heat, which leads to protection of haemolysis of HRBC membrane.

Test for antidiarrhoeal activity, the results presented in (Table 5) showed that the ethanolic extract reduced the mean number of defecation by 24.97% and 41.05% ($P<0.05$ & $P<0.001$) at the doses of 200 mg/kg and 400 mg/kg respectively. After oral ingestion of castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic acid are absorbed in the intestine, the released ricinoleic acid induces a strong laxative effect via prostaglandin receptor EP2, which mediates the effects of ricinoleic acid on the motility of the intestine [38, 39, 40].As we know from literature review, presence of phenolic compounds in medicinal plants, have an inhibition of intestinal motility, antisecretory effects. These observations suggest that extracts reduced diarrhoea by inhibiting ricinoleic acid induced intestinal irritations and contraction [41]. Hence, tannins, flavonoids, steroid and alkaloid may be possible biologically active principles responsible for moderate anti-diarrhoeal activity of *S. grandiflora* leaves at a dose of 200 mg/kg & 400 mg/kg.

Table 5. Antidiarrhoeal activity of the ethanolic extract of leaves of *S. grandiflora* Castor oil induced diarrheal test method on mice.

Sample	Dose	Mean ± SEM Latent period	Defecation	% Inhibition
Distilled water	1 ml/mice p.o	0.79 ± 0.06*	9.9 ± 0.86*	-
Loperamide	50 mg/kg p.o	2.21 ± 0.16**	4.33 ± 0.45**	53.6
EE of *S. grandiflora*	200 mg/kg p.o	1.05 ± 0.07*	7 ± 0.86*	24.97
	400 mg/kg p.o	1.59 ± 0.19**	5.5 ± 0.63**	41.05

Assessment of minimum inhibitory concentrations (MICs), the results of MICs determination of the crude extract of *S.grandiflora* are shown in the (Table 6). The zone around the disc indicated the absence of test bacterial growth and is reported as positive and the absence of zone as negative. The results of antibacterial screening of petroleum ether,

Table 6. Minimum inhibitory concentrations of the crude extract of *S.grandiflora* .

Name of bacteria	MIC (µg/ml)
Escherichia coli	14 ± 0.22
Staphylococcus aureus	11 ± 0.81
Bacillus sublitis	27 ± 0.81
Shigella dysentria	25 ± 0.01
Salmonella typhi	35 ± 0.29
Bacillus cereus	55 ± 0.11
Sarcina lutea	38 ± 0.61

Standard deviation (n=3)

MICs value of extracts varied between 11-55 µg/ml. The highest MIC values of the crude extract was 55 µg/ml against *Bacillus cereus* followed by *Escherichia coli* (14 ± 0.22); *Staphylococcus aureus (*11 ± 0.81), *Shigella dysentria* (25 ± 0.01), *Salmonella typhi* (35 ± 0.29), *Sarcina lutea* (38 ± 0.61), *Bacillus sublitis (27* ± 0.81) whereas the lowest MIC values was ((11 ± 0.81) against *Staphylococcus aureus.* Most MIC values reported in this work were largely higher.

In the study of antimicrobial activity, the ethanolic extract of leaves of *S. grandiflora* was studied against both gram positive and gram negative species at concentrations (100 µg/ml) and the antibacterial activity was compared with the standard kanamycin (35 µg/mL)[42]. Results were recorded as presence or absence of zones of inhibition around the disc. The inhibitory chloroform, carbon tetra chloride, ethanol extract, and water extracts of *S. grandiflora* are presented in (Table 7). The results revealed variability in zone of inhibition of each extract against a given bacteria. Among the various extracts used, petroleum ether extracts of *S. grandiflora* showed the highest activity (zone of inhibition; 18 ± 0.31 mm) against *Bacillus cereus* in comparison to the standard (29 ± 0.14).The lowest activity (zone of inhibition; 9 ± 0.41 mm) of CSF was observed against gram positive species *Staphylococcus aureus.* PSF extract appeared to be most effective extract against *Bacillus cereus*.CTSF extract exhibited moderate antibacterial activity against almost all bacterial strains (8 ± 0.14 - 12 ± 0.67 mm) except *Shigella dysenteriae* at the concentration of 100 µg/ml. The most prominent effects of CTSF were observed on gram positive bacteria,

Bacillus cereus, and *Sarcina lutea.* Antibacterial effects of CSF were more pronounced against gram negative bacteria in comparison to gram positive. CSF was found to inhibit growth of all gram negative microorganisms (9 ± 0.11- 13 ± 0.11 mm) while no inhibitory effects were observed against gram positive bacterial namely *B. subtilis* and *S. lutea*. No antibacterial activity was shown by water extract. The antibacterial activity was comparatively more prominent on the gram negative bacteria than the gram positive bacteria though gram positive species *Bacillus cereus* was the only strain which was sensitive to all extracts. We can claim that the leaves of *S. grandiflora* could be used against both gram positive and gram negative pathogens. From the previous research and literature reviewed it was found that medicinal plants have been shown to have bactericidal, synergistic activity, and the ability to suppress bacterial virulence [43] of phytochemicals that were present in the plant extract of S.grandiflora. The demonstration of antibacterial activity against both positive and gram negative bacteria by this plant may be indicative of the presence of broad or narrow spectrum antibiotic compounds by affecting bacterial protein synthesis.

Table 7. Antimicrobial activities of *S. grandiflora leaf* extract against gram positive and gram negative bacteria.

		Zone of inhibition (in mm) ± SD (n = 3)				
Test organism	**Conc. µg/ml**	**CTSF**	**PSF**	**CSF**	**EE**	**Kanamycin 30 µg/disc**
Gram-positive bacteria						
Bacillus cereus	100	12 ± 0.12	18 ± 0.31	11 ± 0.11	7 ± 0.11	29 ± 0.11
Bacillus subtilis	100	11 ± 0.61	11 ± 0.91	-	10 ± 0.21	27 ± 0.91
Staphylococcus aureus	100	9 ± 0.21	-	9 ± 0.41	-	30 ± 0.31
Sarcina lutea	100	12 ± 0.81	-	-	12 ±.0.11	25 ± 0.61
Gram-negative bacteria *Salmonella typhi*	100	8 ± 0.14	13 ± 0.11	9 ± 0.11	-	36 ± 0.31
Vibreo parahemolyticus	100	10 ± 0.9	11 ± 0.65	10 ± 0.18	-	37 ± 0.11
Escherichia coli	100	12 ± 0.91	-	12 ± 0.88	12 ± 0.33	23 ± 0.18
Vibreo minicus	100	12 ± 0.67	-	10 ± 0.88	10 ± 0.11	28 ± 0.12
Shigella dysentria	100	-	10± 0.29	13 ± 0.11	11 ± 0.23	29 ± 0.19

Correlation of total phenolic compound content with pharmacological activities of *S. grandiflora*: For phenolic compound content a significant but marginal positive correlation (value of, **P < 0.001;*P<0.005; P<0.05 was statistically significant as compared with thrombolytic, membrane stabilizing, antidiarrhoeal and antimicrobial activities of ethanolic extract) was found. This showed a good relationship between phenolic compound

content with the thrombolytic, membrane stabilizing, antidiarrhoeal, and antimicrobial activities of *S. grandiflora*.

4. Conclusion

Qualitative phytochemical screening of various extracts of *S. grandiflora* leaves showed presence of satisfactory amount of phenolic compounds. Thorough analyzing the results, it was revealed that the plant possesses thrombolytic, antidiarrhoeal, membrane stabilizing, and antimicrobial properties mainly due to phenolic compound. It may be assumed and stated that extracts can be considered as good source of antimicrobial, thrombolytic, membrane stabilizing, and antidiarrhoeal remedy.

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