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ARTICLE

Medicinal Properties of the *Sesbania grandiflora* Leaves

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Abstract

Background: The leaves of *Sesbania grandiflora* have been used in local traditional medicine since ancient times. In the present study we investigated, *in vivo* and *in vitro*, the potential health benefits of various fractions of the ethanolic extract of these leaves. **Materials and methods:** Crude ethanolic extract (CEE) of *S. grandiflora* leaves was partitioned into ethyl acetate soluble fraction (EASF), petroleum ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF), and water soluble fraction (WSF). The extracts were evaluated for their thrombolytic, membrane stabilizing (anti-inflammatory), antimicrobial, and antidiarrheal activities. The results were compared to the effects of standard drugs: streptokinase for the thrombolytic, acetylsalicylic acid (ASA) for the membrane stabilizing, kanamycin for antimicrobial, and loperamide for the antidiarrheal activities. **Results:** For thrombolysis, EASF showed the highest % of clot lysis (59.6%) among all fractions, while streptokinase and water resulted in 69.2% and 3.1% clot lysis respectively. With respect to the membrane stabilizing activity, the EASF significantly inhibited the hemolysis of human erythrocytes induced by hypotonic solution (64.3±0.6%) or by heat (57.2±0.7%). The other fractions exhibited no membrane stabilizing effect. By contrast ASA resulted in 73.9±0.3% inhibition of osmotically induced hemolysis and a slightly lower level of inhibition in the case of heat-induced hemolysis (70.1±0.3%). The antidiarrheal activity was evaluated in the mouse model. The unfractionated, CEE reduced the number of defecation episodes by 25.0% at a dose of 200 mg/kg and by 41.1% at dose 400 mg/kg body weight. All extract fractions exhibited significant antibacterial activity, which was higher against Gram negative bacteria than Gram positive bacteria. Since the pharmacological activities of *S. grandiflora* are due to the presence of bioactive compounds we detected and quantified the presence of significant levels of flavonoid and tannin substances. **Conclusion:** Leaves

of *Sesbania grandiflora* have the potential to be used as a remedy for thrombosis, diarrhea, and inflammatory diseases and against few important bacterial pathogens.

Key words: Bangladesh, *Sesbania grandiflora*, thrombolytic, membrane stabilizing, antimicrobial, antidiarrheal

Introduction

Sesbania grandiflora belongs to the family Fabaceae, and in Bangladesh, it is known as "Bagful" due to its medicinal values. 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 (1-6). 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, antihelmintic, antitumor and contraceptive properties. A poultice made from the leaf juice is used in folkloric system as an effective treatment for bruises. The leaf is widely used in detoxification process of manacle.

S. grandiflora leaves are highly nutritious and have been shown to contain significant amounts of proteins, fat, carbohydrates, fiber, and minerals such as iron, calcium, and phosphorus. The young leaves are edible and are quite often used to supplement meals. The plant has also been reported to be a potent antidote for tobacco and smoking-related diseases (1). Numerous reports mention the isolation of sterols, saponin, and tannins from the leaves, flowers, and aerial parts of the plant. These bioactive constituents have potential health benefits and are thought to possess important biological activities such as antibacterial and antifungal (2), antioxidant (3-5), antiurolithiatic (6), anticonvulsant and anxiolytic (7), and hepatoprotective properties. In the rural areas of the Tangail district of Bangladesh, the villagers use a sweetened concentrated juice prepared from the leaves as a remedy for diarrhea. Oral administration of crude ethanolic extracts of *Sesbania grandiflora* reduces the number and duration of defecation episodes. Since we found no published scientific investigation of its antidiarrheal action, we wished to validate the local use of the plant for this purpose.

Materials and Methods

The technical and laboratory methodology are described in detail in Appendix 1 (Supplementary Material). These are summarized below.

Preparation of extract

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 leaves were dried and were ground . to obtain powder of uniform particle size to produce a crude ethanolic extract (CEE) of *S. grandiflora* leaves The concentrated aqueous ethanol extract was fractionated to produce following fractions ethyl acetate (EASF), petroleum ether (PSF), carbon tetrachloride (CTSF), chloroform (CSF), and water soluble (WSF) extracts.

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 as previously described (8). The total flavonoid content was estimated using quercetin as a reference compound (9). The total tannin content of S. grandiflora was determined by Folin-Coicalteu method as previously described (10).

In vitro experiments

The thrombolytic activities of the various extract fractions were evaluated as described by Daginawala (11). The membrane stabilizing activity of the extract was assessed by evaluating their ability to inhibit the hemolysis of human erythrocytes induced osmotically by a hypotonic solution or by heat following the method of Omale (12) and the heat induced hemolysis tests were conducted as previously described by our group (13).

Antidiarrheal activity

Healthy Swiss-Wistar albino mice (body weight: 25-30 g) 8 weeks of age were selected to be used for the evaluation of the in vivo antidiarrheal activity. Loperamide (Square Pharmaceuticals Ltd., Bangladesh) was used as the reference antidiarrheal drug. Antidiarrheal activity of the leaves of *S. grandiflora* was tested using castor oil induced diarrhea in mice (14, 15). Twenty Swiss albino mice were randomly divided into four groups (n=5). The positive control group received loperamide while the test groups received the extract either at a dose of 200 or 400 mg/kg body weight. The controls were given distilled water. The mice were monitored for the presence of diarrhea every 60 minutes for 4 hours after the administration of castor

oil. The time of the first defecation episode and the total number of episodes were noted. Inhibition of defecation was calculated. The acute toxicity of the CEE of *S. grandiflora* leaves was determined in mice by modification of the method of Hilaly et al (16).

Antimicrobial activity

The extracts were evaluated for antimicrobial activity by the standard disc diffusion method. A total of nine bacterial strains were used in the present study, 5 Gram negative and 4 Gram positive. The Gram negative strains included *Escherichia coli*, Proteus mirabilis, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The Gram positive bacteria species included *Staphylococcus aureus*, *Bacillus subtilis*, *Sarcinalutea and Bacillus cereus*. Antibacterial activity was measured as previously described (19,20). The MIC was determined by serial dilution method against *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Bacillus cereus*, and *Sarcinalutea*.

Table 1. Total flavonoid and tannin content of CEE of *S. grandiflora* and its various fractions

Extract Fraction	Flavonoid[a]	Tannin[b]
CEE	None	21.0±0.6
EASF	57.4± 0.6	47.1±0.2
CSF	51.8±1.8	30.1±0.1
PSF	44.4±0.5	39.5±0.0

[a]Total flavonoid content is expressed in terms mg of quercetin equivalents per gram of dry extract.
[b]Total tannin content is expressed in terms mg of tannic acid equivalents per gram of dry extract.

Results

Flavonoid and tannin content

The qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, and tannins in all extract fractions. The same for carbohydrates with the exception of PSF. Reducing sugar was detected only in EASF and CSF, and steroid only in PSF.The total flavonoid content of leaves of S. grandiflora was calculated using the linear equation obtained from the standard curve of quercetin (y = 0.0098x - 0.0364; R2 = 0.9724) and expressed as quercetin equivalents (QAE) per gram of the plant extract. The ethyl acetate soluble fraction (ESF) exhibited the highest flavonoid content while the petroleum soluble fraction (PSF) was found to possess the lowest flavonoid content. The tannin content was determined using the Folin-Coicalteu reagent and is expressed in terms of tannic acid equivalents (mg of TAE/g) (the standard equation Y= 0.0999x - 0.0161; R2=0.9996). The total tannin content was highest in the ESF and lowest in the CEE (Table 1)

Thrombolytic activity

The fibrinolytic enzyme streptokinase (SK) which we used as reference resulted in 69.2% clot lysis while distilled water had a negligible effect on clot integrity (3.1%). Among the extract fractions, the WSF and the CEE had lowest percent clot lysis (23.8 and 33.2% respectively). The remaining fractions resulted in significantly higher percentage (EASF 59.6, PSF 57.4, CSF 51.5). The thrombolytic activity of the various extract fractions was significant (p<001) compared to the effect of distilled water.

Membrane stabilizing activity

All extract fractions provided significant levels of protection against osmotically induced and heat induced hemolysis of human erythrocytes (Figure 1). The EASF exhibited the highest membrane stabilizing effect and the WSF the lowest (Figure1). The reference drug, ASA,

Table 2. Antidiarrheal activity of the CEE compared to distilled water and loperamide in mice challenged with castor oil.

Agent (dose)	Latent Period	number of defecation episodes	Percent inhibition
Water (1 ml)	0.79 ± 0.06	9.90 ± 0.86	0
Loperamide (50 mg/kg)	2.21 ± 0.16**	4.33 ± 0.45**	53.6
CEE (200 mg/kg)	1.05 ± 0.07*	7.00 ± 0.86*	25.0
CEE (400 mg/kg)	1.59 ± 0.19**	5.50 ± 0.63**	41.1

All agents were given orally. Values are expressed as mean ± standard error of the mean (Mean ± SE); * indicates p<0.05 and ** indicates p<0.001, both indicating statistical difference with respect to the effect of distilled water.

resulted in a membrane stabilization level of 73.9±0.3%. A similar trend was observed with heat induced hemolysis.

Antidiarrheal activity

The ethanolic extract reduced the mean number of defecation episodes in a dose-dependent manner as shown in table 2.

Antimicrobial activity

The antibacterial activity of 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). Results were recorded as presence or absence of zones of inhibition around the disc. The inhibitory 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, chloroform, carbon tetrachloride, ethanol extract, and water extracts of *S. grandiflora* are presented in table 3. There was a variation in zone of inhibition of each extract against a given bacteria. Among the various extract fractions, the PSF showed the highest activity (diameter of inhibition zone against *Bacillus cereus* in comparison to the standard. The lowest activity was observed with CSF against gram positive species *Staphylococcus aureus*. PSF appeared to be most effective extract against *Bacillus cereus*. CTSF exhibited moderate antibacterial activity against almost all bacterial strains 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 *Sarcinalutea*. Antibacterial effects of CSF were more pronounced against gram negative bacteria in comparison to gram positive. CSF was found to

Table 3. Minimum inhibitory concentration (Mean ± SD, n = 3) **of the CEE of *S. grandiflora***

Microorganism	MIC (µg/ml)
Escherichia coli	14 ± 0.2
Staphylococcus aureus	11 ± 0.8
Bacillus subtilis	27 ± 0.8
Shigella dysenteriae	25 ± 0.0
Salmonella typhi	35 ± 0.3
Bacillus cereus	55 ± 0.1
Sarcina lutea	38 ± 0.6

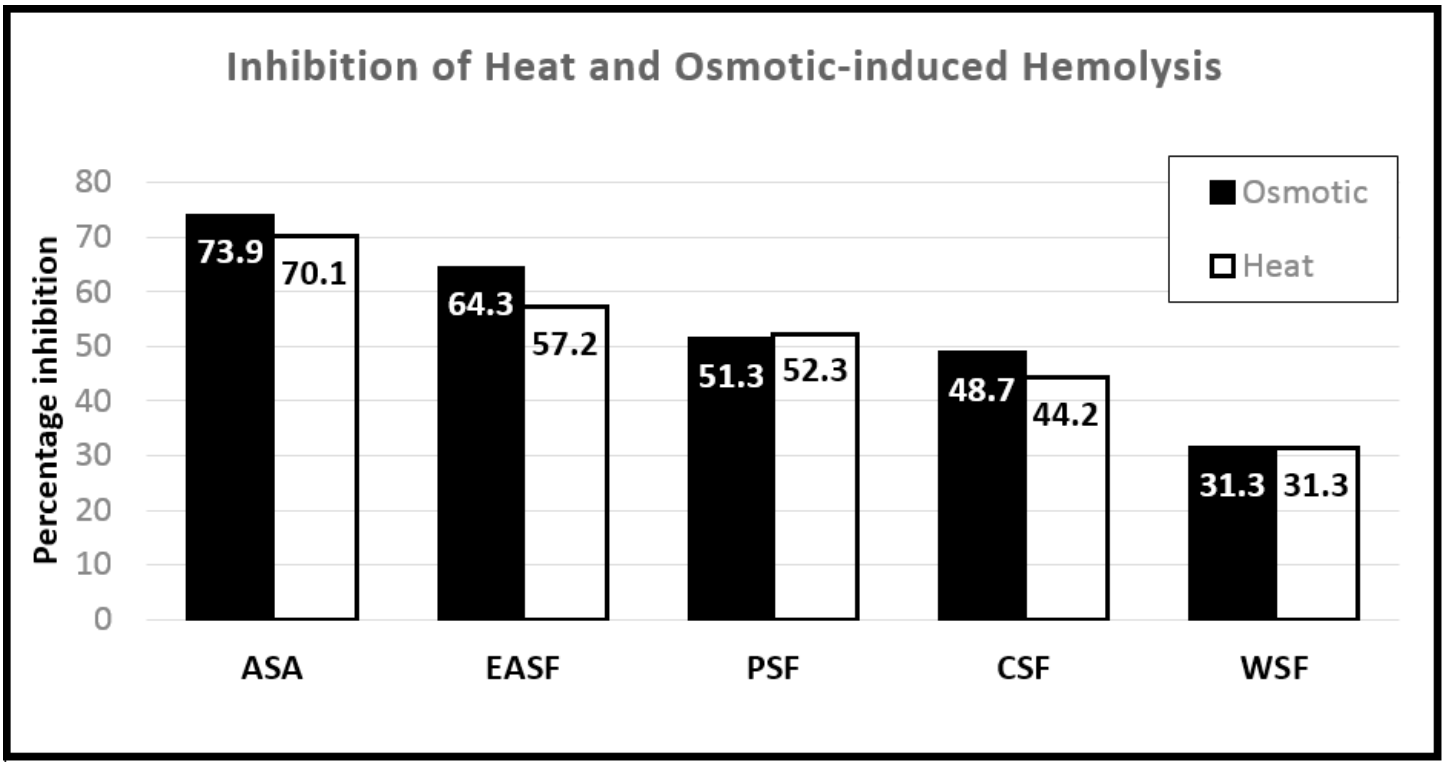

Figure 1: The percent inhibition of hemolysis; osmotically-induced (closed) and heat-induced (open) by various test agents. ASA = acetylsalicylic acid; EASF = ethanol acetate soluble fraction; PSF = petroleum soluble fraction; CSF = chloroform soluble fraction. Values are shown as means.

Table 4. The antibacterial activities of the various fractions of the ethanolic extract of the leaves of *S. grandiflora*.

	CTSF	PSF	CSF	CEE	Kanamycin (30 µg/disc)
Gram Positive Bacteria					
Bacillus cereus	12.0 ± 0.12	18.0 ± 0.31	11.0 ± 0.11	7.0 ± 0.11	29.0 ± 0.11
Bacillus subtilis	11.0 ± 0.61	11.0 ± 0.91	-	10.0 ± 0.21	27.0 ± 0.91
Staphylococcus aureus	9.0 ± 0.21	-	9.0 ± 0.41	-	30.0 ± 0.31
Sarcina lutea	12.0 ± 0.81	-	-	12.0 ± 0.11	25.0 ± 0.61
Gram Negative Bacteria					
Salmonella typhi	8.0 ± 0.14	13.0 ± 0.11	9.0 ± 0.11	-	36.0 ± 0.31
Vibrio parahaemolyticus	10 ± 0.9	11.0 ± 0.91	10.0 ± 0.18	-	37.0 ± 0.11
Escherichia coli	12.0 ± 0.91	-	12.0 ± 0.88	12.0 ± 0.33	23.0 ± 0.18
Vibrio mimicus	12.0 ± 0.67	-	10.0 ± 0.88	10.0 ± 0.11	28.0 ± 12.0
Shigella dysenteriae	-	10.0 ± 0.29	13.0 ± 0.11	11.0 ± 0.23	29.0 ± 0.19

The values (Mean ± SD; n=3) indicate the diameter of inhibition zone (DIZ) in millimeters. The amount of extract fraction used in each case was 100 Mg/ml.

inhibit growth of all gram negative microorganisms while no inhibitory effects were observed against gram positive bacteria namely *B. subtilis* and *S. lutea*. The gram positive species *Bacillus cereus* was the only strain which was sensitive to all extracts. No antibacterial activity was shown by the WSF. The MICs for a number of microorganisms were determined only for the crude ethanolic extract (CEE) of the leaves of *S. grandiflora* (Table 4). The MICs ranged between 11 and 55 µg/ml. The highest MIC was against *Bacillus cereus*) and the lowest was against *Staphylococcus aureus* (Table 4)

Discussion

The leaves of *Sesbania grandiflora* have been used in local traditional medicine since ancient times. In the present study we investigated, in vivo and in vitro, the potential health benefits of various fractions of the ethanolic extract of these leaves. The results of the present study indicate that the leaves of *Sesbania grandiflora* have the potential to be used as a remedy for thrombosis, diarrhea, and inflammatory diseases and also against few important bacterial pathogens. Since all the extract fractions were positive for flavonoid and tannin in the preliminary screening, we have decided to quantify the content of these phytochemicals in the various fractions using the aluminum chloride colorimetric method, which takes advantage of the formation of a flavonoid-aluminum complex (22).

Streptokinase (SK) had been used as an effective and inexpensive thrombolytic agent in cases of myocardial infarction and pulmonary embolism (23,24). Our observations indicate that the thrombolytic activities of at least three of extract fractions compare favorably with that of streptokinase.

Regarding anti-diarrheal action, our results showed that the ethanolic extracts reduced the mean number of defecation episodes in a dose dependent manner. 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 (25). Phenolic compounds found in many medicinal plants are believed to have an inhibitory effect on the motility and the secretory activity of n the intestine. This would explain the antidiarrheal effect we observed in the present study. Biologically active compounds found in the extracts such as tannins and flavonoids may counteract the irritant effect of ricinoleic acid on the epithelium of the intestine (26).

The antibacterial activity of the ethanolic extract of leaves of *S. grandiflora* was studied against both gram positive and

gram-negative species (27). Our results revealed variability in zone of inhibition of each extract against a given bacteria. Among the various extract fractions, the PSF showed the highest activity against *Bacillus cereus* in comparison to the standard. We propose that the leaves of *S. grandiflora* could be used against both gram positive and gram negative pathogens. A number of medicinal plants have been shown to have as bactericidal, synergistic activity and the ability to suppress bacterial virulence (28) of phytochemicals that were present in the plant extract of. Our observations indicate the presence of antibacterial active ingredients in the leaves of *S. grandiflora* against both Gram positive and Gram negative species. The plant has the potential to serve as a pharmaceutical source.

The highest MIC was against *Bacillus cereus* and the lowest was against *Staphylococcus aureus*. Since all the extract fractions were positive for flavonoid and tannin in the preliminary screening, we have decided to quantify the content of these phytochemicals in the various fractions using the aluminum chloride colorimetric method, which takes advantage of the formation of a flavonoid-aluminum complex. The total flavonoid content of leaves of *S. grandiflora* was calculated using the linear equation obtained from the standard curve of quercetin and expressed as quercetin equivalents (QAE) per gram of the plant extract. The ethyl acetate soluble fraction (ESF) exhibited the highest flavonoid content while the petroleum soluble fraction (PSF) was found to possess the lowest flavonoid content. The tannin content was determined using the Folin-Ciocalteu reagent and is expressed in terms of tannic acid equivalents. The total tannin content was highest in the ESF and lowest in the CEE.

In conclusion, qualitative phytochemical screening of the various extract fractions of *S. grandiflora* leaves showed the presence of a significant amount of phenolic compounds. The results of the study indicate that the plant possesses thrombolytic, antidiarrheal, membrane stabilizing and antimicrobial properties. These activities may be attributed mainly to the plant's content of phenolic compound. It is safe to conclude that *S. grandiflora* has the potential a source of pharmaceutical agents that may be safe and effective as antimicrobial, thrombolytic, membrane stabilizing, and antidiarrheal drugs.

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Authors' contributions
All authors contributed significantly in the conception, drafting and revision of this article. They all approved its last version of the manuscript.

Compliance with Ethical Standards
1. Funding: This study received funding from the World University of Bangladesh.
2. Conflict of interest: All authors declare that they have no conflict of interest
3. Ethical approval: Approval was obtained from the ethics committee of the World University of Bangladesh (PTWUB # WE3091). All experimental procedures were performed in accordance with institutional and international policies governing the human and ethical treatment of experimental animals.

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