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Qualitative and quantitative biological analysis of leaves of *Sesbania grandiflora*

Karim Samira, Uddin Jalal, Rahman Laboni Farhina, Khalid Labu Zubair*

Department of Pharmacy, 151/8 Green Road, World University of Bangladesh (WUB), Dhaka, Bangladesh

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ABSTRACT

Objective: To justify the folkloric uses of *Sesbania grandiflora* (*S. grandiflora*) leaves, 90% ethanolic extract and their organic and aqueous partitioning substances were evaluated for their possible antioxidant, antidiarrhoeal and antimicrobial activity *in vivo*.
Methods: Crude ethanolic extract of *S. grandiflora* leaves were being subjected to partitioned into different fractions using solvents of different polarity. Antioxidant, antimicrobial, and antidiarrheal activity was evaluated and subsequently results were corresponded with the conventional standard drugs.
Results: The antidiarrheal activity was assessed using mouse model, where unfractionated crude ethanolic extract showed 25.0% at a dose of 200 mg/kg and 38.1% at dose 400 mg/kg body weight and the reference drug loperamide 53.6% at a dose of 50 mg/kg inhibited diarrhoeal episode. Crude ethanolic extract and extract fractions significantly inhibit the growth of known bacteria cause urinary tract infection. The total phenolic and flavonoid content was found maximum in chloroform soluble fractions (39.47 ± 0.33) and (31.44 ± 0.47) respectively. Extractives showed satisfactory antioxidant potential in DPPH free radical scavenging assay with IC_{50} values found in petroleum ether soluble fractions (18.05 ± 1.90) in comparison with ascorbic acid (17.50 ± 0.20).
Conclusions: Ethanolic extract and their aqueous and organic fractious revealed the leaves of *S. grandiflora* have the potential remedy of diarrhea, known bacteria cause urinary tract infection and potent antioxidant activity which ensure the folkloric use of the leaves of grandiflora.

1. Introduction

Sesbania grandiflora (*S. grandiflora*) belongs to the family Fabaceae and locally known as 'Bakful'. It is a species of tropical climate, short lived, quick growing and soft wooded tree[1]. Almost every single part of *S. grandiflora* is used as folkloric or traditional medicine to treat many diseases such as dysentery, stomatitis, fever, small pox, sore throat, headache, *etc*. The dried leaves are often used to make tea and are considered to have good antibacterial, anthelminthic, antitumor and contraceptive properties[2]. A poultice made from the leaf juice is used in folkloric system as an effective treatment for bruises[3]. Numerous literatures mentioned that *S. grandiflora* leaves and flowers of this plant successfully isolated sterols, saponins, phytopharmacological, and tannins. These bioactive constituents are useful as potential health benefits and have been claimed to possess important biological activities such as antibacterial, antifungal, antioxidant, antiurolithiatic, anticonvulsant, anxiolytic and hepatic protective properties[4]. Based on previous ethnobotanical literatures and traditional medicinal values as judged by local users and healers, we studied the organic and aqueous fractions of *S. grandiflora* leaves extract to find out the antioxidant, antidiarrhoeal and antimicrobial activities.

*Corresponding author: Khalid Labu Zubair, associate professor and chairman, Department of Pharmacy, 151/8 Green Road, World University of Bangladesh (WUB), Dhaka, Bangladesh.
Tel: 01558388956
E-mails: k.samia@yahoo.com (K Samira); zubair.labu@yahoo.com (KL Zubair)

All experimental procedures were followed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as defined in United States National Institutes for Health Guidelines (1985).

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2. Materials and methods

2.1. Plant materials

The leaves of *S. grandiflora* were collected from Tangail district, 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.

2.2. Preparation of extract

The collected plant leaves were shade dried for several days and dehydrated at 37 °C overnight for easy crushing to find uniform

particle sized powder by an electrical grinder. 400 g of the powder was dipped in 2000 mL of 90% ethyl alcohol. Soaked was filtered and the filtrate was condensed by a vacuum revolving evaporator at 40 ˚C. Concentrated aqueous ethanolic extractive was fractionated by the method of Kupchan *et al*.[5]. Obtained fractions were ethyl acetate soluble (EASF), pet. ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF).

2.3. Phytochemical screening

The freshly prepared organic extracts were qualitatively tested for the presence of various phytochemicals. These were identified by characteristic color changes using standard procedures, previously described by Sofowora[6].

2.4. Determination of total flavonoid content (TFC)

To define TFC of the CEE and extractives, aluminium chloride ($AlCl_3$) colorimetric method was used developed by Chang *et al*.[7]. 50 µL of extract solution of CEE, EASF, PSF, CTSF and CSF mixed with 0.3 mL of 5% $NaNO_3$, 0.3 mL of 10% $AlCl_3$ solution. The mixture was kept for 6 min after shake well followed by 2 mL of 1 mol/L sodium hydroxide solution were added and the final volume of mixture were adjusted up to 10 mL with pure water. Before absorbance was noted at 510 nm, the mixture was allowed to stand for 15 min for proper reaction. A standard curve was drawn by taking optical absorbance using dissimilar concentrations of standard quercetin solution at similar conditions.

2.5. Determination of total phenolic content (TPC)

To determine TPC, standard procedure was followed, developed by Dewanto *et al*.[8], where, 300 µL of the crude ethanolic extract and other extractives poured in test tube, contained 2.7 mL of Folin-Coicalteu reagent. Then the solution was kept for 5 min followed by 2 mL of 7.5 % Na_2CO_3 solution added and shaken well. At the end whole the mixture was kept in water bath to maintain at 45 ˚C and cooled for 30 min in dark place. Standard solutions of gallic acid were prepared minus the extract. Absorbance for test and standard solutions were estimated against the blank at 725 nm with an UV/Visible spectrophotometer. The TPC was determined from extrapolation of gallic acid standardization curve.

2.6. Antioxidant studies of S. grandiflora

2.6.1. Antioxidant assay

Assay of reducing power of ascorbic acid, ferric chloride ($FeCl_3$) method was used, developed by Oyaizu[9]. *S. grandiflora* extract and it's extractives showed the reductive ability of Perl's Prussian blue or interns of Fe^{+3} to Fe^{+2} transformations according to the method of Oyaizu[9]. Concisely, simply 2 mL of CEE and extractives of each (10 mg/4 mL of methanol to give 2.5 mg/mL or 5–500 µg/mL) were mixed with 2 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution in a test tube. This mixture was kept at 50 ˚C in water bath for 20 min and cooled followed by addition of 2.5 mL of 10% trichloro acetic acid and then centrifuged at 3000 r/min for 10 min to collect 2.5 mL of supernatant. An aliquot (1.5 mL) of distilled water, 1 mL of 0.1% (w/v) fresh ferric chloride were mixed well with 2.5 mL of supernatant and kept for 10 min. The positive control (ascorbic acid) at concentration of (20–200 µg/mL) was prepared in similar manner (without extract) and obtained results were compared to test sample. The absorbance was measured at 700 nm. The standard curve of ascorbic acid's graph was plotted for concentration of tannic acid µg/mL *vs.* absorbance ($y = 0.002x + 0.0143$; $R^2 = 0.9951$. A higher absorbance value of the reaction mixture indicated a higher reducing power.

2.6.2. DPPH radical scavenging assay

To conduct an antioxidant assay, free radical DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) was used, according to the method described by Brand-Williams *et al*.[10]. In brief, the reaction mixture consisted of 2 mL of CEE and its extractives at a concentration of 5–500 µg/mL were added to 3 mL of a 0.02 mg/mL (20 µg/mL) methanolic solution of DPPH followed by mixture was vortexed thoroughly and incubated for 30 min in dark place at room temperature. Observed, changes in color, the more decolourization is, the more reducing ability is (from deep violet to light yellow) then absorbance was measured against a blank (without sample) at 517 nm. A low absorbance of the reaction mixture indicated a high free radical scavenging activity. Results were compared with the percent of inhibition at various concentration of positive ascorbic acid (Table 1). The percent of inhibition of oxidation power of DPPH was calculated as the following equation:

[% Inhibition = 100 × (1 – AS/AB)]

where, AB is the absorbance of the control and AS is the absorbance of the extract/standard.

2.7. Evaluation of the antidiarrheal activity

2.7.1. Experimental animals and drugs

Healthy Swiss-Wistar albino mice (body weight 25–30 g) were selected randomly for the evaluation of antidiarrheal activity *in vivo*. All experimental procedures were followed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as defined in United States National Institutes for Health Guidelines (1985). Loperamide (Incepta Pharma) was used as the reference antidiarrheal drug.

2.7.2. Castor oil-induced diarrhea

The ethanolic crude extract of *S. grandiflora* was found effective in castor oil-induced diarrhea in mice model[11,12]. First we selected such mice which were capable to produce diarrhea in preliminary screening. Then selected mice were divided into four groups, each group consisted of five mice. Defecation was induced by intraperitoneal administration of 0.5 mL of castor oil at a dose of 10 mL/kg body weight. During the study, negative control group received only distilled water (0.5 mL/mice) while the positive control group received reference standard drug

for diarrhea (50 mg/kg body weight), while the test groups received the CEE at a dose of 200 mg and other groups at a dose of 400 mg/kg. CEE and drug were given intraperitoneal 1 h before giving the diarrhea inducing agent. The time of first and the total numbers of defecation episode was noted every 60 min for 4 h. Inhibition of defecation was calculated.

% Inhibition = 100 × (C – EC)

where C is mean number of defecation episodes in the control group and E is mean number of defecation episodes in experimental group.

2.7.3. Acute toxicity test

To determine acute toxicity of the CEE of *S. grandiflora* on experimental mice, acute toxicity test was used according to El Hilaly *et al*.[13] with slight modification. The aim of acute toxicity studies was to create the therapeutic window between the pharmacologically effective dose and the lethal dose of *S. grandiflora* on the selected mice for experiment. Mice were kept without food for 16 h and simply divided into five mice each groups. Doses of crude ethanolic extract (200, 400, 800, 1600 and 3200 mg/kg) were giving intraperitoneal to all mice and then allowed foods; after that their activities were closely and constantly observed for the first 3 h then every 4 h for the next 48 h. Within 48 h any death casualty did not appear, but some changes were found such as ataxia, diarrhea, and weight loss. On the basis of behavior it was clear that the CEE of *S. grandiflora* leaves were safe at all tested doses.

2.8. Antimicrobial activity

The CEE and extractives of *S. grandiflora* potentials were evaluated on the bacteria caused urinary tract infection by the standard disc diffusion method. A total of eight bacterial strains were used in the present study, including *Escherichia coli* (*E. coli*), *Proteus mirabilis* (*P. mirabilis*), *Serratia*, *Klebsiella*, *Pseudomonas fluorescens* (*P. fluorescens*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterobacter* and *Staphylococcus Saprophyticus* (*S. saprophyticus*). These pathogens were obtained from the Department of Microbiology, University of Dhaka. The bacterial strains were maintained on nutrient agar slants tubes at 4 ℃ overnight.

2.8.1. Determination of minimum inhibitory concentration (MIC)

The MIC was determined by serial dilution method using nine sterile tubes[14,15] for *E. coli*, *P. mirabilis*, *Serratia*, *Klebsiella*, *P. fluorescens*, *Enterobacter*, *P. aeruginosa* and *S. saprophyticus*. Serial dilution was prepared from 250 mg/mL of the plant extract using nutrient agar to make 250, 125, 62.5, 31.25, and 15.625 mg/mL were specifically inoculated with 0.1 mL of standardized inoculums (10^9 CFU/mL). The tubes were incubated aerobically at 37 ℃ for 18–24 h. Cephalosporin (0.1 mg/mL) and gentamycin (0.1 mg/mL) were used as controls. The lowest concentration (highest dilution) of the extract produced no visible bacterial growth (no turbidity). The procedure was repeated in triplicates and compared with the extract free gentamycin and cephalosporin standards.

2.8.2. Antibacterial activity

The CEE and fraction of grandiflora leaves were screened for their antibacterial activity of various known bacteria caused UTI and the growth of inhibition was compared to the conventional drug kanamycin (30 µg/disc) *in vitro* disc diffusion technique[16]. Nutrient agar was sterilized at 121 ℃ for 15 min and cooled to a 45–50 ℃ followed by inoculated with the 0.1 mL test microorganisms in test tubes, organism (10^9 CFU/mL) were spread on sterile agar plates[17]. About 1 mL of plant extract was added in the paper discs (6 mm diameter, Whatman No. 1 filter paper, containing 100 µg/mL plant extract), then the dried separate paper disc was placed aseptically on the organism contained Petri disc agar surface with the help of sterile forceps followed by keeping at 4 ℃ for overnight and then incubation at 37 ℃ for 16 h to promote bacterial growth. The inhibition zone formed was measured in nearest millimeter and the assay was performed in triplicate for each extract sample.

2.9. Statistical analysis

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

3. Results

The results of qualitative phytochemical screening of CEE and various extracts of *S. grandiflora* leaves showed presence of important plant secondary metabolites. All the prepared extracts showed the presence of alkaloids, carbohydrates, tannins, phenol and flavonoids while steroids were detected only in petroleum soluble fractions.

3.1. Flavonoid and phenolic content

The qualitative phytochemical screening exposed the presence of alkaloids, flavonoids, and phenol in all extract fractions. All fractions (CTSF, CSF, CEE and AQSF) of *S. grandiflora* leaves contained carbohydrates with the exception of PSF. Reducing sugar was detected only in EASF and CSF, and steroid only in PSF. The CSF (31.44 ± 0.47) exhibited the highest flavonoid content while the PSF (23.34 ± 0.11) was found to possess the lowest flavonoid content. The total phenol content was highest in the CSF (39.47 ± 0.33) as well and lowest in the CEE (20.98 ± 0.49) (Table 1).

Table 1
Summary of various determinants of free radical scavenging, total phenolic, flavonoid content and reducing capacity of CEE and extractives of *S. grandiflora*.

Extracts radical capacity	Total flavonoid scavenging activity (mg of QAE /g of dry extract)	Total phenol scavenging activity (mg of GAE/g of dry extract)	Total reducing content (µg/mL of extract)	DPPH free content (IC_{50} µg/mL)
EASF	27.37 ± 0.25	27.08 ± 0.22	0.653 ± 0.013	21.16 ± 0.80
PSF	23.34 ± 0.11	30.09 ± 0.11	0.621 ± 0.033	18.05 ± 1.90
CSF	31.44 ± 0.47	39.47 ± 0.33	0.443 ± 0.022	26.76 ± 1.10
CEE		20.98 ± 0.49	0.541 ± 0.053	23.98 ± 1.20
AA	–	–	0.765 ± 0.015	17.50 ± 0.20

Results are expressed as mean ± SD (n = 3). AA: Ascorbic acid.

3.2. Activity of reducing power

In this study, we observed that concentration-dependent increase in the absorbance of reaction mixture for all the solvent extracts and the AA at 700 nm increased, indicating reducing ability. The antioxidants potentiality present in the CEE and fractions of *S. grandiflora* caused their reduction of Fe^{3+} to the ferrous form. EASF fractions showed the highest reducing power (absorbance; 0.653 ± 0.013 µg/mL) among all the tested concentrations followed by PSF (absorbance; 0.621 ± 0.033 µg/mL), while the CSF showed the lowest reducing power (absorbance; 0.443 ± 0.022 µg/mL). Absorbance of EASF was compared to that of AA (absorbance; 0.765 ± 0.015 µg/mL) (Table 1). The observed reducing ability of the plant extracts could attribute to the presence of hydrophilic polyphenolic compounds. So it can be proposed that the reducing power of plant extracts correlate properly with the phenolic content.

3.3. DPPH free radical scavenging activity

The antioxidant activity of the extracts was determined using by the method described by Braca[18]. In this investigation among the extractives, PSF showed the highest scavenging activity with IC_{50} value (18.05 ± 1.90 µg/mL) as compared with the AA (17.50 ± 0.20 µg/mL), followed by CEE with IC_{50} value (23.98 ± 1.29 µg/mL). The lowest scavenging activity was shown by CSF with IC_{50} value (26.76 ± 1.10 µg/mL) and data are presented in the Table 1. IC_{50} value of CEE, PSF, CSF, EASF and AA were determined by plotting the percentage of inhibition against log of sample concentration and corresponding straight line equations obtained.

3.4. Antidiarrheal activity

For the evaluation of antidiarrhoeal activity, mouse model was used. The ethanolic extract limited the mean number of defecation episode in a dose-dependent manner (Table 2). The unfractionated CEE limited the number of defecation episodes by 25.0% at a dose of 200 mg/kg and by 38.1% at dose 400 mg/kg body weight which was compared by castor oil provoked diarrhoea and standard loperamide exhibited 53.6%.

Table 2
Antidiarrhoeal activity for crude ethanolic extract of *S. grandiflora* leaves.

Agent	Dose	Latent period	Number of defecation episodes	% Inhibition
Distilled water	0.5 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 (TG1)	200 mg/kg	1.05 ± 0.07*	7.00 ± 0.86*	25.0
CEE (TG2)	400 mg/kg	1.59 ± 0.19**	5.50 ± 0.63**	38.1

All agents were given orally. Values are expressed as mean ± SE; *: $P < 0.05$, **: $P < 0.001$, both indicating statistical significant with respect to the effect of control.

3.5. Assessment of MICs

The MICs against *E. coli*, *P. mirabilis*, *Serratia*, *Klebsiella*, *P. fluorescens*, *P. aeruginosa*, *S. saprophyticus* and *Enterobacter* were determined only for the CEE of the leaves of *S. grandiflora*, and results were corresponding with the control. The MICs ranged between 13 and 33 µg/mL. The highest MIC was (33.00 ± 0.61) µg/mL against *E. coli* and the lowest was (13.00 ± 0.91) µg/mL against *Serratia* (Table 3).

Table 3
MICs of the CEE of *S. grandiflora.*

Name of bacteria		MIC (µg/mL)
1	*E. coli*	33.00 ± 0.61
2	*P. mirabilis*	23.00 ± 0.19
3	*Serratia*	13.00 ± 0.91
4	*Klebsiella*	30.00 ± 0.41
5	*P. fluorescens*	22.00 ± 0.11
6	*P. aeruginosa*	21.00 ± 0.33
7	*S. saprophyticus*	28.00 ± 0.63
8	*Enterobacter*	29.00 ± 0.93

Values were expressed as mean ± SD ($n = 3$).

3.6. Antimicrobial activity

The antibacterial activity of the ethanolic extract of leaves of *S. grandiflora* was studied against bacteria caused urinary tract infection at a concentration (100 µg/mL) and activity was compared with the standard kanamycin (35 µg/mL)[19]. The results of antibacterial screening of CTSF, PSF, CSF, CEE and AQSF of *S. grandiflora* are presented in (Table 4). Growth of inhibition of each extract against known bacteria was not same. At a concentration of 100 µg/mL among the various extract fractions, the PSF showed the maximum sensitivity (19.00 ± 0.35) against *E. coli* whereas the lowest sensitivity was observed with CSF against *S. saprophyticus* (7.01 ± 0.10). *E. coli*, *P. fluorescens*, *Enterobacter*, *S. saprophyticus* were sensitive to all extractive. No antibacterial activity was shown by the AQSF (Table 4).

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

Bacteria	CTSF	PSF	CSF	CEE	Kanamycin (30 µg/disc)
E. coli	13.00 ± 0.13	19.00 ± 0.35	10.00 ± 0.12	8.00 ± 0.12	29.50 ± 0.10
P. mirabilis	10.00 ± 0.51	10.00 ± 0.90	7.90 ± 0.14	10.00 ± 0.24	28.00 ± 0.91
Serratia	9.80 ± 0.20	-	8.00 ± 0.91	-	30.00 ± 0.38
Klebsiella	10.00 ± 0.84	8.90 ± 0.11	-	10.00 ± 0.12	26.00 ± 0.51
P. fluorescens	12.00 ± 0.51	9.00 ± 0.90	8.00 ± 0.15	10.00 ± 0.24	29.00 ± 0.91
Enterobacter	11.00 ± 0.13	18.00 ± 0.35	11.00 ± 0.12	9.00 ± 0.12	26.50 ± 0.11
S. saprophyticus	7.01 ± 0.10	17.00 ± 0.30	7.01 ± 0.10	9.00 ± 0.02	24.50 ± 0.11

4. Discussion

Since all the fractions were ensured for flavonoid and phenolic content in the preliminary screening of these biochemical. We decided to investigate the pharmacological potential of *S. grandiflora* leaves. The outcomes of the study directed that the leaves have the antioxidant potential and also a good of remedy for diarrhoea and sensitive to known pathogenic microbes cause UTI.

For outcome of this investigations of antidiarrhoeal episodes, it is cleared that the ethanolic crude extract limits the mean number of defecation episodes in a dose dependent mode. In brief, oral ingestion of castor oil provoked to release ricinoleic acid by lipases in the intestinal lumen, and this unsaturated omega-9 fatty acid of ricinoleic acid is absorbed in the intestine, subsequently exerts its laxative effect via prostaglandin receptor EP2 and facilitated the effect of ricinoleic acid on the motility of the intestine[20]. Phenolic compounds found in numerous medicinal plants are believed to

have an inhibitory effect on the intestinal motility and the secretory activity of the intestine. Biologically active compounds found in the extract such as phenols and flavonoids may counteract the irritant effect of ricinoleic acid on the epithelium of the intestine. We can also claim based on our investigation that pharmacological potential of different organic extract of *Sesbania* may interfere to release or actions of autacoids like histamine, serotonin, prostaglandins, *etc*.

In acute toxicity test, no toxic effects were observed at a graded doses of the CEE (200, 400, 800, 1 600 and 3 200 mg/kg). Hence, there were no lethal effects in any of the groups.

Antibacterial activity of the crude ethanolic extract and its fraction of *S. grandiflora* was studied against bacteria caused UTI. Extracts show variable growth of inhibition against some given bacteria and then the growth inhibition was compared with the predictable standard drug kanamycin. Kanamycin is a conventional bactericidal drugs (inhibit bacterial cell wall synthesis). It acts on 30S ribosome by anticodon recognition, so defective protein synthesis occurs. We can claim that the extracts of *S. grandiflora* could be used against both stains but mostly Gram-negative pathogens fruitfully. According to previous studies and review literature, number of medicinal plants have been shown to anti-infective properties[21]. Leaves of *S. grandiflora* may possess the same properties, which already found in our study. So we can have demanded that the presence of antibacterial active ingredients in the leaves of *S. grandiflora* inhibits the growth of both Gram (+) and Gram (–) pathogens.

The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for a visible deep purple color. The degree of color change is proportional to the concentration and potency of the antioxidants. Outcome of our study revealed that, changes in color (the more decolourisation is, the more reducing ability is; from deep violet to light yellow) followed by low absorbance of the reaction mixture indicated a high free radical scavenging activity.

Phytochemical study of the various extract fractions of *S. grandiflora* leaves showed the existence of a significant amount of phenolic compounds that ensure us the plant possesses aforesaid pharmacological potentials. On the basis of investigations, it is justified the folkloric use of *S. grandiflora* leaves have the potential of antioxidant activity and remedy of microbial infection, diarrheal disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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