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Research Article

PHYTOCHEMICAL, ANTIMICROBIAL EVALUATION AND DETERMINATION OF TOTAL PHENOLIC AND FALVONOID CONTENTS OF *SESBANIA GRANDIFLORA* FLOWER EXTRACT

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ABSTRACT

Sesbanial grandiflora used in traditional klowledge of ayurveda for various diseases and infections. The acetone (70%) extraction was carried out on the flowers of the plant. The phytochemical groups were identified by the tests of characterization, and then quantified by the tests of proportioning of total phenolics and flavonoides. The total flavonoids and phenolic contents were also quantified in acetone extract of flower part. This activity is related to phenolic compounds contained in the extracts. The total polyphenolic content in the flower extract found to be 49.1+0.96 mcg/mg and flavonoid content was 12.86+0.72 mcg/mg. Extracts also expressed a good antibacterial activity. The flowers extract FE 200 showed highest inhibition against *P.* aerugunosa (25.00 ± 0.60 mm), *S. aureus* (21.00 ± 0.50 mm) and E*. coli* (19.00±0.60), which are especially responsible for ophthalmic infection, conjunctivitis.

Keywords: *Sesbania grandiflora*, Total phenolics and flavonoids, Antimicrobial, conjunctivitis.

INTRODUCTION

Sesbania grandiflora (Fabaceae), commonly known as Agati is a widely available plant; it is an open branching tree tall up to 15m and 39cm in diameter belongs to family Fabaceae[1]. It is native to India, Australia, Indonesia, Malaysia, Myanmar, Philippines. The chemical constituents found are galactommannans, linoleic acid, beta-sitosterol and carbohydrates[2]. The major contributors of phenolic substances in S.grandiflora are simple phenolics acids. Apart from this the other bioactive compounds reported in this plant are saponins[3]. Traditionally the plant has been used for the treatment of headache, in fever, as a tonic, in catarrh, as an astringent etc[4-5]. Generally bark is used as astringent and used for treatment of small pox, ulcers in mouth and alimentary cannal, infantile disorders of stomach, scabies etc. The juice of leaves of the *Sesbania grandiflora* have been reported to have anxiolytic and anticonvulsant anthelmintic demulcent, expectorant, antipyretic, in treatment of bronchitis, cough, vomiting, wounds ulcers, diarrhoea, dysentery etc[4]. The flowers have been reported to have anti microbial activity.

Ethnobotanical investigations in the central region of Burkina Faso have shown that some species of *Sesbania* are used frequently and widely in traditional medicine to treat gastrointestinal infections, cardiovascular diseases and have antibacterial and anti-viral activities[6-7]. In this fact, the aim of the present work is to evaluate the phytochemical composition and relationships between total phenolic contents and antibacterial potentials from *S. grandiflora*, particularly for ophthalmic conjunctivitis.

In order to determine the therapeutic properties of the plant, we begin with simple tests of phytochemical characterization. This characterization was supplemented by the proportioning of total phenolics and flavonoides. Lastly, the antibacterial activities are determined by research by bacterial inhibiting activity and minimal inhibition concentration.

MATERIALS AND METHODS

Chemicals such as quercetin and Gallic acid were obtained from Sigma Ltd., USA, Folin Ciocalteu reagent (Merck Co.), Sodium carbonate; aluminium nitrate and Sodium acetate were required for the experiment. Nutrient broth procured from the Hi-media, Mumbai.

Collection of Biological/Plant materials

The samples were collected during the months from October to December 2011. The studies were conducted from December 2011 to February 2012. The flowers of *Sesbania grandiflora* were collected at the site of Shirpur, Satpuda Hills, Maharashtra, India. The vegetable specie was identified by the botanists of the University. These flowers were washed thoroughly with water and allowed to shade dry for ten days at the laboratory at a temperature of surroundings 30ºC, safe from the light, pulverized and preserved in plastic sachets safe from the light.

Preparation of extract

The shade dry flowers were firstly powdered coarsely with the help of grinder. A 100gm of powdered flowers were extracted with organic solvents by using Soxhlet apparatus. These flowers are successively extracted with petroleum ether, ethyl acetate and 70% acetone. The obtained extract was further filtered with Whatmman filter paper and then allowed to dry.

Preliminary Phytochemical Screening

The plants may be consisting of many chemical constituents like alkaloids, glycosides, carbohydrates, volatile oils, tannins, saponins, flavonoids etc. These chemical constituents are called as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of these secondary metabolites acetone (70%) extracts was subjected to chemical tests[8].

Total polyphenolic content

Total phenolic content of hydroacetone extract of Sesbania grnadiflora was determined using Folin–Ciocalteu reagent[9]. In this method, the blue colour formed due to the polyphenol present in the extract was measured at 660 nm using UV spectrophotometer. Sample solution of flower extract was prepared by dissolving 10 mg of the each extract in 100 ml of methanol to give 100 µg/ml solutions. The extract (0.1ml) was mixed with the Folin-Ciocalteu phenol reagent (0.2 ml), water (2 ml) and sodium carbonate (15 % w/v, 1 ml), and absorbance was measured at 660 nm using spectrophotometer (Shimadzu 2405) after 2 h incubation period at 50 ºC for 10 min. All the experiment was performed in triplicate. The total phenolic content is expressed as µg Gallic acid equivalents (GAE).

Total Flavonoid content

Total flavonoid content of hydrocetone extract of *Sesbania grandiflora* was determined using reported method[9]. Sample solution was prepared by dissolving 10 mg extract in 100 ml of methanol to give 100µg/ml solution. Sample solution of flower extract (0.5 ml), ethanol (1.5 ml), $Al(NO_3)_3$ (0.1 ml, 10%), CH_3COONa

(0.1 ml, 1 M) and water (2.8 ml) were thoroughly mixed and kept at ambient temperature for 40 min. The absorbance of reaction mixture was measured at 415 nm using spectrophotometer (Shimadzu 2405). All the experiment was performed in triplicate. Total flavonoid content was calculated according to a standard curve established with quercetin. The total flavonoid content was expressed as µg Quercetin equivalents (QE).

Antibacterial activity

Extracts of *Sesbania grandiflora* are tested on the bacteria[10, 13-17]. The antibiotics of reference are Penicillin, Ampicillin and Ciprofloxacin.

Bacterial strains

Some strains of bacteria from the American Type Culture Collection (ATCC, Rockville) were tested: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923. Among the strains bacteria, *Escherichia coli* was Gram-negative bacteria; *P. aeruginosa* and *Staphylococcus aureus* was Gram positive bacteria. We test those strains particularly because there are the causative agents for ophthalmic infection, conjunctivitis.

Determination of minimum inhibitory concentration

The MIC The extract of *Sesbania grandiflora* was initially dissolved in DMSO and tested at the different concentration of 50 to 250mcg/ml. The test microorganisms used are *Pseudomonas aeruginosa, Staphyllococcus aureus and E. Coli*. Sterile NA plates were prepared and the inoculums of test microorganisms were spread uniformly. Wells were prepared by using sterile borer having the diameter of 6mm. About 100µl of test solution; standard antibiotic solution and solvent control were added in each well separately. The plates were kept at 4^0C for 1 hour for the diffusion of the test solution and then place them in the incubator for 24hrs at 37^0C. Each MIC experiment was repeated three times. Inhibition of bacterial growth was judged by rose or yellow color. The MIC is defined as a lower concentration of the extract at which the bacteria do not demonstrate the visible growth.

Preparation of inoculums

The active cultures required for the experiment were prepared by transferring one loop full of microorganisms from the stock cultures to the test tubes containing nutrient broth and were incubated for 24 hrs at 37^0C in incubator.

Preparation of nutrient agar

Generally nutrient agar is used as the media for the growth of the bacteria and Potato dextrose agar media is used for the growth of fungi. These media were prepared by dissolving the given amount in the distilled water, sterilised it with the help of autoclave at 121^0C for 15 min.

Determination of zone of inhibition

The zone of inhibition was determined by the agar well diffusion method by using the nutrient agar plates seeded with the testing microorganisms like *Staphylococcus aureus, Pseudomonas aeruginosa, E. Coli.* The NA plates were prepared by pouring the 20ml of sterilised nutrient agar solution in to the sterilised petri plates, the plates were allowed to solidify. The bacterial suspension was swabbed on the solidified NA, make hole in solidify NA with the help of borer having diameter 6mm. Pour the 0.5ml of testing solution of different concentration separately into separately cup and allow diffusing for 10min in refrigerator. Plates were kept in incubator at 37^0C for 24hrs. We took the penicillin and Ampicillin as a standard antibiotic.

Minimum Bactericidal Concentration (MBC)

MBC was determined for each set of wells. After incubation, the concentration at which no visible growth was noted as minimum bactericidal concentration [11].

RESULTS AND DISCUSSION

There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infection. However, the development of new antibiotics should be continued as they are of prime importance to maintain the effectiveness of antimicrobial treatment. In developing countries, the WHO estimates that about three quarters of the population relies on plant based preparations used in their traditional medicinal system and as the basic need for human primary health care. [12]

Preliminary phytochemical screening

Preliminary phytochemical screening of acetone (70%) extract of flower revealed the presence of chemical constituents like Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Amino acids which were confirmed by performing the chemical tests which are the confirmatory test for that respective chemical constituent as shown in Table 1.

Total polyphenolic and flavonoid content

S. grandiflora are the good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress. The floavonoid and polyphenolic were reported to be responsible for the antioxidant activity and also crude flavonoid from all flowers has the highest anti-microbial properties. The Table-2 indicates the amount of polyphenolic and flavonoids compounds present in the acetone (70%) extract of flower with respect to gallic acid and quercetin respectively. The total polyphenolic content in the flower extract (FE) found to be 49.1+ 0.96 mcg/mg and flavonoid content was 12.86+ 0.72 mcg/mg.

Antimicrobial activity

Our earlier studies had shown the successful candidature of flower extract of *Sesbania grandiflora* for antibacterial potential [13-18]. Results of determination of the bacterial inhibiting activity and the minimal inhibitory concentration of the extracts. Tables 2 and 3, respectively indicate the results of the determination of the bacterial inhibiting activity and the inhibiting minimal concentration of the extracts. All the extracts were active on all the strains of bacteria, with diameters of inhibition most important, compared to antibiotics by reference, for which strains were sensitive. The antibacterial actions of the extracts were compared with ciprofloxacin as positive control. Certain extracts were active on strains resistant to Penicillin. Indeed, the extracts inhibited the growth of *S. aureus*, *E. coli*, resistant to penicillin, the extracts were active. Also on *S. aureus,* resistant to Ampicillin, the extracts were active. The inhibiting minimal concentrations of the bacteria by the extracts indicate that the extracts generally act with low dose: surroundings 12.5 mg/mL of extract, even if these concentrations remain high compared to penicillin or with ampicillin, 2 mg/mL. The acetone extract of flower was active on the strains of bacteria, we taken for testing with diameters of inhibition. We had taken the different concentrations of the extract in DMSO. The flower extract of *S. grandiflora* shows the more intense activity on *P. aeruginosa* than the remaining two organisms S. aureus and *E. Coli*. The extracts contain several chemical compounds while the antibiotics of reference are purified, containing only the inhibiting molecules.

Minimal Inhibitory Concentration and Minimum Bactericidal Concentration

In most cases the MIC was found 0.65 mg/ml against most bacteria. *E. Coli*. was appeared the most resistant bacterial strain as it has highest MIC values in comparison with other species. Based on the minimum inhibitory concentration results, the minimum bactericidal concentration (MBC) was performed against *P. aeruginosa,* S. aureus and *E. Coli.* There was no visible growth of any of these microorganisms. Hence *Sesbania grandiflora flower extract was* found to have significant antibacterial activity against the microorganisms tested. The results of the present study support the traditional usage of *Sesbania grandiflora* and it can be recommended for usage as antimicrobial agents in new drugs for the therapy of infectious disease caused by pathogens, especially conjunctivitis.

Table 1: Tests of characterization of flower extract of *Sesbania grandiflora*

S. No.	Chemical Constituents	Testing reagent	Result
1	Flavonoids	Shinoda test	+
2	Tannins	Ferric chloride test Lead acetate test	+
3	Carbohydrates	Molisch's test Fehling's test	-
4	Steroids	Salkowski reaction	+
5	Alkolides	Dragendorff's reagent Mayer's reagent Hager's reagent Wagner's reagent	-
6	Amino acids	Ninhydrin test	-
7	Saponins	Foam test	+

+ sign indicates positive and – sign indicates negative

Table 2: Antibacterial activity of *Sesbania grandiflora* flower extract by well diffusion method (Diameter of zone in mm)

S. No.	Concentration (mcg/ml) of S.G. flower extract (FE)	*P. aerugInosa*	*S. aureus*	*E.coli*
1	FE 20	10±0.4	9±0.3	7±0.4
2	FE 50	11±0.4	11±0.3	9±0.4
3	FE 100	15±0.5	14±0.5	13±0.4
4	FE 150	21±0.5	17±0.5	16±0.5
5	FE 200	25±0.6	21±0.5	19±0.6
6	Penicillin	16±0.4	-	-
7	Ampicillin	18±0.12	-	-
8	Ciprofoxacin	44±0.14	24±0.6	30±0.5

Values are mean±SEM of three separate determinations.

Table 3: Minimal inhibition concentration values of flower extract of *Sesbania grandiflora*

S. No.	Concentration (mcg/ml) of S.G. flower extract (FE)	*P. aerugInosa*	*S. aureus*	*E.coli*
1	FE 20	-	-	-
2	FE 50	2.5	2.5	2.5
3	FE 100	-	-	5
4	FE 150	0.65	0.65	1.5
5	FE 200	-	-	-

CONCLUSION

Our results indicate that the extracts of the flower part of the plant of *Sesbania grandiflora* contain polyphenols, compared to Quercetin used as reference. This acetone (70%) extract was also able to inhibit the bacterial growth. The spectrum of action of the extracts is broad because it covers the Gram negative and positive bacteria and also some bacteria which are resistant to two antibiotics of reference tested. Based on the results, it can be concluded that the *Sesbania grandiflora* flower extracts have great potential as antimicrobial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms, especially conjunctivitis. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antibacterial activity and the underlying mechanisms.

Conflict of interest

None of the authors have any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. This research article is a part of Ph D Research Work of Mrs. Kalpana B. Mundhe-Wagh.

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