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MEDICINAL AND HEALTH-PROMOTING POTENTIAL OF SPERGULA PENTANDRA (CARYOPHYLLACEAE): PHYTOCHEMICAL PROFILING, ANTIMICROBIAL EVALUATION, AND SUSTAINABLE THERAPEUTIC APPLICATIONS

Farman Ullah Khan[*1,2], Fawad Ahmad[3], Hazrat Bilal[1,4], Romana Bibi[1], Muhammad Ibrahim[5], Abdullah[5], Mohibullah[6], Ihsan Ullah Khan[7], Azam Khan[1], Bushra Daraz[1,b], Shah Faisal[1], Tariq Aziz[8]

[1]*Department of Chemistry, University of Science and Technology Bannu-28100, KPK Pakistan*
[b]*Government Girls Degree College, Mandan, Bannu, KPK Pakistan*
[2]*Qiddiya Investment Company, Riyadh, Kingdom of Saudi Arabia*
[3]*Resident Cardiology, MTI, Khyber Teaching Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan*
[4]*Government High School Haider Khel, NWTD, Elementary and Secondary Education Department, Khyber Pakhtunkhwa, Pakistan*
[5]*B.E. in Chemical Engineering; M.Sc. in Sustainability and Environmental Management, University of Derby, UK, Saudi Arabian Parsons Limited, Riyadh, Saudi Arabia*
[6]*Department of Forest, Mardan Forest Division, Khyber Pakhtunkhwa, Pakistan*
[7]*Department of Forest, Abbottabad Forest Division, Khyber Pakhtunkhwa, Pakistan*
[8]*Faculty of Civil Engineering and Mechanics, Jiangsu University, 212013 Zhenjiang, China*

farmandphil@yahoo.com/ muhammad.ibrahim@parsons.com, [*1,2]farmandphil@yahoo.com , [3]drfawaddawar039@gmail.com, [1,4]bilalmphil2013@gmail.com [1]romanabibi13@gmail.com, [5]Muhammad.ibrahim@parsons.com, [5]Abdullah.khan@jacobs.com [6]muhibdawar2020@gmail.com [7]ihsandawar88@gmail.com, [1]azamdurrani45@yahoo.com, [1,b] bushradaraz@gmail.com, [1]kingfasial0310@gmail.com, [8]tariq@ujs.edu.cn

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Corresponding Author: *
Farman Ullah Khan

Abstract

Spergula pentandra has been historically used for medicinal purposes in North-west Pakistan. Due to the importance of the plant, it was crucial to analyze, recognize, and both quantitatively and qualitatively evaluate the physiochemical and phytochemical components (anthraquinones, alkaloids, flavonoids, glycosides, terpenoids, saponins, phenols, and tannins) it contains, along with their antimicrobial activities. Phytochemical examination of plant samples showed the presence of alkaloids, flavonoids, terpenoids, anthraquinones, glycosides, and saponins, though tannins are absent. The results indicated antibacterial activity at varying degrees against Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus. The specified species has shown quite promising results against the tested bacterial and fungal strains. Crude hexane extract of Spergula pentandra has showed good antifungal activity and found active against both the Aspergillus niger and Aspergillus fumigates with a diameter of zone of inhibition (6 and 5 mm) used in the study while aqueous fraction was inactive against both A. niger and A. fumigates respectively.

INTRODUCTION

Plant-based medicine (herbal origin derived drug) is extensively employed for therapeutic purposes because of their reasonably safer side to synthetic and alternatives and are certainly accessible and cheaper in the market [1].

These plants are used as a whole or their parts as oral solution, inhaler, skin lotion and are supposed to work as medicine [2]. The curative value of such herbal plants is mainly hidden in some chemical core bodies that create a distinct physiological action on the human body when used. These bioactive chemical components are alkaloids, tannins, terpenoids, flavonoids, and phenolic contents [3].

Plant extracts have been studied in detail by many researchers to screen out the bioactive portions with possible biological study specifically antimicrobial activities to corroborate the various factors related to it. The plants extract efficiency on different bacteria and fungi were investigated in detail by number of researchers throughout the world.

It was investigated that ethanol and aqueous extracts of different plants applied in allopathic remedies are latent sources of antitumor, antiviral and antimicrobial mediators [4].

Crude herbal extract has significant potential against biological evaluation in initial steps as compared to pure isolated compounds from medicinal herbs [5].

Caryophyllaceae, the prime family of dicotyledonous plants, is also famous as pink family, which encompasses 85 genera and some 2,100 species. This family is mainly used for medicinal and ornamental purposes.

Spergula is a genus of Caryophyllaceae containing about 11 species distributed worldwide, especially in northern hemisphere. In Pakistan, it grows in different regions and so far two species of *Spergula* is also characterized in Pakistan [6].

The genus *Spergula* is traditionally used in indigenous Chines medicines. *Spergula* species have been used as therapies in phyto-medicines, alternative medicine since time distant past. Some young species have edible uses and are locally used as anti-diarrheal. Their seeds are rich in oil and contain saponins. The local communities that are consistently using indigenous Chinese medicine are still using some species of Spergula like dianthus, arenaria, gypsophila, psammosilene, and silene for pharmaceutical purposes [7].

This medicinal importance of the afore-mentioned herb prompted us to assess it for physiochemicals, phytochemicals and antimicrobial activities.

EXPERIMENTAL

Collection of Plant Materials

Spergula pentandra was assembled from district Bannu of Khyber Pakhtunkhwa, Pakistan in August 2013 and was identified by a plant taxonomist Prof. Dr. Sultan Mehmood, Department of Botany, UST Bannu, KPK Pakistan. The sample obtained was subsequently washed with Millipore grade water and then allowed to dry in shade. The sample after drying was ground powdered and stored in sample bags for further use. At herbarium of the Botany department, UST Bannu, the voucher specimen of the sample was deposited.

Physicochemical analysis/ Fluorescence analysis

The reported method was used to determine the drying base weight loss (%), ash content; total, water-soluble, acid-insoluble, and residue on ignition [8].

The efficiency of different solvent systems to extract the leaf powder were investigated, results are tabulated in Table 1 and 2.

Phytochemical screening (Quantitative Determination)

Flavonoid determination

50 g of the plant extract was extorted with 200 mL of aqueous methanol (80%) thrice at ambient condition. The collected solution was filtered with Whatman filter paper No. 42 to obtain the clear filtrate solution, which was dried, and weighed [9]. The results obtained are given in Table 3.

Saponin determination

The suspension was made by adding the sample (50 g) in 20% ethanol (200 mL) and subjecting it to stirring and heating at 55°C. The extraction was repeated several times. The volume of collective extracts was reduced to 40 mL by rotary evaporator and transported to separating funnel and added 20 mL of diethyl ether, shaken vigorously to form ether and aqueous layers. The ether layer was discarded, and the

water layer was repeatedly washed with diethyl ether to obtain the pure product which was mixed with 60 mL of n-butanol. Aqueous sodium chloride solution (5 %, 10 mL) was used twice for washing down of *n*-butanolic extracts. The final solution was heated to evaporate the solvents and then weighed the final product. The percentage of saponin content was calculated by the method as reported by Ladan Z *et al*., 2014 [10]. The results obtained are presented in Table 3.

Alkaloid determination

50 g sample was added to 200 mL acetic acid/ethanol (20/80) and allowed to set for 4 h. Again, the same process was repeated three times. The collective extract was heated under pressure to obtain its concentrated solution. NH_4OH solution (conc.) was added to the extract until the precipitation was complete. The desired precipitation was obtained by filtration, dried and weighed [10]. The results obtained are presented in Table 3.

Primary detection of phytochemicals (Qualitative establishment)

Various crude fractions of *S. pentandra* were assessed for chemical compounds including flavonoids, alkaloids, phenolic compounds, saponins, tannins, anthraquinones, and triterpenes using formerly described methods [11].

Test for anthraquinones

1 mL of *S. pentandra* extract was boiled with H_2SO_4 (20 mL) and then filtered it. 10 mL of chloroform was added to the filtrate, shake vigorously and then allow to set to get two layer. The chloroform layer was pipetted to a test tube to which 2 ml of dil. NH_3 was added to observe the change in intensity and colour of solution [12] as summarized in table 4.

Test for reducing sugars (Fehling's test)

Ethanol extract (0.5/ 5 mL of water) was put in boiling Fehling's reagent (A and B) contained in a test tube and any colour change was observed [12] and the result obtained is presented in Table 4.

Test for flavonoids

For the determination of flavonoids, three procedures have been adopted. In first procedure, 5 mL of dil. NH_3 was mixed with aqueous filtrated fraction, which develops yellow color. The disappearance in yellow colour after addition of I mL of H_2SO_4 (conc.) confirms the presence of flavonoid materials. Secondly, 1% aluminum solution was added drop wise to the filtrate which results in yellow colour, showing the flavonoids in the samples. In 3rd procedure, an extract portion was mixed with ethyl acetate (10 mL) followed by heating for 3 min in boiling water. After heating the solution was filtered and 4 mL of the resultant filtrate was mixed with 1 mL of NH4OH (dil.) which results in yellow coloration that confirms its flavonoids contents [13] (Table 4).

Test for terpenoids

Salkowski test was applied to confirm terpenoids contents in the extracts. In a test tube 2 mL of $CHCl_3$ and 3 mL of conc. H_2SO_4 added subsequently in 0.5 mL of each extract and then allow to set to form a layer. The formation of a reddish-brown color appeared at the interface confirms the terpenoids in the sample [12] (Table 4).

Test for saponins

The aqueous solution of plant extract (0.5/ 5 mL distilled water) shaken briskly and allow to set examined for a stable froth. 3 drops of olive oil were added to frothing, shake it properly to form emulsion, which confirms the occurrence of saponins [12] (Table 4).

Test for tannins

For tannins, the extract of plant in a test tube (0.5mL/10 mL of distilled water) was boiled for a while and then filtered. 0.1% $FeCl_3$ solution was added in the filtrate and appearance of either brownish green or blue-black coloration assuring the existence of tannins [12] (Table 4).

Test for alkaloids

10 mL acid alcohol was mixed with 0.5 mL of plant extract, boiled and then filtered. After filtration, 2 ml of dil.NH_3 and 5 ml of chloroform was added to the 5 ml of the filtrate, shake vigorously. 10 mL of acetic acid was added to extract $CHCl_3$ and divided into two fractions. To one fraction, Mayer's reagent and to the other Draggendorff's reagent was added. The

beginning of a cream or reddish-brown precipitate with Mayer's reagent and Draggendorff's reagent respectively was considered as confident indication for the existence of alkaloids in our samples [12] (Table 4).

Confirmative test for glycosides (Keller-Killianitest) determination

The plant extract (0.5 ml) was diluted with 5 ml distilled water and then mix with acetic acid (2 ml) having one drop of $FeCl_3$ solution and under layered with 1 mL of conc. H_2SO_4. A brown or violet ring formation may appear at the interface clearly divulged the presence of a deoxysugar, which is typical of cardenolides while in the CH_3COOH part a greenish ring may observed above the brown ring and then steadily extend to the covered layer demonstrated the being there of glycosides [12] (Table 4).

Antibacterial and antifungal activity

Plant material and preparation of crude extract

The grounded powder (100 g) of plant material was extracted with n-hexane, chloroform ethyl acetate, methanol and water. The fluid type extract was then filtered and NaCl solution was then added to form precipitates which are separated, air dried and transported to glass bottles for further process [10].

Preparation of standard bacterial suspension

Surface possible counting technique was used for average number of bacteria like *Klebsiclla pneumonia*, *Escherichia coli* and *Staphylococcus aureus* per mL of the stock suspension and about (108 to 109) colony making units per mL was utilized [14].

Well agar diffusion method was conducted for antibacterial activity of *S. pentandra* extracts. 60 ml of the sterilized agar as nutrient was mixed with bacterial stock suspension completely. 20 ml inoculated food agar was transferred into germ-free petri dishes. The agar was left for some time for settlement and with the help of spotlessly clean cork borer No 8, wells of 10 mm diameter in four number was prepared in each of these plates. The whole wells were filled with 0.1 ml of each extracts using micro titer-pipette and consented to disseminate at r.t for 2 h. The plates were kept warm at 37 °C for 24 h. The experiment was conducted twice. Concurrently adding up of the individual solvent in its place of extract was brought out as controls. After incubation period, inhibition zones in mm were det ermined and mean value was evaluated [15].

Anti-fungal activity and fungal strains

The media used for antifungal activity was disinfected yeast and mould extract agar. The fungi *Aspergillus fumigates* and *A. niger* were incubated for two days and three days at 25 °C respectively. The fungal (*Aspergillus niger, Aspergillus fumigates)* strain was acquired from Biotechnology Department at UTB Bannu. For the conduction of smooth experiment, sizable wells of 7 mm were excavated in the medium by means of sterile metallic borer. Stock solutions of both crude and fractions were ready in DMSO at conc. of 20 mg/ml and a volume of 200 µL from respective stock solutions was put into the individual wells. The petri dishes as well control wells containing standard antibiotic (fluconazole) as positive control were incubated at 37 °C for 24 h and anti-fungal activity was measured by the inhibition in zones diameter. To measure the zone of inhibition, the inhibition zones of crude and fractions were compared with the zones of inhibition of Fluconazole; a standard drug [15].

RESULTS

The percentages of all the parameters on ignition were found using typical methods of proximate analysis. The extractive values (%) in polar and nonpolar solvents were also measured and the results are tabulated in table 1.

For fluorescence analysis, *Spergula pentandra* powder and the extracts in many solvents were checked under visible and UV light (365 nm). For phytochemicals confirmation, the grounded material was reacted with various chemical reagents [8]. These showed changes in color, and the results are presented in table 2.

The plant *S. pentandra* was also subjected for quantitative assessment of phytochemicals (alkaloids, flavonoids and saponins). The literature methods were adopted in quantitative determination of phytochemicals.

Table 3 displays the concentration of alkaloids found to be 6.01% followed by flavonoids which is 4.9% and saponins 4.3% respectively. It has been reported that the therapeutic value of medicinal plants are due to these constituents. They are considered potent

antimicrobial, analgesic, diuretic etc and active against human pathogens [3].

Similarly, preliminary phytochemicals screening of the crude extracts of *S. pentendra* showed that anthraquinone metabolites were present in all the tested crude fractions while tannins were absent in all fractions of *S. pentandra*. The hexane fraction showed positive tests only for anthraquinone and terpenoids while ethyl acetate has shown positive tests for saponins, anthraquinone and terpenoids. Similarly chloroform extract reveals the presence of flavonoids, saponins, anthraquinone and terpenoids and methanolic extract has confirmed the presence of all the above-mentioned phytochemicals except tannins. In aqueous fraction, tests for glycosides and tannins were found negative and the rest were determined positively as tabulated in table 4.

The antibacterial activity of *Spergula pentendra* is represented in table 5. The results indicated showed antibacterial activities at variable degrees against *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus*. Results displays high activity of 18 mm measured in the methanolic extract of *S. pentendra* against *Klebsiclla pneumonia* followed by chloroform (17 mm) and aqueous extract (16 mm) while the activity exhibited by rest of the *S. pentandra,s* fractions were in between 5 -15 mm while less activity of 5 mm was verified in ethylacetate fraction against *Escherichia coli*.

Similarly, the various fractions of the titled species were also screened for its antifungal activities. Crude hexane extract of *Spergula pentendra* has showed good antifungal activity and found active against both the *Aspergillus niger and Aspergillus fumigates* with a diameter of zone of inhibition (6 and 5 mm) used in the study while aqueous fraction was inactive against both *A. niger* and *A. fumigates* as illustrated in table 6.

Table 1. Physio-chemical characters of Spergula pentandra

Physiochemical	% (*Spergula pentandra*)
Moisture material	0.37
Ash materials	0.69
Acid inexplicable ash	0.08
ash (Basic insoluble)	0.07
Ash insoluble in H_2o	0.24
Acid soluble Ash	0.72
Ash material soluble in Base	0.67
Ash (Water soluble)	0.65

Table 1: UV-Vis study (Fluorescence characters) of Spergula pentandra powder and its extract

S.No	Plant material + solvents	Normal light	UV/Vis light
1	entire Plant	Grey	Grey
2	n-hexane fraction	Light green	Reddish brown
3	Whole plant+H_2SO_4 (1:1)	Reddish brown	Dark brown
4	*S. pentendra* + 1 N NaoH	Brown	Reddish brown
5	$CHCl_3$ fraction	Reddish black	Pink
6	Combined methanolic extract	Green	Pink
7	Ethyl-acetate fraction	Green	Pink
8	Aqueous portion	Pink	Light crimson

Table 3. Quantitative study of flavonoids, saponins and alkaloids (g kg-1) in Spergula pentandra

Serial No.	Analysis	Wt of plant residue in g	Wt of crude materials	Percentage (%)
	Flavonoids contents	30	2.7	4.9
	Saponins substances	30	2.2	4.3
	Alkaloids materials	30	3.5	6.01

Table 4. Pre-phytochemical test of S. pentandra

Serial No.	Assessment	n-hex fraction	ethyl-acetate portion	chloroform extract	methanol extract	aqueous extract
1	Flavonoids	-	-	+	+	+
2	Saponins	-	+	+	+	+
3	Alkaloids	-	-	-	+	+
4	Anthraquinone	+	+	+	+	+
5	Terpenoids	+	+	+	+	+
6	Glycosides	-	-	-	+	-
7	Tannin	-	-	-	-	-

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Key: (+) present., (-) absent

Table 5: Fractions of Spergula pentandra used for antibacterial activities

Bacterial Strains	Zone of inhibition(mm)					Standard Drug (Doxycycline 30 µg/ml
	n-hexane fraction	chloroform fraction	methanol portion	ethyl-acetate fraction	aqueous part	
E.coli	9	9	7	5	6	30
K.pneumonia	15	17	18	12	16	30
S.aureus	8	12	12	11	14	30

Table 6: Antifungal activities of Spergula pentandra,s various fractions

Fungal Strains	Zone of inhibition(mm)					Standard Drug(Fluconazole (100 µg/disc))
	n-Hexane Fraction	Chloroform Fraction	Methanol Fraction	Ethyl-Acetate Fraction	Aqueous Fraction	
A.niger	6	0	0	0	0	100
A.fumagatus	5	4	0	2	0	100

DISCUSSIONS

The presence of various active constituents as revealed by the phytochemical screening (table 3,4) supports the ingenuity of the plant extracts [12].

The results of the current study on the antimicrobial aptitudes of *Spergula pentendra* against *Staphylococcus aureus, Klebsiella Spp.* and *Escherichia coli* have revealed that the crude extracts of the plant inhibited the growth of majority of the test isolates. There is an indication that the extracts possess substances that can inhibit the growth of some micro-organisms.

From the above collected facts and results, phytochemical composition and antimicrobial activities of *Spergula pentendra* are investigated here for the first time.

The different secondary metabolites present in the title specie here may be the main cause of antimicrobial activities. However, differences were noticed in results between antibacterial and antifungal activities of the *S. pentendra* extracts. These different potencies in results could be due to the variation in the chemical biodiversity of these extracts as literature revealed that secondary constituents of plants exhibit many biological and pharmacological properties including antibacterial and antiviral [16].

Conclusion

Spergula pentandra collected form Bannu, KPK, Pakistan has been evaluated for its phytochemicals, physiochemical and antimicrobial properties. It has been assumed that these extracts will contain various types of active secondary metabolites. However, further studies are suggested to be performed to explain the mechanism lying with these effects. According to reported literature, no study has so far been reported on this medicinal herb from Bannu areas. The results of the current study endorse the traditional use of plant *S. pentandra* in the treatment of infections caused by bacteria as well as fungus. Furthermore, this study is also a good addition in scientific databases to be utilized by local people and herbal practitioners.

Statistical study

All the *in vitro* experimental results were given as mean± of three similar measurements. Results were processed by Excel and MS word (2007) and bio state.

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