ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 6, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF *DROSERA SPATULATA* VAR. *BAKOENSIS* - AN INDIGENOUS CARNIVOROUS PLANT AGAINST RESPIRATORY TRACT INFECTIOUS MICROBES

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Received: 29 July 2016, Revised and Accepted: 04 August 2016

ABSTRACT

Objective: In the present study, phytochemical constituents of *Drosera spatulata* var. *bakoensis* have been evaluated, and antimicrobial activity was screened against respiratory tract infectious microbes.

Methods: The phytochemicals present in *D. bakoensis* by qualitative phytochemical assays and aqueous, ethanol, methanol extracts of thick roots, open flower, and hair of *D. bakoensis* against this pathogenic bacteria and fungi showed a high zone of inhibition which estimated by disc diffusion method as well as minimum inhibitory concentration (MIC) manifestation by the broth microdilution assay followed minimum bactericidal concentration (MBC) and biofilm inhibitory concentrations (BICs) were determined against *Staphylococcus aureus, Klebsiella pneumoniae, and Staphylococcus pneumoniae* the causative organisms of pulmonary infections, mainly effects the nasal pharynx, trachea, and lungs as well as *Aspergillus niger.*

Results: The values of MIC, MBC, and BIC obtained were between 0.3-0.9, 0.36-2.25, and 0.12-0.37 mg/mL, respectively. In the antibacterial and antifungal activity, results revealed that ethanol and methanol extracts significantly showed activity against the tested respiratory disease causing bacteria and antifungal properties to the zone of inhibition showed more than aqueous extracts at very low concentrations.

Conclusion: The plant extracts of *D. bakoensis* have high potential even at low concentrations values against bacteria and fungi cultures, and these results validated by the presence of high amounts of alkaloids, quinones, anthraquinones, and flavonoids in the plant extracts which have tremendous impact against respiratory infectious microbes.

Keywords: *Drosera spatulata* var. *bakoensis,* Antibacterial activity, Phytochemical analysis, *Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus pneumoniae, Aspergillus niger* minimum bactericidal concentration, minimum inhibitory concentration, biofilm inhibitory concentration.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9i6.14382

INTRODUCTION

Active compounds in carnivorous plants play an important role against the infectious diseases. Carnivorous plants are rich in these active compounds, which have medical importance [1]. Carnivorous plants are depended most of their nutrients from trapping and digestion of insects and other arthropods using enzyme secretion mechanism. Carnivorous plants grow in places where the soil consists of very less amount of nitrogen percentage [2]. Many theories stated that true carnivore plant evolved independently six times in five different orders of flowering plants [3,4]. Carnivorous plants are now represented by more than a dozen genera. These include about 630 species that attract and trap prey, produce digestive enzymes, and absorb the resulting available nutrients [5].

In these carnivorous plants, *Drosera* genus is most popular due to its great medical, pharmacological, and commercial significance [6]. *Drosera spatulata* var. *bakoensis* have flower structure and small roots in the soil, at the initial stages, it appears in pure green and slowly it turned in dark red. From ancient days, *D. bakoensis* plant extracts used for the treatment of respiratory diseases such as whooping cough, bronchitis, asthma, and pulmonary diseases [7,8]. In Indo-China countries, they are using as a homemade medicine for treatment various infectious diseases [6]. However, there is limited research work has done on indigenous carnivorous plants for antimicrobial properties such as *D. bakoensis*, which have high impact in the area of respiratory related diseases. There is no significant practical evidence on the *D. bakoensis* as medicine for respiratory related diseases.

In the present study, we established antimicrobial, antifungal activity against selected microbes and phytochemical screening of indigenous carnivorous plant, i.e., *D. bakoensis.* The main intension of this research is to prove how practically *D. bakoensis* plant extracts effective medicine for treatment of asthma, whooping cough, and other respiratory related diseases. The phytochemical screening will reveal the metabolites, which are present in this *D. bakoensis* plant extracts, with this work we can able to support the possibilities for initiation of *D. bakoensis* plant extracts against respiratory infections.

METHODS

Collection and authentication of plant material

D. bakoensis plants were collected from the nitrogen deficient soil lands located at Thottambedu Mandal, near Srikalahasti, Chittoor district, Andhra Pradesh, India. The area is located at 13°45'N 79°42 E near the bank of the Swarnamukhi River. The identity of the plant was confirmed by the Department of Botany, College of Science, Sri Venkateswara University, Tirupati, Chittoor District, Andhra Pradesh. The plant height was observed at 0.02 m, roots deep in the soil are 0.005 m, and the plant flowered part diameter measured at 0.018 m (Fig. 1).

Preparation of sample extracts

From the collected *D. bakoensis,* plant sample roots, flower, and hair separated carefully from micro plant dissector tool. Samples were thoroughly washed with distilled water until disassociation of soil molecules (Fig. 2). Then, these plants parts were dried separately in the presence of sunlight for 15 days and then completely grind to a fine powder.

Fig. 1: *Drosera spatulata* var. *bakoensis in* N deficient soil (in field)

Fig. 2: Collected samples of *Drosera spatulata* var. *bakoensis* (in lab)

Soaking, filtration, and concentration

About 50 g of powder separately soaked in 200 mL of aqueous, ethanol, methanol at a ratio of 1:4 (powder/solvent). The mixture was agitated in a rotary shaker at 220 rpm for 24 hrs and then centrifuged at 5000 rpm for 15 minutes were in supernatant was poured into the air-tight plastic container [9]. The filtrate was evaporated by a vacuum dryer at 40°C overnight to get the dried extracts to remove residual solvents and then preserved in the refrigerator at 4°C for 48 hrs. The solid powder was suspended in the respective solvents at a concentration of 0.1-5 mg/mL.

Microorganism collection and media preparation

The test microorganisms *Staphylococcus pneumoniae* (Microbial Type Culture Collection and Gene bank [MTCC] 655), *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC 3384), and *Aspergillus niger (*MTCC 282) were obtained from culture repository of MTCC, India. Mueller-Hinton agar [10] (2 g/L beef extract, 17.5 g/L acid hydrolysate of casein, 1.5 g/L starch, 17 g/L agar final pH 7.3±0.1 at 25°C). Mueller-Hinton broth (MHB) medium (17.50 g/L Acid Casein Peptone (H), 1.50 g/L Corn Starch, 2.00 g/L beef infusion, final PH 7.4±0.2 at 25°C) used for bacterial culture. Sabouraud dextrose agar [10] (40.00 g/L dextrose mycological, 10.00 g/L peptone, 15.00 g/L agar final pH 5.6±0.2 at 25°C). Sabouraud dextrose broth (SDB) (5 g/L enzymatic digestion of casein, 5 g/L enzymatic digestion of animal tissue, 20 g/L dextrose final PH 5.6±0.2 at 25°C) used for fungi culture. Medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The sterilized agar medium was mixed well and poured into 0.1 m Petri plates (25-30 mL/plate).

Determination of antibacterial activity

Culture inoculation

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of MHB for bacteria and SDB for fungi that were incubated without agitation for 24 hrs at 37°C and 25°C, respectively. The cultures were achieved optical densities corresponding to 10^6 colony-forming units/mL for bacteria and 10^6 spore/mL for fungal strain. Using spread plate method [11], cells were spread over the surface of a solid agar Petri plates with a sterile, L-shaped bent rod while the Petri dish was spun on a "lazy-susan" turntable.

Disc diffusion method for dose-dependent antibacterial activity

Root, hair, and flower of *D. bakoensis* extracts (water, ethanol, and methanol) were checked for the dose-dependent antibacterial and antifungal activity. Antimicrobial activity of each extract was determined using a modified Kirby-Bauer [12-14] disc diffusion method. Extracts were tested using Whatman No. 3 filter papers were punched into 0.005 m sterilized filter paper discs. Discs were impregnated with different concentrations of the test samples using micropipette and allowed to dry and placed onto inoculated plates (30 minutes incubation). Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The plates were kept at 4°C for 2 hrs before incubation at 37°C for 24 hrs with the test microbial and fungal agents. Control discs containing sterile water, ethanol, methanol solvents (negative control), and standard antibiotic discs (positive control) were impregnated. The antibacterial activity was performed in triplicates. The diameter of the zone of inhibition around the disc after incubation was measured and recorded.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was defined as the lowest concentration of the extract, in which the microorganism did not show visible growth. MIC estimated according to the broth microdilution method [15] for all extracts of *D. bakoensis* plant. Around an aliquot of 180 µL of MHB dispensed in 96 well plates followed by 20 µL bacterial inoculums containing 10^6 UFC/mL and 10^6 spore/mL for the fungal strain. All stock extracts solutions were dispensed in corresponding positions in concentrations from 10 to 1000 µg/mL. The microplates were incubated at 37°C for 24 hrs. Kanamycin (10 mg/mL) was used as positive control and dimethyl sulfoxide as a negative control. The MIC value of the extract was determined as the lowest concentration that completely inhibited bacterial growth. Experiments were conducted in triplicates. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC [16]. Positive and negative cultures were also prepared.

Biofilm inhibitory concentrations (BICs)

The BIC was determined as the lowest concentration at which no visible growth was observed in the supernatant fluid. According to Johnson *et al*. (2002) [17] and Nostro *et al*. (2007) [18], effects of sub inhibitory concentrations of the all *D. bakoensis* plant extracts on established biofilms were evaluated. Bacteria and fungi cultures were grown in BHI in a polystyrene flat-bottomed microtiter plate to form biofilm after 24 hrs of incubation at 37°C. The supernatant bacteria and fungi cells were removed with a micropipette, and the wells were washed with saline solution 0.85% for three times and filled with 200 mL 2-fold dilutions of the *D. bakoensis* solvent based plant extracts, ranging from the MIC to a 16-fold dilution of the MIC. Kanamycin (10 mg/mL) was used as positive control. The plates were incubated for 24 hrs at 37°C.

Data analysis for comparison between samples, data were analyzed by the Student's *t*-test and the one-way analysis of variance (ANOVA). In all cases, $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS package.

Phytochemical analysis

Phytochemical analysis of all the solvent extracts was done according to the procedure of the Indian Pharmacopoeia (1985). The following tests are performed to root, hair, and flower based plant extracts of *D. bakoensis.*

Test for alkaloids (Dragendorff reagent)

About 2 mL filtrate added to six drops of dragendorff reagent [19] orange precipitation was indicated the presence of the respective alkaloids.

Test for tannins (Braymer's test) [20]

About 2 mL of extract was treated with 10% alcoholic ferric chloride solution and observed for the formation of blue or greenish solution.

Test for flavonoids (alkaline reagent test)

About 2 mL of extracts were treated with a few drops of 20% sodium hydroxide solution. Formation of intense yellow, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for steroids (Liebermann-Burchard reaction) [20]

About 2 mL filtrate, 2 mL acetic anhydride, and 2 mL concentration H_2SO_4 added together. Blue-green ring indicated the presence of steroids.

Test for phenols (lead acetate test)

About 5 mL of filtrate and 3 mL of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds.

Test for saponins (foam test)

About 2 mL of extract was added 6 mL of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for cardiac glycosides (Keller Kelliani's test)

About 5 mL of each extract was treated with 2 mL of glacial acetic acid, and a drop of ferric chloride solution was added to it. By the addition of concentration H_2SO_4, a brown ring at the interface was formed. It indicates the presence of cardiac glycosides.

Test for terpenoids (Salkowski's test)

About 1 mL of chloroform was added to 2 mL of plant extract followed by a few drops of concentrated sulfuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for sterols (Liebermann-Burchard test)

About 1 mL of extract was treated with drops of chloroform, acetic anhydride, and concentration H_2SO_4 and observed for the formation of dark pink or red color.

Test for quinones

About 1 mL concentrated sulfuric acid was added to 1 mL of the plant extract. Formation of red indicated the presence of quinones.

Test for phlobatannins (precipitate test)

Deposition of a red precipitate when 2 mL of extract was boiled with 1 mL of 1% aqueous hydrochloric acid were taken as evidence for the presence of phlobatannins.

Test for anthraquinones [20]

About 1 mL of the extract was boiled with 10% HCl for few minutes in water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. A few drops of 10% ammonia were added to the mixture and heated. Formation of rose pink indicates the presence of anthraquinones.

Test for amino acids and proteins (1% ninhydrin solution in acetone)

About 2 mL of filtrate were treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Test for carbohydrates (Molisch's test)

Few drops of Molisch's reagent were added to 2 mL portions of the various extracts and followed by addition of 2 mL of concentration H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of a red or dull violet at the interphase of the two layers was a positive test.

RESULTS AND DISCUSSION

D. bakoensis plant extracts dosages tested against *S. pneumoniae* results reported that methanol flower extract (MF) showed high zone inhibition. In the past studies, *Droseea rotundifolia* L. methanol extracts showed the best inhibition activity against *S. aureus* [21]. Results reported that ethanol flower extract (EF) showed a high zone inhibition against *S. aureus*. For *K. pneumoniae*, results showed that EF showed a high zone inhibition. Plant extracts with varying dosages tested against *A. niger*, results showed that MF showed a high zone inhibition approximately. The antimicrobial activity of different samples of *D. rotundifolia* L. ethanol extract against different species of bacteria is reported previously [22], comparing with that, the present study showed the best results among the *Drosera* genus plants.

The antibacterial activity at different doses of *D. bakoensis* extracts was tabulated in Tables 1-10. The dose-dependent antimicrobial activity of all the extracts was reported in Figs. 3-11, respectively.

The aqueous root extracts of *D. bakoensis* showed the same zone of inhibition till 1 mg/mL concentration against all micro organisms, but varied activity was observed from 3 to 5 mg/mL concentration with a maximum zone of inhibition against *K. pneumoniae* followed by *S. aureus*.

The aqueous hair extracts of *D. bakoensis* showed nearly same zone of inhibition against *S. aureus* and *K. pneumoniae* but from 3 to 5 mg/mL concentrations, maximum zone of inhibition was observed against *S. aureus* followed by *K. pneumoniae*.

The aqueous flower extracts of *D. bakoensis* showed nearly same zone of inhibition against *S. aureus* and *S. pneumoniae;* maximum zone of inhibition was observed against *S. aureus* followed by *S. pneumoniae*.

The ethanol root extracts of *D. bakoensis* showed nearly same zone of inhibition against *S. aureus* and *S. pneumoniae;* 0.65-2.65 mg/mL concentration showed maximum zone of inhibition against *S. aureus* followed by *S. pneumoniae*.

The ethanol hair extracts of *D. bakoensis,* 0.75-2.65-mg/mL-concentration showed maximum zone of inhibition against *S. aureus* followed by *S. pneumoniae*.

The EFs of *D. bakoensis* showed maximum zone of inhibition against *S. aureus* followed by *S. pneumoniae* and *K. pneumoniae.*

The methanol root extracts of *D. bakoensis* showed nearly same zone of inhibition against *K. pneumoniae* and *S. pneumoniae;* 0.85-2.85-mg/mL concentrations showed maximum zone of inhibition against *K. aureus* followed by *S. pneumoniae*.

The methanol hair extracts of *D. bakoensis* showed nearly same zone of inhibition against *K. pneumoniae*, S. *pneumoniae,* and *S. aureus*.

The MFs of *D. bakoensis* showed nearly same zone of inhibition against *S. pneumoniae* and *S. aureus*.

Table 1: Antibacterial, antifungal activity *D. bakoensis* plant extracts controls

Standard antibiotic (30 µg/disc)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
Positive control				
Kanamycin	21.2	14.8	19.2	18
Penicillin	12.6	19.2	17.4	16.5
Tetracycline	13.8	16.7	14.6	15.5
Negative controls				
Aqueous	-	-	-	-
Ethanol	-	-	-	-
Methanol	-	-	-	-

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus nige*, -: No zone of inhibition

Table 2: Antibacterial, antifungal activity *D. bakoensis* plant AR at various concentrations

AR (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
1.0	8.3	9.6	8.8	7.5
1.5	10.4	10.8	11	8.6
3.0	11.5	12.1	12.4	10.2
4.5	12.1	13.5	14.2	11.7
5.0	14.2	15.2	16.6	13.2

D. bakoensis: Drosera spatulata var. *bakoensis, S. Pneumoniae: Staphylococcus Pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* AR: Aqueous root extract

Table 3: Antibacterial, antifungal activity of *D. bakoensis* plant AH at various concentrations

AH (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
1.0	7.8	8.2	8.4	6.2
1.5	9.4	10.3	10.5	8.2
3.0	11.5	12.5	12.1	10.1
4.5	13.6	13.7	14.4	12.3
5.0	15.8	16.5	15.7	13.7

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* AH: Aqueous hair extract

Table 4: Antibacterial, antifungal activity *D. bakoensis* plant AF at various concentrations

AF (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
1.0	11.2	12.4	9.8	7.5
1.5	13.7	14.2	11.7	9.2
3.0	15.8	16.6	13.8	11.7
4.5	17.6	18.3	15.9	13.6
5.0	19.8	20.3	17.6	15.9

D. bakoensis: Drosera spatulata var. *bakoensis, S. Pneumoniae: Staphylococcus Pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* AF: Aqueous flower extract

MIC was tabulated in Table 11. According to literature results, it is considered a strong activity when MIC values are between 0.05 and 0.50 mg/mL, moderate activity for MIC values between 0.6 and 1.50 mg/mL, and weak activity above 1.50 mg/mL (Aligiannis *et al*., 2001). The active extracts were tested in concentrations of correspondent to MIC, 1/2 MIC, 1/4 MIC, 1/8 MIC, and 1/16 MIC. The values of MIC obtained were between 0.3 and 0.9 mg/mL. The result confirmed plant extracts have strong activity. Ethanol, methanol based flower, hair, and root extracts showed strong MIC against *S. pneumoniae, S. aureus*, and *K. pneumoniae*. Only EFs showed strong MIC against *A. niger*.

Table 5: Antibacterial, antifungal activity *D. bakoensis* plant ER at various concentrations

ER (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.65	12.5	11.7	10.5	10.4
1.15	13.4	12.4	11.8	12.0
1.65	15.2	14.6	13.7	14.5
2.15	17.8	16.8	15.9	16.2
2.65	19.4	18.7	19.5	18.4

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* ER: Ethanol root extract

Table 6: Antibacterial, antifungal activity *D. bakoensis* plant EH at various concentrations

EH (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.65	11.8	12.2	10.4	9.8
1.15	13.2	14.6	12.4	11.5
1.65	15.8	16.8	14.8	13.2
2.15	17.5	18.2	16.7	15.6
2.65	19.7	20.1	18.2	17.4

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* EH: Ethanol hair extract

Table 7: Antibacterial, antifungal activity *D. bakoensis* plant EF at various concentrations

EF (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.65	12.4	14.2	10.5	10.7
1.15	14.8	16.7	12.7	11.9
1.65	16.8	18.9	15.4	13.5
2.15	18.6	21.4	18.9	15.4
2.65	21.1	24.2	22.2	18.7

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* EF: Ethanol flower extract

Table 8: Antibacterial, antifungal activity *D. bakoensis* plant MR at various concentrations

MR (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.85	10.4	11.2	12.1	9.6
1.35	13.2	12.7	14.4	11.3
1.85	15.9	14.2	16.1	13.2
2.35	17.9	16.4	18.0	15.8
2.85	19.2	18.6	20.1	17.2

D. bakoensis: Drosera spatulata var. *bakoensis, S. Pneumoniae: Staphylococcus Pneumoniae, S.aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* MR: Methanol root extract

MBC concentration of plant extracts against bacteria and fungi is tabulated in Table 12, showed that ethanol flower, hair, and root extracts

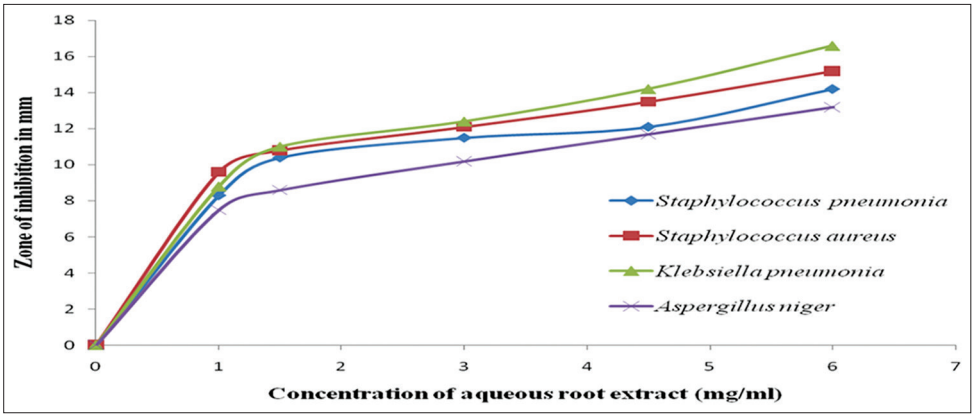

Fig. 3: Antibacterial, antifungal activity *D. bakoensis* plant aqueous root extract at various concentrations

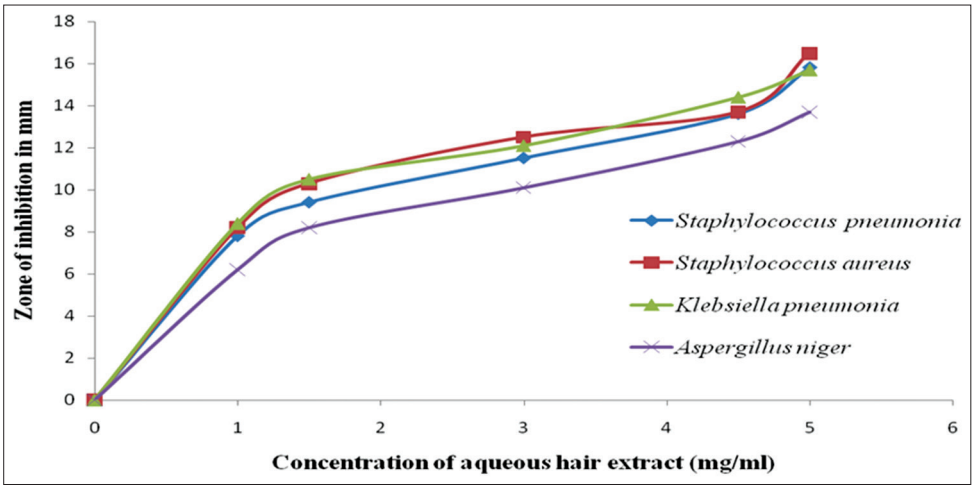

Fig. 4: Antibacterial, antifungal activity *D. bakoensis* plant aqueous hair extract at various concentrations

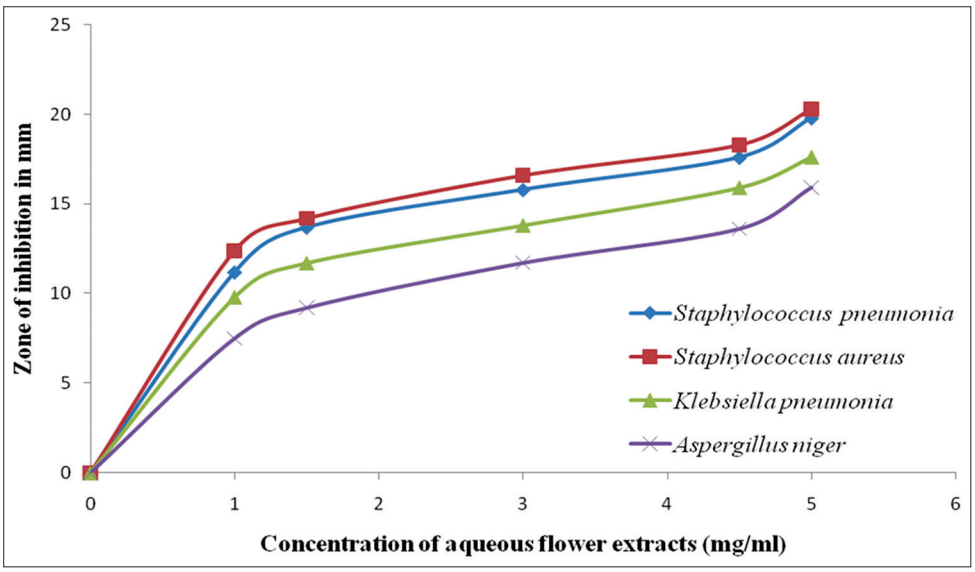

Fig. 5: Antibacterial, antifungal activity *D. bakoensis* plant aqueous flower extract at various concentrations

showed very MBCs all over the extracts against bacteria and fungi culture. Values of MBC obtained were between 0.36 and 2.25 mg/mL.

BIC concentration of plant extracts against bacteria and fungi is tabulated in Table 13; showed that strong BIC values reported in all ethanol based root, hair, and flower extracts followed by ethanol extracts. All BIC values found were lower than 1/4 MIC of the respective extract. The values of BIC obtained were between 0.12 and 0.37 mg/mL. When compared to the positive control, MIC, MBC, and BIC values obtained are still low. However, combinations of this hair, root, and flower extracts will give significant results compare antibiotics, and it has wide scope in the pulmonary infections. Ethanol based root, hair,

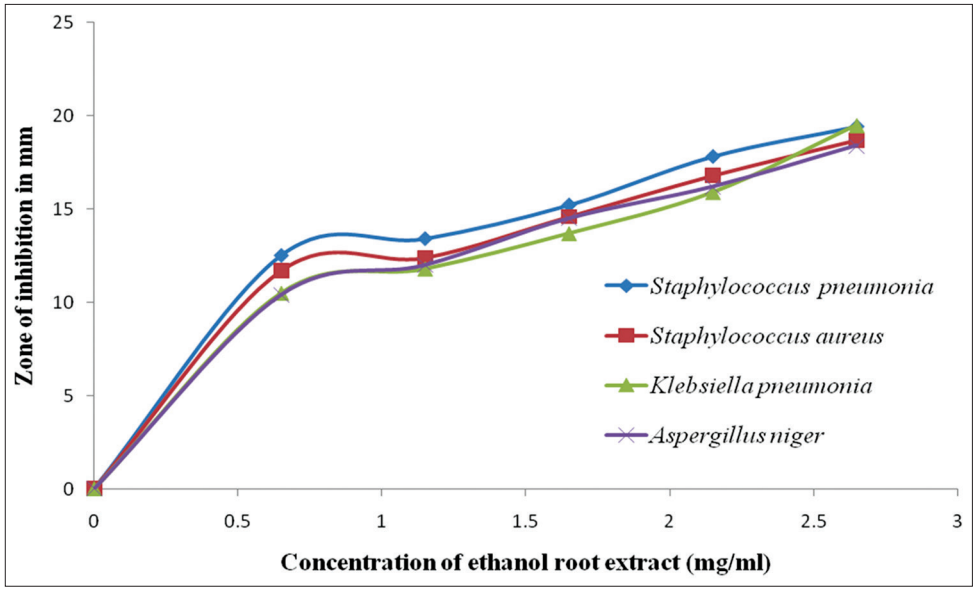

Fig. 6: Antibacterial, antifungal activity *D. bakoensis* plant ethanol root extract at various concentrations

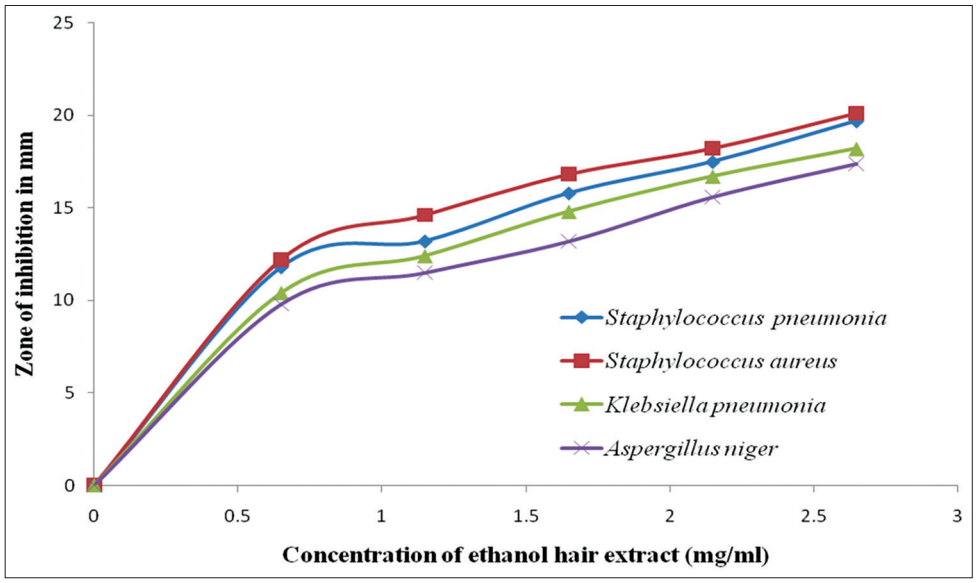

Fig. 7: Antibacterial, antifungal activity *D. bakoensis* plant ethanol hair extract at various concentrations

Table 9: Antibacterial, antifungal activity *D. bakoensis* plant MH at various concentrations

MH (mg/mL)	Zone of inhibition (in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.85	10.5	11.2	9.5	8.7
1.35	12.4	13.5	12.2	10.7
1.85	14.7	15.7	14.5	12.2
2.35	16.2	17.2	16.8	14.7
2.85	18.5	19.4	18.6	16.4

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* MH: Methanol hair extract

Table 10: Antibacterial, antifungal activity *D. bakoensis* plant MF at various concentrations

MF (mg/mL)	Zone of inhibition(in mm)			
	S. pneumoniae	*S. aureus*	*K. pneumoniae*	*A. niger*
0.85	13.8	13.2	11.4	11.2
1.35	15.2	15.8	13.6	13.4
1.85	17.4	17.4	15.7	15.8
2.35	19.7	19.3	17.9	17.9
2.85	22.4	22.2	19.5	19.6

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger.* MF: Methanol flower extract

and flower extracts showed high inhibition zones and lowest MIC, MBC, and BIC values against bacteria and fungi cultures.

Drosera species contain physiologically active compounds such as flavonoids and naphthoquinones [22]. Similarly, the extract of *Drosera peltata*, which contains naphthoquinones, plumbagin, exhibited antimicrobial activity against oral bacteria [23]. Early studies reveal that *Drosera* genus plants having napthaquinones and flavonoids [24] have been reported to possess antimicrobial and anti-inflammatory properties, which are efficacious in the treatment of oral infectious

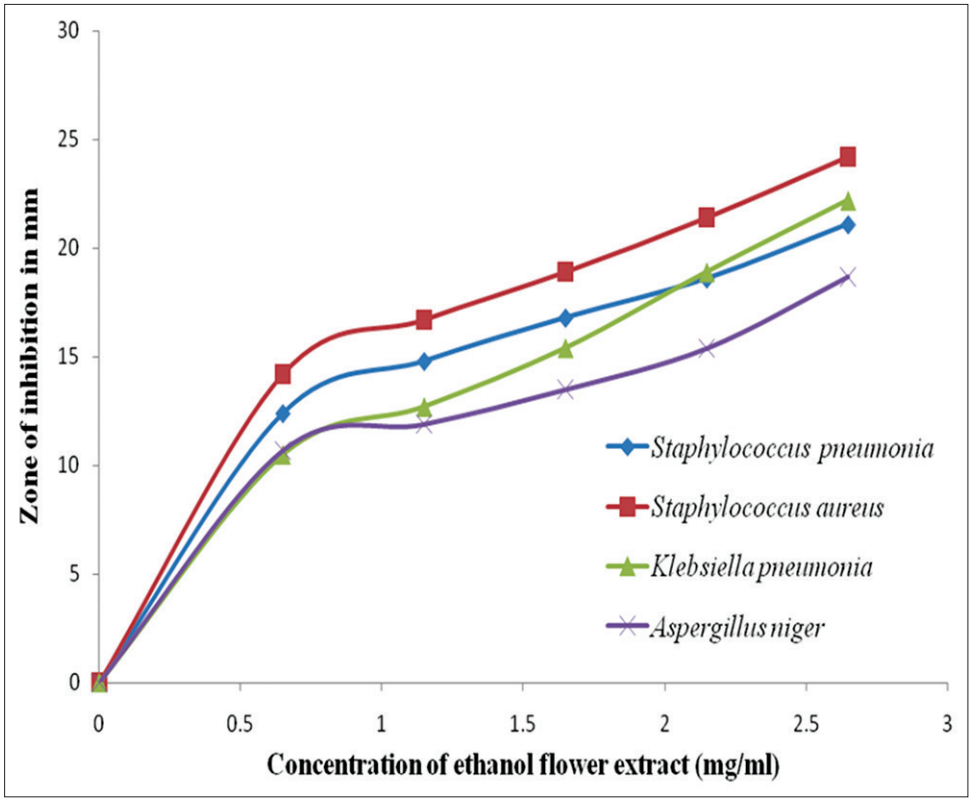

Fig. 8: Antibacterial, antifungal activity *D. bakoensis* plant ethanol flower extract at various concentrations

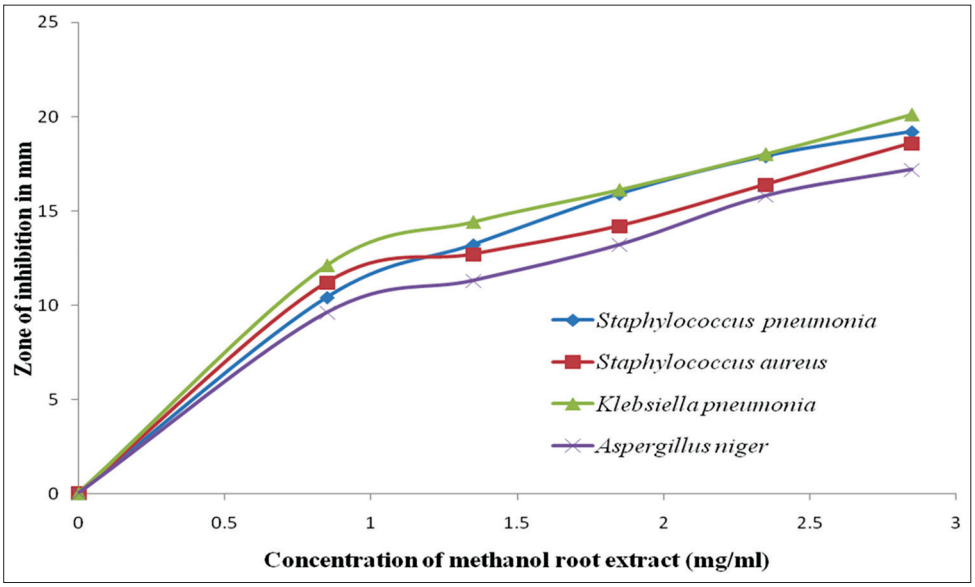

Fig. 9: Antibacterial, antifungal activity *D. bakoensis* plant methanol root extract at various concentrations

Table 11: MIC of *D. bakoensis* plant extracts against bacteria and fungi

Bacteria and fungi	MIC (mg/mL)									Positive control kanamycin
	AR	ER	MR	AH	EH	MH	AF	EF	MF	
S. pneumoniae	0.85	0.50	0.65	0.70	0.40	0.60	0.65	0.35	0.55	0.080
S. aureus	0.90	0.45	0.75	0.75	0.35	0.55	0.55	0.30	0.50	0.050
K. pneumoniae	0.80	0.55	0.70	0.65	0.45	0.50	0.60	0.45	0.50	0.085
A. niger	0.90	0.60	0.80	0.85	0.65	0.70	0.80	0.50	0.65	0.090

D. bakoensis: Drosera spatulata var. *bakoensis, S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger,* MIC: Minimum inhibitory concentration, AR: Aqueous root extract, ER: Ethanol root extract, MR: Methanol root extract, AH: Aqueous hair extract, EH: Ethanol hair extract, MH: Methanol hair extract, AF: Aqueous flower extract, EF: Ethanol flower extract, MF: Methanol flower extract

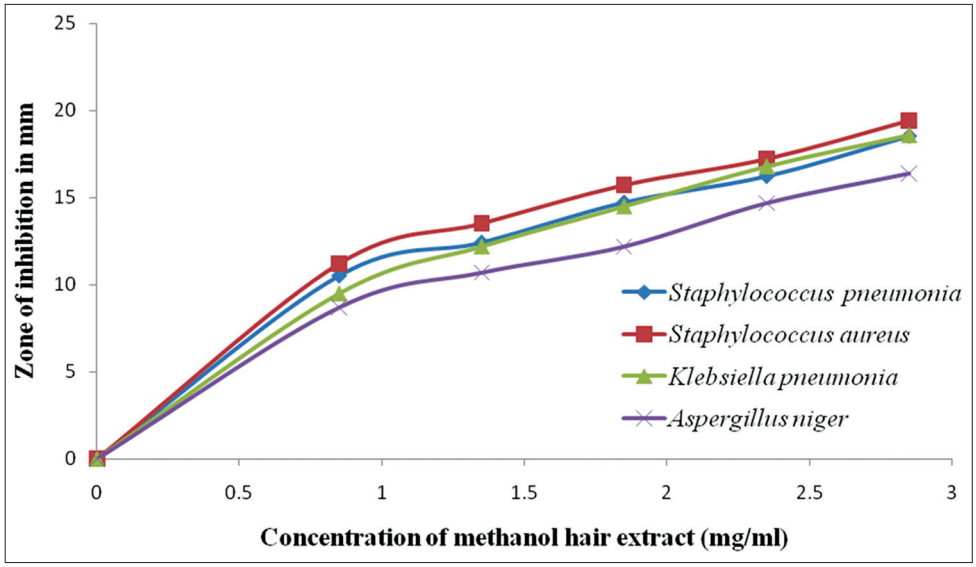

Fig. 10: Antibacterial, antifungal activity *D. bakoensis* plant methanol hair extract at various concentrations

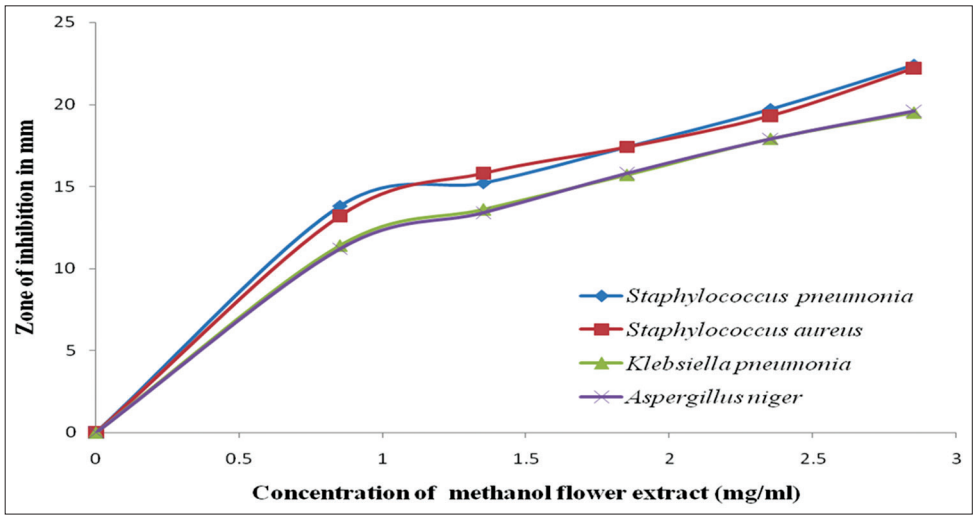

Fig. 11: Antibacterial, antifungal activity *D. bakoensis* plant methanol flower extract at various concentrations

Table 12: MBC concentration of *Drosera* plant extracts against bacteria and fungi

Bacteria and fungi	MBC (mg/mL)									Positive control kanamycin
	AR	ER	MR	AH	EH	MH	AF	EF	MF	
S. pneumoniae	1.70	0.75	1.07	1.40	0.60	1.02	1.30	0.52	0.93	0.24
S. aureus	1.35	0.54	0.90	1.12	0.42	0.77	1.10	0.36	0.70	0.10
K. pneumoniae	1.60	0.68	1.05	1.30	0.56	0.80	1.20	0.51	0.80	0.30
A. niger	2.25	0.99	1.40	2.12	0.91	1.22	2.00	0.70	1.13	0.36

S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger, MBC: Minimum bactericidal concentration, AR: Aqueous root extract, ER: Ethanol root extract, MR: Methanol root extract, AH: Aqueous hair extract, EH: Ethanol hair extract, MH: Methanol hair extract, AF: Aqueous flower extract, EF: Ethanol flower extract, MF: Methanol flower extract

diseases. Comparing with this previous data, *D. bakoensis* plant having high amounts of alkaloids, quinones, anthraquinones, and flavonoids in both methanol and ethanol extracts of root, hair, and flower. Comparatively root extracts of methanol, followed by ethanol extracts contain a high quantity of the above active principles. This is because of their solubility difference in the solvents. Phytochemical screening performed for the presence of 14 phytochemicals in all extracts of *D. bakoensis* showed in Table 14.

CONCLUSION

In the present study, the results showed that the presence of high amounts of alkaloids, flavonoids, quinones, anthraquinones, and terpenoids in *D. bakoensis.* In the antibacterial and antifungal activity, ethanol and methanol extracts significantly showed activity against the tested respiratory disease causing bacteria and antifungal properties to the zone of inhibition showed more than aqueous extracts at very

Table 13: BIC of plant extracts against bacteria and fungi

Bacteria and fungi	BIC (mg/mL)									Positive control kanamycin
	AR	ER	MR	AH	EH	MH	AF	EF	MF	
S. pneumoniae	0.25	0.16	0.22	0.22	0.13	0.20	0.16	0.12	0.14	0.12
S. aureus	0.37	0.22	0.30	0.25	0.17	0.19	0.18	0.14	0.16	0.04
K. pneumoniae	0.26	0.18	0.23	0.21	0.15	0.17	0.20	0.16	0.12	0.14
A. niger	0.28	0.21	0.24	0.26	0.16	0.23	0.21	0.18	0.16	0.18

S. pneumoniae: Staphylococcus pneumoniae, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, A. niger: Aspergillus niger, BIC: Biofilm inhibitory concentration, AR: Aqueous root extract, ER: Ethanol root extract, MR: Methanol root extract, AH: Aqueous hair extract, EH: Ethanol hair extract, MH: Methanol hair extract, AF: Aqueous flower extract, EF: Ethanol flower extract, MF: Methanol flower extract

Table 14: Result of phytochemical screening of roots, hairs, and flowers of *D. bakoensis*

Phytochemicals	*D. bakoensis* root extracts			*D. bakoensis* hair extracts			*D. bakoensis* flower extracts		
	AR	ER	MR	AH	EH	MH	AF	EF	MF
Alkaloids	++	++++	+++	++	+++	++	++	+++	+++
Flavonoids	++	+++	+++	++	++	++	+++	++++	++++
Phenols	+	++	++	+	+	+	+	++	++
Steroids	-	++	+	-	+	+	-	++	+
Tannins	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-
Cardic glycosides	-	-	-	-	-	-	-	-	-
Terpinoids	+	++	++	++	+++	+++	+	++	++
Sterols	-	+	+	-	-	+	-	+	+
Quninoes	++	++++	+++	+++	++++	++++	+++	++++	+++
Phlobatannins	-	-	-	-	-	-	-	-	-
Anthraquinones	++	++++	+++	+++	++++	++++	+++	++++	+++
Carbohydrates	+	+	+	-	-	-	++	++	++
Proteins and aminoacids	+	+	+	++	++	++	+++	+++	+++

AR: Aqueous root extract, ER: Ethanol root extract, MR: Methanol root extract, AH: Aqueous hair extract, EH: Ethanol hair extract, MH: Methanol hair extract, AF: Aqueous flower extract, EF: Ethanol flower extract, MF: Methanol flower extract. +: Less, ++: Moderate, +++: High, ++++: Very high, -: Absence of phytochemicals

low concentrations. It was correlated the reports of Schell (1984) [25] that medicinal use of *Drosera* sp. extracts since the 16th century as an important antitussive for different respiratory diseases including tuberculosis. Comparatively flower ethanol and methanol extracts showed a high antibacterial and antifungal activity against respiratory pathogens followed by root extracts and hair extracts. It has further confirmed that the use of the plant in folklore medicine in rural areas for treatment of asthma, whooping cough, and other respiratory related diseases. So, to combat these resistant microbes, more effective pharmaceutical preparations are required. Over the time *S. aureus, K. pneumoniae,* and *S. pneumoniae* is gaining resistance to the antibiotics. *S. pneumoniae is* one of the causative organisms of hospital-acquired pneumonia [26]. *S. pneumoniae* is mainly affecting the nasal pharynx, trachea, and lungs [27] occur in the lungs, where they cause necrosis, inflammation, and hemorrhage within the lung tissue. Hospital-acquired infections rely on the urinary tract, the lower respiratory tract, biliary tract, and surgical wounds to set up colonization [28]. Aspergillosis refers to the spectrum of disease caused by *A. niger* species. Chronic pulmonary aspergillosis is a slowly progressive destructive disease of the lung, leading to loss of respiratory function [29].

Further research in the present study may be highly useful and show the way to pharmaceutical preparations by these carnivorous plants to combat against respiratory-related infections such as whooping cough, bronchitis, asthma, and pulmonary diseases.

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