Physiochemical Screening and Antimicrobial Potential of *Otostegia limbata* Benth

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Summary: *Otostegia limbata* (Benth.) has been used in treatment of gums diseases, dental disorders, healing of wounds, hypertension, eye inflammation, and most importantly as anticancer. The present investigation deals with the physiochemical screening of crude extract of *O. limbata* and antimicrobial activities of its various fractions. The results showed the presence of alkaloids, catecholic tannins, phenols, sugars, flavonoids, terpenoids, and saponins. Antimicrobial activities of four fractions of *O. limbata* namely *n*-hexane, chloroform, ethyl acetate, and *n*-butanol were performed by using disc diffusion method against *Salmonella setubal*, *Pseudomonas pickettii*, *Staphlococcus aureus*, and *Micrococcus luteus* to evaluate its therapeutic value. All the fractions showed significant antibacterial activities but none of the fractions showed antifungal activity against *Aspergilus niger* and *Aspergilus flavus*.

Keywords: Antimicrobial activities, Disc diffusion, *Otostegia limbata*, Physiochemical screening.

Introduction

Otostegia limbata belongs to family Lamiaceae. A number of plants belonging to Lamiaceae have been screened for their potential medicinal uses which also exhibit antimicrobial activities [1]. Previously, the antibacterial activity of methanolic extracts of the same plant has been investigated against *St. aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [2]; however, in this present investigation various solvents were used to extract four fractions namely *n*-hexane, chloroform, ethyl acetate and *n*-butanol of the plant and all of these were tested against some selected human pathogens.

Family Lamiaceae comprising 220 genera and nearly 4000 species around the world is a unique family of angiosperms. *Otostegia* is one of the most important genera of this family comprising 20 species [3]. *O. persica* showed antibacterial activity against Gram-positive bacteria such as *St. aureus*, *St. epidermidis*, *Enterococcus faecilis* etc and antioxidant activity of its methanolic extract has also been reported [3, 4]. *O. limbata* and *O. aucheria* species are found in northern areas of Pakistan. *O. limbata* has been used in the treatment of gums diseases, dental disorders, wound healing, opthalmia, hypertension, eye inflammation, infections, as a tonic, and anticancer agent [5-7]. Antibacterial activity of methanolic and ethanolic extracts of *O. limbata* has already been reported. In present investigation antibacterial as well as antifungal activities of four fractions of *O. limbata* are reported and its physiochemical analysis is performed.

Results and Discussion

Results in terms of zone of inhibition of antibacterial as well as antifungal screening of different fractions of *O. limbata* are represented in Table-1 and 3, respectively, while MIC values of antibacterial activity are in Table-2. All fractions showed good antibacterial activities against Gram positive strains which is in agreement with study on methanolic and ethanolic extracts of *O. limbata* that also showed good antibacterial activity against Gram positive strains like *St. aureus*, *E. faecalis* and *St. epidermidis* with zone of inhibition value of 18 mm, 14 mm and 17 mm respectively as reported in the literature [5].

Table-1: Zone of inhibition (mm) values of different fractions of *O. limbata.*

Fractions	*Staphlococus aureus*	*Micrococcus luteus*	*Salmonella setubal*	*Pseudomonas pickettii*
OL *n*-hexane	10±0.87	09±0.75	11±1.40	9.5±0.75
OL chloroform	15±0.45	11±0.61	14±0.77	10±0.58
OL *n*-butanol	12±1.36	13±1.09	10±1.01	8±0.66
OL ethyl acetate	14±0.55	11±1.04	NR	9±0.75
Standard (Kanamycin)	20	18	21	20

OL: *O. limbata*.
Each value is taken for three experiments and mean (±) standard deviation is given

Antibacterial activity of four fractions of *O. limbata* against Gram-positive as well as Gram-negative bacterial strains is represented (Fig. 1), where *O. limbata* (OL) chloroform fraction gave highest activity against *St. aureus* with 15 mm zone

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of inhibition and OL ethyl acetate fraction did not show antibacterial activity against *S. setubal*. Moderate antibacterial activity of OL *n*-hexane was obtained against all four bacterial strains with the zone of inhibition in the range of 9-11 mm. OL chloroform fraction gave higher antibacterial activity against *St. aureus* while showed lower antibacterial activity against *M. luteus* and *P. pickettii* with zone of inhibition of 10 mm and 11 mm, respectively. OL *n*-butanol fraction gave low to moderate activity against all four bacterial strains. OL ethyl acetate fraction gave good antibacterial activity against *St. aureus* with zone of inhibition 13mm and resistant against *S. setubal*.

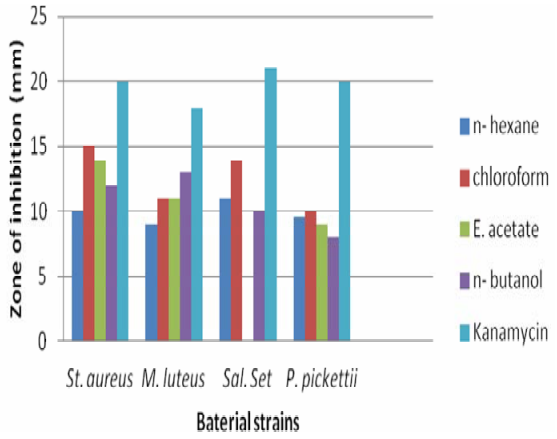

Fig. 1: Antibacterial activity of different fractions of *O. limbata.*

Lowest concentration of sample at which the bacterial growth is inhibited is taken as its MIC value. Lower the MIC value higher will be the antibacterial activity of that fraction. So in the present investigation OL chloroform fraction showed good antibacterial activity with MIC value of 0.18 mg/ml for *St. aureus*, *M. luteus* and *S. setubal* (Table-2). Similarly *n*-butanol fraction also showed good antibacterial activity with MIC value of 0.18 mg/ml for *St. aureus*, *S. setubal* and *P. pickettii*. Results indicated that all fractions of *O. limbata* showed good antibacterial activity against Gram-positive bacterial strains instead of Gram-negative strains (Fig. 2).

Table-2: MIC (mg/ml) values of different extracts of *O. limbata.*

Fractions	*Staphlococus aureus*	*Micrococcus luteus*	*Salmonella setubal*	*Pseudomonas pickettii*
OL *n*-hexane	0.75	1.5	3	0.37
OL chloroform	0.18	0.18	0.18	1.5
OL *n*- butanol	0.18	1.5	0.18	0.18
OL ethyl acetate	0.75	0.18	NR	0.18

OL: *O. limbata*, NR: No Result

In this investigation antifungal activity of four fractions of *O. limbata* were checked against two fungal strains namely *A. niger* and *A. flavus*. None of the fractions showed any antifungal activity against these two fungal strains (Table-3).

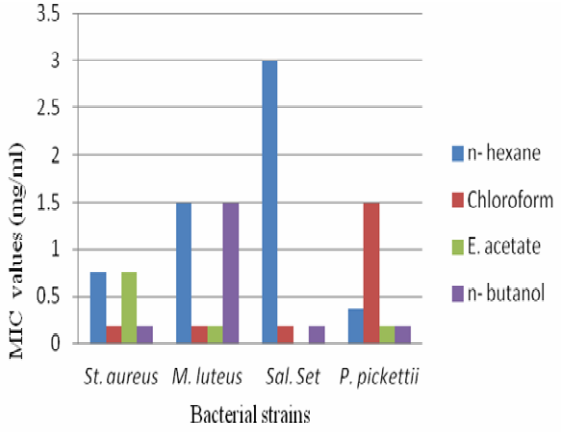

Fig. 2: MIC values of antibacterial activity of *O. limbata.*

Table-3: Zone of inhibition (mm) of different fractions of *O. limbata.*

Fractions	*Aspergilus niger*	*Aspergilus flavus*
OL *n*-hexane	NR	NR
OL chloroform	NR	NR
OL *n*- butanol	NR	NR
OL ethyl acetate	NR	NR
Standard (Nystatin)	21	24

NR: No Result

Results of Physiochemical Analysis

Physiochemical analysis of *O. limbata* showed the presence of alkaloids, catecholic tannins, phenols, sugars, flavonoids, terpenoids and saponins while gallic tannins and phlobatannins were absent (Table-4). These chemical constituents are responsible for anti-oxidant, anti-inflammatory, anti-microbial, hemolytic and analgesic activities of plants [8-10]. On the basis of results of physiochemical analysis, it is suggested that *O. limbata* has huge therapeutic value and further work on the isolation and purification of these bioactive constituents can be performed.

Table-4: Physiochemical analysis of *O. limbata.*

Phytochemicals	*O. limbata Response*
Alkaloids	+
Catecholic tannins	+
Gallic tannins	-
Phylobatannins	-
Phenols	+
Sugars	+
Flavonoids	+
Terpenoids	+
Saponins	+

Experimental

O. limbata was collected from different areas of Hazara division mostly Galiyat region. Plant was shade dried, ground into fine powder and soaked in methanol for fifteen days. Filtrate obtained after filtration was subjected to vacuum rotary evaporator to get crude extract and the process was repeated thrice. Crude extract was then converted into four fractions from non-polar to highly polar as *n*-hexane, chloroform, ethyl acetate, *n*-butanol fractions. All four fractions of *O. limbata* were then subjected to antimicrobial activities.

Antimicrobial Activity Test

For antibacterial activity four bacterial strains were used namely *Salmonella setubal* (*S.set*) and *Pseudomonas pickettii* (*P.picketti*) (Gram-negative) and *Staphlococus aureus* (*St.aur)* and *Micrococus luteus* (*M.leu*) (Gram-positive). Antifungal activity was studied against *Aspergilus niger* and *Aspergilus flavus*.

Media

Mueller Hinton agar (Merck, Germany) was used for antibacterial activities and Sabouraud Dextrose agar (SDA, Merck, Germany) medium was used for antifungal activities. Dilutions of test samples were carried out in Dimethyl sulfoxide (DMSO).

Agar Disc Diffusion Method

Agar disc diffusion method [11-13] was used for antibacterial screening of fractions of *O. limbata*. Agar solution was made and sterilized in autoclave at 120°C. A 15ml of agar medium was poured onto the surface of 100 mm size sterilized petri dishes and allowed to settle. Fractions were dissolved as 3mg/ml of DMSO and incorporated on filter paper discs of 6mm size with the help of micropipette and allowed to dry in laminar flow. The standard antibiotic used was Kanamycin. Blank discs were made by dipping disc in DMSO solvent. The test discs, standard as well as blank discs were placed on the surface of petri dish equidistantly. The plates were incubated for 24 h at 37 °C and zone of inhibition (ZOI) was measured using transparent ruler and process was repeated thrice. For minimum inhibitory concentration (MIC) values, serial dilutions of fractions were made as 1.5 mg/ml, 0.75 mg/ml, 0.37 mg/ml, and 0.18 mg/ml.

MIC values were taken as the lowest concentration of fractions at which growth inhibition of bacterial strains was observed after incubation for 24 h at 37 ºC [14]. Same procedure was followed for antifungal activity with incubation time of 72 h where standard used for antifungal activity was Nystatin.

Physio-Chemical Analysis

For qualitative analysis of *O. limbata* different physiochemical tests were performed.

Test for Alkaloids

0.2 g of plant extract was warmed with 2 % sulfuric acid solution in a test tube and filtered into another test tube. Then few drops of Dragendorff's reagent was added to filtrate resulted in Orange red precipitate indicating presence of alkaloids [15].

Test for Terpenoids

2 ml of chloroform was added to plant extract (0.5 g) in a test tube. Then concentrated sulfuric acid was added to this mixture that resulted in reddish brown interface confirming the presence of terpenoids [15].

Test for Steroids

0.5 g of plant extract was taken in a test tube into which 2 ml each of sulfuric acid and acetic anhydride were mixed. As a result, change in color from violet to blue was observed, indicating presence of steroids [16].

Test for Flavonoids

1-2 ml of aqueous filtrate of plant extract was taken in a test tube and 5 ml of ammonia solution was added. Then small amount of conc. sulfuric acid was added that resulted in yellow coloration confirming flavonoids [16].

Test for Saponins

2 ml of plant extract solution and 2 ml of distal water was taken in a test tube. The mixture was well shaken, as a result; froth appeared indicating the presence of saponins [17].

Test for Phenols

Few drops of alcohol were added in 2 ml solution of plant extract in a test tube. Then 3-4 drops of ferric chloride were added and result indicated the presence of phenolic compounds [18].

Test for Tannins

1 mg of plant extract was dissolved in 1.5 ml of distilled water then 3 drops of ferric chloride solution were added. Blackish green color appeared indicating presence of catecholic tannins [18, 19]. Tests for gallic tannins and phlobatannins were negative.

Conclusion

Physiochemical analysis and antimicrobial activities in the present investigation suggested *O. limbata* to be highly important therapeutically and that it can be used in drug industry. Results of antibacterial activities of all four fractions were higher in present investigation than antibacterial activities of crude methanolic extracts reported in literature. As zone of inhibition (ZOI) values for antibacterial activities obtained was up to 15 mm, further research on bioactive constituents responsible for its antimicrobial activities is required to find the full spectrum of its antimicrobial constituents.

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