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Biological Efficacy of the Extracts and Pure Compound of *Gentiana olivieri*

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Abstract
Biological activity of the extracts of *Gentiana olivieri* and its pure compound Gentianine was evaluated by performing antibacterial and antifungal assays. The antibacterial screening observed by Agar well diffusion method revealed the activity of all the fractions against four of the ten organisms employed. These fractions were also found to be significantly active against seven of the ten fungi tested.

Introduction
The mountains of Balochistan are inhabited by a large variety of wild herbs and shrubs. The plants of genus *Gentiana* have been the subject of interest from medical point of view. It is being used from centuries in traditional folk medicine for the treatment of skin diseases, ulcer and abscesses. It is also used for arthritis, blood pressure and fever supposed to be caused by liver and other diseases (Perry, 1980). In Japan G. *septemfid* are used as stomachic or stimulants of appetite. The literature survey reveals that so far no significant scientific research has been carried out on the pharmacological and biological properties of *Gentiana olivieri.* Griseb. In our previous publication reduction in blood pressure has been reported (Mansoor, 1998). The present work deals with the antibacterial and antifungal activity of the pure alkaloid and extracts of *Gentiana olivieri.*

Experimental Material: The fresh plant material of *Gentiana olivieri* was collected from Chiltan heights near Quetta, in April 1992. After drying and grinding, crude ethanolic extract was prepared and its alkaloids were purified by the method described by Mansoor (1996). The aqueous fraction was prepared by extracting the grinded plant with water.

Antibacterial Assay: The antibacterial activity of the pure compound and the extracts of *G. olivieri* were determined by agar well diffusion method in a replicate of three. Identified pure isolates of gram positive and gram negative bacteria were collected from the pathological laboratory of Civil Hospital Research Cell, Quetta. A twenty four hour old culture of the bacteria containing 10^4 and 10^6 cells was inoculated and evenly distributed on Mueller Hinton agar plates. Wells were dug in the medium with the help of a sterile 5 mm borer. 100 µl of 100 and 200 mcg of the fractions in methanol were added to their respective wells. Control wells receive 100 mcg of tobramycin and ampicillin. The plates were incubated for 48 hrs and the diameter of the zones of inhibition were measured in mm. Results were expressed in mean values.

Antifungal Assay: Sabroud Dextrose Agar (SDA) was poured in sterile culture tubes when the temperature of the assay medium was 45°C. One ml of the pure compound or crude extract of the required concentration of 400 µg/ml was added to each tube. Control tube received only 1 ml of dimethyl sulfoxide (DMS0) instead of the test substance. The tubes were then allowed to solidify in a slanted position at room temperature. The above mentioned seven days old fungal cultures were inoculated on the solid medium with the help of a sterile fungal needle. The slants were incubated at 30°C for 7-10 days, after which the samples were observed and measured in mm for growth inhibition. This inhibition was compared with the standard tube.

Results and discussion
Antibacterial Activity: Table 1 indicates the activity of gentianine, ethanolic extract and aqueous extract of *Gentiana olivieri* against ten bacterial organisms employed. No activity was exhibited by any fraction of the concentrations of 100 µg/100 µl except for the ethanolic extract of *G. olivieri* which was active against *Pseudornonas aeruginosa* and inhibited their growth to 5.5 mm (mean). Of the three fractions tested at the concentration of 200 µg/100 µl, all the three fractions were found to exhibit antibacterial activity against four of the ten organisms employed.

The most susceptible organisms appear to be *P. aeruginosa* and *V. cholera* as compared to the standard antibiotic ampicillin and tobramycin. These fractions were also significantly active against *C. boydii* and *E. coli* but were completely inactive against other six bacterial species employed.

The most amazing observations were the almost similar results observed in the pure and other fractions used. This might be due to the large amount of Gentianine present in the plant or it may either be due to the greater activity of Gentianine present in the extract as compared to the other compounds.

The fractions were quite active against four of the five investigated gram negative bacteria. No activity was observed against the gram positive bacteria. *G. oliveri* is known for centuries for it's therapeutic importance (Perry, 1980). Since no laboratory research work was ever done on it's pharmacological actions, therefore its exact effect on the bacteria was unknown. These antibacterial properties provide sufficient justification for the possible use of gentianine and their extracts for the developments of drugs to treat infections and reduce human sufferings due to toxicity of synthetic drugs. Its antibacterial effect on *V. cholera, E. coli* and *S. boydii* proves that it could be used

Table 1: Antibacterial activity of gentianine and its crude extract

Compound tested 200 μg 100 μl	Gram negative					Gram positive				
	1	2	3	4	5	6	7	8	9	10
Gentianine	-	7	6	7	7	-	-	-	-	-
Ethanolic extra	-	7	8	6	6	-	-	-	-	-
Aqueous Extract	-	7	6	8	7	-	-	-	-	-
Ampicillin 100 μg	12	10	9	12	9	8	8	9	9	10
Tobramycin 100 μg	11	11	8	11	10	7	7	10	10	12

Zone of inhibition in mm; Each value is a mean of three; No activity indicated by-Ampicillin, Tobramycin: Standard Antibiotics. 1 = *Escherechia coli;* 2 = *Pseudomonas aeruginosa;* 3 = *Pseudomonas microbius;* 4 = *Shigella boydii; 5 = Vibrio cholera;* 6 = *Corynebacterium diptheriae; 7 = Corynebacterium hoffmani; 8 = Enterobacter cloacea; 9 = Streptococcus Faecalis* 10 = *Staphylococcus aureus*

Table 2: Antifungal activity of gentianine and its crude

Ethanolic Extract fungi tested	Activity with gentianine	Activity with crude extract
Allescheria boydii	18 ± 1	20 ± 1
Aspergillus niger	No activity	No activity
Candida albicans	No activity	No activity
Curvularia lunata	14 ± 0	16 ± 0
Drechslera rostrata	18 ± 2	18 ± 1
Epidermophyton floccosum	18 ± 1	20 ± 1
Fusarium moniliforms	16 ± 0	16 ± 1
Microsporum canis	16 ± 1	16 ± 1
Nigrospora oryzae	22 ± 0	22 ± 1
Pleuretus ostreatus	No activity	No activity

Zone of inhibition in mm ± SEM*; Each value is a mean of three. *Standard error of mean

as an antidiarrheal drug. *V. cholera* cause virulent infection of the intestinal tract, while *S. boyclii* causes bacillary dysentery (Thomas, 1988). *E. coli* is an important cause of gastroenteritis in children (Robins-Browne, 1987). In literature the use of Gentiana species has been described as stomachic in folk medicine (Perry, 1980). Therefore, its mode of action should further be studied.

Anti Fungal Activity: Table 2 enlists the effect of gentianine and the crude extracts of *G. olivieri* on ten different fungal species. Amongst the ten fungi tested, the samples of gentianine and crude ethanolic extract exhibited antifungal activities against seven fungi employed. These activities indicate that the fungi are more vulnerable to the attack of gentianine and its crude extract than bacteria. The most susceptible organism found to be was *Nigrospora oryzae,* a plant infecting fungus. Besides this good inhibition was also recorded with *Allescheria boydii, Drechslera rostrata* and *Epidermophyton floccosum.* The effect of *G. olivieri* was purely of an antifungal nature, since the normally acting antifungi do not inhibit the growth of *Epidermophyton floccosum* (Shameel and Aftab, 1993). Similar observations were made in our antifungal experiment. However, the crude extract showed greater inhibition as compared to its pure-alkaloid. This might be due to the fact that crude extracts also contain large amount of fatty acids and oils. Essential fatty acids are well known for their marked anti-fungal activities.

This important property of *G. oliveri* could be incorporated into medicaments as a source of fungicide for human beings as well as plants. These antifungal and anti bacterial properties might be the reason for its use in folk medicine and skin disease. Traditionally, its use to poultice for boils and carbuncles have been in use for centuries but until di present studies was not proven scientifically and experimentally.

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