Antimicrobial and toxicological studies of *Ochthochloa compressa* plant

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Abstract: The present study demonstrates the biological activity of *Ochthochloa compressa*, since extensive literature survey has shown no documented biological activity of this plant. Ethanolic extract of whole plant was prepared and evaporated under reduced pressure by rota vapor. The crude extract was further fractionated into n- hexane, dichloromethane, ethyl acetate and n-butanol soluble fractions. These fractions were screened for antifungal, phytotoxic, cytotoxic and antibacterial activities. Dichloromethane soluble fraction showed significant phytotoxicity whereas n-hexane, ethyl acetate, and n-butanol soluble fractions showed non-significant phytotoxicity. Similarly, ethyl acetate soluble fraction was the only fraction, which showed significant cytotoxic activity. There was no antibacterial but moderate antifungal activity was shown by these fractions against selected strains of bacteria and fungi. This is the first report on the biological activity of *O. compressa*.

Keywords: *Ochthochloa compressa*, phytotoxicity, biological activity.

INTRODUCTION

The medicinal plants used as medicine were considered to be important due to a large number of factors such as unavailability, expensiveness and unwanted effects of the western medicines (Okafor *et al.,* 1999). *O. compressa* belongs to the flowering plants family, Poaceae.It is classified within the Order of Poales (Michelangeli *et al.*, 2003) It is used as the source of food both for domestic as well as wild life (Mensah, 1990; Lowe, 1989). It is distributed in Pakistan, Northwest India and from Arabia to Northeast Africa (Nasir *et al.*, 1982). The plant seeds and fruits are used as stomachic in camels (Sher and Aldosari, 2013). The *O. compressa* was searched for biological and phytochemical studies as no literatures on these studies have been found and the plant is completely unexplored.

MATERIALS AND METHODS

Plant material

O. compressa was collected from Baghdad Campus (District Bahawalpur) Pakistan. The plant was identified by Dr. Muhammad Arshad (Late), Cholistan Institute of Desert Studies (CIDS) The Islamia University of Bahawalpur, where a voucher specimen number 2520/CIDS/IUB was deposited.

Extraction

A total of 20kg plant material was obtained and was shade dried in the month of April 2010. Dried plant material was grounded and extracted successively in an Ethanol solvent for 20 days. Filtrate was concentrated under reduced pressure by Rotavapor-R20 at 35^{o}C and was finally obtained as solid. The concentrated ethanolic crude extract obtained from above process was subjected to the extraction with n-Hexane, Dichloromethane, Ethyl acetate and n-Butanol (twice with each solvent) respectively. Filtrates so obtained were concentrated under reduced pressure by Rotavapor-R20 at 35^{o}C. The filtrate was subjected towards water (Distilled) and a fraction was obtained having water-soluble compounds. The entire chemicals used in the above procedure were purchased from Merck Group Frankfurter Strabe 25064293 Darmstadt Germany.

Antibacterial activity

Six species of pathogenic bacteria were screened against the plant crude extract and its various soluble fractions of *O. compressa*. The antimicrobial activity of the *O. compressa* is carried out by using pathogenic strains consist of gram-negative bacterial species such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexeneri* and *Salmonella typhi* and gram-positive bacterial species such as *Bacillus subtilis* and *Staphylococcus aureus*. Mueller Hinton Agar (MHA) was used to maintain microbial cultures at 4^{o}C having a subculture period of fifteen (15) days. Stock solution of microbes containing a count of 108-109 colony creating units c.f. u/ml was established with a help of surface viable counting technique (Miles and Misra, 1938). Every time, preparation of a 2hour old microbial stock suspension was carried out and the conditions of experiment such as aeration and temperature were developed constantly until the antimicrobial evaluation was done. The Diffusion method of the Agar Well was employed for the approximation of antimicrobial actions (Okeke *et al.*, 2001). Melted Mueller-Hinton agar media was completely mixed with 100 micro liters of standardized stock suspension of microbes (10^{8}) and discharged this mixture into the Petri plates (sterile). Three 8 mm wells were made in each Petri plate by means of a sterile cork borer

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no. 4. After that woof the wells were filled with100 µl of each of the sample comprising of 250µg of ethanolic extract of *O. comprressa*, respectively. As control, the third well was filled with 100µl of a broad spectrum antibiotic comprising of 5µg of amikacin. Then the process of following bacterial growth was allowed at 37°C overnight. The percentage of bacterial inhibition was estimated by formula given below:

Percentage Inhibition = 100 (X – Y) / X

In which,

X = absorbance in control with bacterial culture

Y = absorbance in test sample

The result was mean (±SEM) of triplicate experiment. All experiments were done under strict aseptic conditions.

Antifungal assay

In-vitro anti-fungal bioassay of the crude extract, n-hexane, DCM, Ethyl acetate and n-butanol soluble fractions were performed by Agar Tube Dilution method (Atta-ur-Rehman *et al*., 2001). Crud extract and its various soluble fractions along with reference standards Amphotericin B and miconazole were evaluated against clinical specimens of *Fusariumsolani, Trichophyton longifusus, Aspergillus flavus, Candida glabrata, Candida albicans*, and *Microsporumcanis.* Clinical specimens were obtained from Hussain Ebrahim Jamal Research Institute of Chemistry Karachi, Pakistan. A control experiment was performed for the indication of fungal growth with test substance (medium supplemented with appropriate amount of DMSO) and for reference standards miconazole and Amphotericin Bwere used. The extracts (24 mg) dissolved in sterile DMSO (1.0 ml), functioned as stock solution. Sabouraud Dextrose Agar (SDA) (4 ml) was dispensed into screw cap tubes, which were subjected to autoclaved at 121°C for 15 minutes and then cooled to 50°C. Stock solutions (66.6 µl) of each extract and its various fractions were mixed with the non-solidified SDA media producing the concentration of extract/ml of SDA up to400µg. All the tubes were inoculated with a slice (a diameter of 4mm) of inoculum detached from seven day old culture of fungi. An agar surface streak technique was used non-mycelial growth. The observation was done after seventh day of incubation at 28±1°C.

Phytotoxicity assay

Lemna minor L. (*Lemnaceae*) is a miniature aquatic *Thaloid monocot. Lemna* assay is a quick measure of phytotoxicity of the material under investigation. An inorganic medium (E. Medium) of pH 5.5-6.0 was prepared. Containers to be tested; 10 vials per dose (500, 50, 5 ppm) and for control were prepared in following steps as:15 mg of extract and its various soluble fractions was weighed and dissolved in 15ml solvent, then 1000, 100, and 10 µl solutions were added to the solvent was vaporized overnight followed by addition of 2ml of E. Medium and then a rosette containing single plant. Vials were positioned in a glass dish occupied with 2 cm of H_2O, and vessel was closed with stopcock grease and glass plate. Dish containing vials was positioned for seven days at 26°C under fluorescent and incandescent light in growth chamber. Number of fronds in each vial were calculated and documented on day 3 and day 7.Growth inhibition was calculated in percentage with reference to negative control with the help of the following

Formula:

$$\text{Growth regulation (\%)} = 100 \frac{\text{Number of fronds in test flasks}}{\text{Number of fronds in negative control}} \times 100$$

Data was analyzed as percent of control with ED_{50} computer program to define FI_{50} values and 95% confidence interval (Atta-ur-*Rehman et al.,* 2001).

Cytotoxicity

Brine shrimp eggs were incubated for 24 hours in a petri dish having artificial seawater. Larvae that are hatched were transferred to a second petri dish, incubated for another 24 hours for molting to second in star. This process is necessary for attaining a homogenous population of second in star larvae. Ethanolic extract and its various soluble fractions having a maximum concentration of 50µg/ml were solubilized in DMSO. Then Larvae were calculated into clusters of 10. After that positioned artificial seawater in 1ml of to which the sample to be investigated in DMSO had been added. Survival was assessed at 25°C for 24 hours after incubation process by noticing with the help of dissection microscope (Atta-ur-Rehman *et al*., 2001).

STATISTICAL ANALYSIS

To evaluate differences between the studied groups, one way analysis of variance (ANOVA) with Least significant difference (LSD) post hoc test was used to compare the group means and $P<0.05$ was considered statistically significant. Statistical Package for Social Sciences (SPSS), version 18.0 was used for statistical analysis

RESULTS

Dried then powdered whole plant was extracted at room temperature with solvents likeethanol, n-hexane, DCM, Ethyl acetate and n-butanol. Ethanolicextract and its various soluble fractions were investigated for; Anti-bacterial bioassay against *Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Salmonella typhi* and *Shigella flexeneri*; Anti-fungal bioassay against *Microsporumcanis, Candida albicans, Fusariumsolani, Candida glabrata, Trichophyton longifusus* and *Aspergillus flavus*,; Brine-Shrimp toxicity and Phytotoxicity against *Lemna minor L*. were performed. None of ethanolic extract and its various soluble fractions showed antibacterial activity against the selective clinical specimens. However, ethyl acetate

soluble fraction of *O. compressa* revealed significant phytotoxic and cytotoxic activities at highest concentration of *O. compressa.* Results are shown in tables 1-3.

DISCUSSIONS

Antibacterial activity

None of the crude ethanolic extract and its soluble fractions displayed antibacterial activity against selective strains of**,** *Shigella flexenari, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa.* Our results suggest that there are no such chemicals that can affect the growth of selected bacterial species.

Antifungal activity

In antifungal assay, growth in the media comprising of crude extract and its various soluble fractions was determined by calculating the growth inhibition (%) and linear growth (mm) by comparing with negative control as reference. The antifungal activities results of crude extract and its soluble fractions are shown in the table (table 1). For standardization, Miconazole as positive control was used against all fungal species except for *A. falavus* where Amphotericin B was used as standard drug. None of the crude extracts and its soluble fractions showed antifungal activity against *Candida albicans* and *C. glabrata* species of fungus, which propose that in crude extracts of *O. compressa*, no such antifungal agents are present against both of these species. Crude ethanolic

Table 1: Antifungal activities results of different crude extracts of *O. compressa*

	Standard	MIC	% Inhibition by different fractions (mm)				
Fungus	Drug	µg/ml	Ethanolic crude extract	n-Hexane soluble fraction	Dichloro methane soluble fraction	Ethyl acetate soluble fraction	n-butanol soluble fraction
C. albicans	Miconazole	110.8	0	0	0	0	0
A. flavus	Amphotericin B	20.2	35	10	35	5*	10
M. canis	Miconazole	98.4	25	20	25	25	30
F. solani	Miconazole	73.25	20	15	20	15	15
C. glabrata	Miconazole	110.8	0	0	0	0	0

Table 2: Phytotoxicity results of *O. compressa* crude extracts

Name of fraction	% Growth inhibition at various concentrations		
	1000 µg/ml	100 µg/ml	10 µg/ml
Ethanol crude Extract	15	5	0
n-hexane soluble fraction	15	5	0
Dichloromethane soluble fraction	31**	21*	15*
Ethyl acetate soluble fraction	57*	10**	5**
n-butanol soluble fraction	15	5	0

Table 3: Results of Cytotoxicity by various crude extracts of *O. compressa*

Name of fraction	Dose	No. of survivors (30)	LD_{50} µg/ml	Upper Limit	Lower Limit	G value
	1000	17				
Ethanolic crude extract	100	22	1808	51886	437	0.46
	10	27				
	1000	17				
n-hexane soluble fraction	100	19	1146	45937	336	0.37
	10	28				
	1000	16				
Dichloromethane soluble fraction	100	22	1027	8915	393	0.26
	10	29				
	1000	15*				
Ethyl acetate soluble fraction	100	20	1175	3288	291	0.49
	10	26				
	1000	17				
n-butanol soluble fraction	100	22	1957	1969	388	0.59
	10	26				

extract of *O. compressa* showed 35%, 25% and 20% fungal growth inhibition against, *Fusarium solani*, *Microsporum canis* and *Aspergillus flavus* respectively. Minor to moderate antifungal activities were also demonstrated by n-hexane soluble fraction against species of *Fusarium solani, Microsporum canis* and *Aspergillus flavus* (table 1). Similar results were observed in case of Dichloromethane, n-butanol and Ethyl acetate soluble fractions of *O. compressa.* It is obvious from the antifungal activities of crude extract and its various soluble fractions that ethanolic crude extract and Dichloromethane soluble fraction were more potent against *Aspergillus flavus* whereas soluble fractions ofn-butanol, ethyl acetate and n-hexane showed activity against *Microsporum cani.* However, none of the crude extracts and its various soluble fractions exhibited significant fungal growth inhibition against either specie. *Microsporumcanis*, a zoophilic dermatophyte most commonly produces *tineacapitis* and *tineacorporis. Tinea corporis* in patients with advanced HIV infection can extend over large areas of the body (Giordani *et al.*, 2001). The standard drug used was Miconazole. Azole antifungals are generally the most effective agents but are among the most expensive. *Aspergillus flavus* is the common causative organism of all forms of aspergillosis. The major drug of proven value is intravenous Amphotericin B. From many years ago, fungal infection attack is common due to increase in immune compromised population for example organ transplant recipients, HIV/AIDS and cancer patients. This element can be linked to the emergence of resistance to antibiotics and toxicity produced due to prolong used of these drugs (Fostel *et al.,* 2000). This has been the reason for an extended newer drugs searchto treat opportunistic fungal infection (Balandrin *et al.,* 1985). Plants produce different compounds are of importance as a source of safer or more effective substitutes for synthetically produced antimicrobial agents (Gillani *et al.,* 1994).

Phytotoxic activity

The results of phytotoxic activities of crude extracts of *O. compressa* are shown in table (table 2). Ethanolic extract and n-hexane, Dichliromethane and n-butanol soluble fractions showed some phytotoxicity at various concentrations, but, results were non-significant. However, Ethyl acetate soluble fraction was the only fraction which showed significant phytotoxicity at higher concentration (1000 µg/ml).

Cytotoxic activity

The results of cytotoxic activities of ethanolic crude extract and its various soluble fractions of *O. compressa* are provided in the table (table 3). The ethanolic crude extract and n-hexane, DCM and n-butanol soluble fractions showed non-significant Cytotoxicity, whereas the Ethyl acetate soluble fraction showed significant Cytotoxicity at highest dose of 1000µg/ml. The percentage mortality increased with increasing *O. compressa* extract concentration. LD_{50} value ranges from 1027-1957 µg/ml. A variation in the concentrations narrates different variety and concentration of cytotoxic substances. The brine shrimp lethality assay (BSLA) has been used regularly in the primary selection of the crude extract and its various soluble fractions as well as isolated compounds to calculate the toxicity towards brine shrimplarva's, which might also be an indication of possible cytotoxic properties of the test materials. Brine shrimp larva's have previously been utilized in various bioassay systems like anesthetics, pesticide residues analysis, dinoflagelate toxins, mycotoxins, stream pollutants, morphine-like compounds, carcinogenicity of phorbol esters and toxicants in marine environment (Nonita *et al.,* 2010).

CONCLUSION

The readings suggest that crude ethanolic extract and its soluble fractions of *O. compressa* has revealed that plant has antifungal properties against *Fusarium solani, Microsporum canis* and *Aspergilus flavus*. Ethanolic crude extract and n-hexane, Dichloromethane and n-butanol soluble fractions showed phytotoxic and cytotoxic activities and Ethyl acetate soluble fraction shown significant Cytotoxic and Phytotoxic activities.

For future studies, there is need to isolate individual compounds by using HPLC and other advanced techniques. Obtained compounds should further be analyzed for their chemical structures and used to evaluate for their potency against fungus, cell and plant culture. Such results might lead us to find out some novel compounds from *O. compressa*.

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REFERENCES

Atta-ur-Rehman, Choudhary MI and Thomsen WJ (2001). Bioassay techniques for drug development, Taylor & Francis, Harwood, United States, 28-30.

Balandrin MF, Klock JA, Wurtele ES and Bollinger WH (1985). Natural plant chemicals: Sources of industrial and medicinal materials. *science*, **228**: 1154-1160.

Fostel J and Lartey P (2000). Emerging novel anti-fungal agent, *Drug Discov. Today*, **5**: 25.

Gilani AH, Janbaz KH, Lateef A and Zaman M (1994). Cu+ channel bloking activity of Artemisia scoparia extract. *Phytother. Res.,* **8**: 161-165.

Giordani R, Trebaux J, Masi M and Regli P (2001). Enhanced anti-fungal activity of ketoconazole by Euphorbia characias latex against *Candida albicans*. *J. Ethnopharmacol*., **78**: 1-5.

Lowe J (1989). The flora of Nigeria grasses, Ibadan University Press, Ibadan, Nigeria, 326.

Mensah JK (1990). Epidermal morphology in relation to the taxonomy of grasses. *J. Plant Anat. Morph.*, **1**: 1.

Michelangeli FA, Davis JI and DW (2003). Phylogenetic relationships among Poaceae and related families as inferred from morphology, inversions in the plastid genome and sequence data from the mitochondrial and plastid genomes. *Am. J. Bot*., **90**: 93.

Miles AA and Misra SS (1938). Estimation of bactericidal power of blood. *J. Hyg*., **38**: 732-748.

Nasir E and Ali SI (1982). Flora of Pakistan, F1, Pak. **143:** 81.

Nonita, Peteros and Mylene Uy (2010). Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. *J. Med. Plant Res*., **4**: 407-414.

Okafor JC (1999). Identification, Utilization and Conservation of Medicinal plant in Southeastern Nigeria. Issue 3 of issues in Africa Biodiversity, Biodiversity support programme. Michigan, Indian, p.1.

Okeke MI, Iroegbu CU, Eze EN, Okoli AS and Esimone CO (2001). Evaluation of extracts of the root of *Landol phiaowerrience* for antibacterial activity. *J. Ethnopharmacol*., **78**: 119-127.

SherH, and Aldosari A (2013). Ethnobotanical survey on plants of veterinary importance around Al-Riyadh (Saudi Arabia). *Afr. J. Pharm. Pharmacol.*, **7**: 1404-1410.