The Effect of Cadmium and Chromium Concentration, on Biological Activity of *Marsilea minuta*

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Summary: Antibacterial activity of the *Marsilea minuta* was investigated that was collected from four different places of the same stream. The activities were correlated with the chromium and cadmium ions contents of the stream. The concentration of cadmium and chromium was determined by using the atomic absorption spectroscopy. The metal contents were extracted from the plants and crude extract by using wet digestion process. The antibacterial activity was determined by using disc diffusion method. The change in biological activities with the change in concentrations of metal ions is discussed in this communication.

Introduction

Plants are important in terms of producing food and shelter, cleaning and refreshing air as well as in fixation of solar energy. Plants and herb are also important in term of their medicinal properties as well as fixation and detoxification of the pollutants and contaminates [1-6]. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated that only one percent of 2, 65,000 flowering plants on earth have been studied exhaustively for their chemical composition and potential against important medicinal value [1]. The mechanism of the fixation of toxic material is complicated and has many routs. In case of the toxic metal ions the process of chelation is important [7]. The chelation is facilitated by some of the peptides and complex chemicals [8, 9]. Aquatic plants have the potential to take and accumulate toxic metal ions [10]. These plants accommodate and detoxify the toxic ions through certain cellular changes [11]. It is believed that these changes are also responsible for the change in activity or composition and even concentration of the bioactive and medicinally important components of the plants. Using this theory *Marsilea minuta* was selected for this work. *Marsilea minuta* is an aquatic fern and belongs to the family *Marsilacae*. There are literature reports for the bioactivity of the plants of this family, *Marsilea quadrifolia* is reported for its cytotoxic, antibacterial and antioxidant activities [12]. The accumulation of metal ions by the *Marsilea minuta* is also reported [13].

Results and Discussion

The activity of the crude ethanolic extract of *Marsilea minuta* was investigated against six bacteria. The hypothesis for these investigations was that *Masilea minuta* produce bioactive materials, the concentration of which may change with changes in concentration of heavy metals and other pollutants. The results of the activity and the concentration of toxic metals are given in Tables-1 and 2. It can be observed that as the concentration of chromium and cadmium changes there is also change in activity. This might be due to two reasons, the concentration of these heavy metals may change the concentration of bioactive material which results changes in activity or the presence of these ions or there compounds make the bioactive material less available for the bioactivity. Therefore changes in activity are observed. It can be seen from the Table-1 that the antimicrobial activity from less polluted area is greater than those of the polluted area. Another reason is the deactivation of the bioactive materials which may be due to chelation or deactivation in the presence of other compounds. It can be seen from the results that ethanolic extracts has no chromium but contains small quantities of cadmium. Therefore both possibilities are there: The chelation of bioactive material with the cadmium and the deactivation of bioactive material by the compounds of chromium.

Table-1: Antimicrobial Activities of the ethanolic extracts of *Marsilea minuta* samples *cereus.*

Codes	*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus cereus*		*Klebssiella pneumoniae*		*Salmonella typhi*		*Staphylococcus aureus*	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
M-1-S	11.5	12.5	10.5	37.0	8.5	11.0	7.5	30.0	9.0	40.0	8.5	22.0
M-2-S	8.5	12.5	8.0	37.0	7.0	11.0	6.0	30.0	7.5	40.0	7.5	22.0
M-3-S	6.0	12.5	7.5	37.0	6.0	11.0	6.0	30.0	6.5	40.0	6.5	22.0
M-4-S	6.0	12.5	7.0	37.0	6.0	11.0	-	30.0	6.0	40.0	6.0	22.0

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Table-2: Chromium and cadmium contents of the *Marsilea Minuta* samples.

S. No.	Code no.	Ethanolic extract		Plant sample	
		Cd mg/mL	Cr mg/mL	Cd mg/mL	Cr mg/mL
1	M-1-S	0.024 ± 0.0067	0.000 ± 0.0063	0.053 ± 0.0163	0.228 ± 0.0173
2	M-2-S	0.022 ± 0.0043	0.000 ± 0.0063	0.059 ± 0.0164	1.434 ± 0.0153
3	M-3-S	0.023 ± 0.0055	0.003 ± 0.0063	0.090 ± 0.0093	1.464 ± 0.0145
4	M-4-S	0.023 ± 0.0062	0.000 ± 0.0063	0.090 ± 0.0125	1.464 ± 0.0136

Experimental

Material and Method

Plant Material

Marsilea minuta was collected from Badray stream coming from district Buner and passing through Swabi city of the Khyber Pakhtunkhwa province of Pakistan. The plant was collected in the month of February from four spots of the same stream. The samples were named as M-1-S, M-2-S, M-3-S and M-4-S. M-1-S is the sample obtained from a location about 6 Kilometer upstream from the city where there are no effluents from the residential area. M-2-S is the sample from a spot about one kilometer downstream in the city where the effluents directly come to the stream. Each of the M-3-S and M-4-S are samples from a location at a distance of about one kilometer from each other and are at the out skirts of the city.

Plant Material Extraction

The whole of the plant was collected from the all the four spots. These samples were processed separately. The extract was obtained by soaking 200 g of each sample in 1 liter of dry ethanol for one week. It was filtered and transferred to the flask of rotary evaporator for the evaporation of ethanol. The extract obtained was used for the determination of metal ions as well as biological activity.

Bioassay

The antibacterial assay of the ethanolic extract was carried out using disc diffusion method [14]. Solutions of known concentration (1 mg/6 µL) of the test samples were made by dissolving measured amount of the test samples in DMSO (dispersed sample on the disk). Dried and sterilized filter paper discs were then impregnated with known amounts of the test substances using micropipette. Discs containing the test materials were placed on agar medium. Standard antibiotic discs and blank impregnated with solvent used as the positive and negative control. We have used azithromycin for *Staphylococcus aureus* and ciprofloxacin as positive control for each of the *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebssiella pneumoniae* and *Salmonella typhi*. The blank, control and test discs were kept at low temperature for 24 h for maximum diffusion. Afterward the discs were incubated for 24 h at 37 °C for the growth of the organisms. A clear zone of inhibition was observed for the test material and control. The solution of extract was applied in 6 μL doses. The antibacterial activity was determined by measuring the diameter of the zone of inhibition in millimeter. All the experiments were carried out in triplicate and the results were the mean of the triplicates [14].

Extraction of the Metal Ions and Atomic Absorption Spectroscopy

Both the ethanolic extracts and plant were analyzed for the cadmium and chromium contents using atomic absorption spectroscopy. The metal ions were extracted from both type of samples by wet digestion. 5 g of each sample was weighed and digested with concentrated HNO_3, H_2SO_4 and H_2O_2 (2:6:6) [15]. On completion of the digestion, the flasks were brought to dryness. These were diluted with doubled distilled water and analyzed by Perkin Elmer atomic absorption spectrophotometer Analyst 700.

Conclusion

The chromium and cadmium contents of *Marsilea minuta* was found to vary with the pollution load. It was higher at polluted areas and less at the non polluted areas. The biological activity of the plants is a function of the concentration of the metal ions. It was found to decrease with increase in pollution. These findings are significant for correlating the metal ions concentration with the composition of the bioactive and other important compounds of the plants. It also gives direction for the biosynthesis of compounds in significant quantities by the inhibition of competing process or the acceleration of the process of interest by the addition of some chemicals.

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