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Phytochemical Analysis of Organic Compounds as an Indicator Metal Uptake in Selected Aquatic Plants

Muhammad Waris[1]*, Farah Maqsood[2]

[1]*Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan*
[2]*US-Pakistan Center for Advanced Studies in Water, Mehran University of Engineering and Technology, Jamshoro-Pakistan*

ABSTRACT

Plants contain numbers of potential chelating agents such as amino acids, organic acids, peptides and proteins etc. These chelating compounds are essential for the physiological processes in plants. Besides, these ligands are found in both Hyperaccumulator and non-hyperaccumulator Aquatic plants and play a vital role in metal uptake from contaminated soil. Many organic compounds have found to bind and accumulate trace and toxic metals in hyperaccumulating Aquatic plants. Three aquatic plants (Typhadomingensis, Phragmitesaustralis and Tamarixaphylla) selected for the phytochemical screening of alkaloids, amino acids, carbohydrates, diterpenes, flavonoids, phenols, proteins, quinones, steroids, terpenoids and tannins in the extract of water and petroleum ether. This research includes the data that may be useful for the proper classification and confirmation of those Aquatic plants containing active chelating agents for the uptake of trace and toxic metals from the soil.

Key words: *Phytochemical screening;Physicochemicalstudy; Chelating agents; Aquatic Plants*

Introduction

Plants have a natural tendency to take up trace and toxic metals by ligands, such as organic acids, amino acids, proteins and peptides [1]. These plants play an essential role in the uptake, storage and recycling of metals by ligands. It is a worldwide accepted fact that the assessment of toxicants in the environment is most important to evaluate the route of exposure of particular toxicant, which will be helpful for those institutes/organization and scientific community for solving the environmental threats and directly or indirectly impact on human [2]. The uptake of metals depends on the ligands which present in the Root, Shoot and leaves of the plants. Mainly atoms like S, N and O function as ligand atoms in the form of chemical groups like –SH, –S-S, –NH2, =NH, –OH, –OPO3H, or >C=O [3].

Organic ligands are low molecular weight Amino acids considered solubilization agents of metals linked to some mineral fractions of soils because they form stable soluble complexes with metallic ions [4]. Organic acids play a role as detoxification agents water and the soil. It can bind elements such as metals. Heavy metals tolerant plants have higher concentrations of organic acids in sensitive plants [5]. Oxalic, malic, malonic, citric, and fumaric acids are low molecular weight organic acids which involved in metal absorption by plant roots [3]. The proteinogenic amino acid function as osmolyte, radical scavenger, electron sink, stabilizer of macromolecules and cell wall component [6]. In situations where roots take up an excess of cations (particularly K+), the negative charge required to balance this often provided by organic acids, such as malate, malonate, citrate and aconitate[7].

The current study aims to find the different organic ligands which have potential to uptake trace and toxic metal, from soil by qualitative analysis. Moreover, physicochemical parameters (Moisture, ash content, pH of 1 % and 10 % soulution) of slelected Aquatic plants (*T. domingensis, P. australis*, and *T. aphylla*).

Materials and methods

Sample collection and pre-treatment

Three Aquatic plants (*T. domingensis, P. australis*and *T. aphylla*) were collected from District Hyderabad, Sindh

Pakistan during February 2020, and are characterized by the Department of Plant Sciences, Sindh Jamshoro University, Pakistan. The plant was dry indoor soil, using mortar and pestle powder. For phytochemical analysis the powdered sample was placed in air-tight bag.

Preparation of Water and petroleum ether extracts

10 g powdered sample of each plant was extracted by soxhlet extraction method with 50 ml petroleum ether and maceration process was used for water extraction (50 ml water). The whatmann No. 42 filter paper was used to extract the residue and the clear filtrate was evaporated to dryness to form the crude extract and processed for phytochemical screening at 4°C.

Physicochemical analysis

For physicochemical parameters such as moisture, ash and pH (1 % and 10 % aqueous) of triplicate samples for each plant were used according [8].

Physicochemical parameters

Moisture content: 1.0 g powdered sample of each plant taken in a dry and flat Petri dish and samples were dried in an electric oven at 105°C for 2 hours and measured the % weight loss.

Ash content: Weighed 2.0 g of a duplicate sample of each plant taken straight into crucibles and put for six hours in a muffle furnace at 550°C. Change in dry weight of plant sample was used to measure % of the total ash content before and after ashing.

Samples formulation pH: The pH of different solutions in 1 % w/v (1 g: 100 ml) and 10 % w / v (10 g: 100ml) soluble portions of water for each plant sample was calculated using standard pH meter for simple glass electrodes.

Phytochemical Screening of Aquatic plants

Phytochemical screening of petroleum ether and water extract performed by laboratory methods to analyze the presence of various phytochemical components (alkaloids, amino acids, carbohydrates, diterpenes, flavonoids, phenols, proteins, quinones, steroids, terpenoids and tannins).

Alkaloids: Few drops of Wagner's reagent (aqueous iodine and potassium iodide solution) added in 1 ml of plant extract (1.5 % HCl). The presence of brown precipitate suggested that the presence of Alkaloids.

Amino acids: In a clean test tube, 2 ml of the filtered extract with few drops of ninhydrin solution was taken and the solution was boiled in the water bath for 1-2 min. The purple colour suggested that amino acids are present.

Carbohydrates: The extract of each plant treated with Benedict's reagent (a complex mixture of sodium carbonate, sodium citrate, and copper (II) sulfate pentahydrate) followed by 5 minutes of gentle heating in a water bath. Confirmation of carbohydrates demonstrated by the presence of Orange-red precipitates.

Diterpenes: Few drops of water were added to each plant extract and treated with 1 ml of freshly prepared copper-acetate solution. The presence of diterpenes suggested by the observation of emerald green colour.

Flavonoids: Few drops of the solution NaOH applied to each extract of Aquatic plants. Upon adding a few drops of dilute acid, the intense yellow colour observed colour disappeared that indicates the presence of Flavonoids.

Phenolics: A small amount of each plant extract taken in a 2ml distilled water test tube and a few drops of 10 per cent ferric chloride solution applied. Blue or green colour produced indicates phenols.

Proteins: Each plant extract treated with only a few drops of conc. HNO3. The appearance of yellow colour was indicative of protein presence.

Quinones: Only a few drops from conc. HCl was applied to plant extract, and yellow colour or precipitate formation indicated the existence of quinones.

Steroids: The mixture of 2 ml CH3COOH anhydride and 2 ml CHCl3 applied to the plant sample extract in 0.5 ml and treated with 2 ml of conc. H2SO4. The colour observed shifting from violet to blue or green indicates steroid presence.

Terpenoids: The filtered mixture of the extract of 2 ml of plant and 2 ml of CHCl3 and treated with a few drops of concentrated H2SO4, shaken vigorously and allowed to stand. The golden yellow colour reveals the presence of terpenoid.

Tannins: Few drops of 5 % aqueous solution of ferric chloride added to every plant's extract. The Tannins also confirmed the presence of bluish-black colour.

Results and discussion

The preliminary phytochemical study of three native Aquatic plants (*T. domingensis, P. australis*, and *T. aphylla*) using petroleum ether and Water extract. This research involves a preliminary screening of phytochemicals, such as alkaloids, amino acids, carbohydrates, diterpenes, flavonoids, phenols, proteins, quinones, steroids, terpenoids, and tannins, typically contained in Aquatic plants.

Physicochemical and phytochemical analysis of Aquatic plants

The physicochemical parameters of selected aquatic plants calculated based on the World Health Organization (WHO) recommended methodologies. The *T. domingensis*, *P. australis*, and *T. aphylla*moisture % values found to be 6.92±1.53, 10.5±1.99 and 8.52±0.63, respectively. The percentage of *T. domingensis*, *P. australis*, and *T. aphylla* ash content found at 8.81±0.92, 5.21±0.66 and 13.1±0.52, respectively. For quantitative criteria, ash values used to find out consistency, validity those values are relevant [9]. Role of pH also is a crucial factor for complex formation and stability. Most chelating agents are unstable at low pH, whereas at high pH metals tend to form insoluble hydroxides which are less accessible to chelating agents [2]. The pH of *T. domingensis*, *P. australis*, and *T. aphylla*for 1 % w/v formulations noticed to be 7.51±0.68, 7.96±0.64 and 7.62±0.42 and for 10 % w/v formulations pH observed 7.15±0.63, 7.43±0.53 and 7.39±0.73, respectively. Such values are not indicating any variation in the pH of water-soluble portions of the entire plant. The extractive values are useful for the calculation of the chemical components in the crude product and also for the evaluation of other soluble components in a specific solvent (Table 1) [10].

This study has indicated that the presence of phytochemicals considered as active chelating chemical constituents which have potential to uptake toxic metals from contaminated soil. The results of the qualitative analysis of aquatic plants indicated that the *T. domingensis*is rich in amino acids, carbohydrates, flavonoids, phenolic, proteins and tannins. Whilst the phytochemical screening of *P. australis*showed the presence of alkaloid, amino acid, carbohydrates, diterpene, phenolic, protein, tannin and terpenoid. In the case of *T. aphylla*, the phytochemical screening confirmed the presence of alkaloids, amino acids, carbohydrates, phenolic, proteins and quinones, respectively (Table 2). Chelating agents are present in aquatic plants, such as fatty acids, amino acids, proteins, and peptides. The absorption of metals depends on the ligands present in the Aquatic plant's Root, Shoot, and Leaves [2]. For these molecules, metal binding, antioxidant defence, and signalling are important functions. Amino acids such as Histidine, and other particularly phytochelatins and glutathione play an essential role in the soil for the uptake of metals [6]. Low molecular weight

Table 1: Physicochemical Parameters of *T.domingensis, P. australis*and*T.aphylla.*

Parameters	*T. domingensis*	*P. australis*	*T. aphylla*
Moisture %	6.92±1.53	10.5±1.99	8.52±0.63
Ash %	8.81±0.92	5.21±0.66	13.1±0.52
pH of 1% w/v formulation solution	7.51±0.68	7.96±0.64	7.62±0.42
pH of 10% w/v formulation solution	7.15±0.63	7.43±0.53	7.39±0.73

Table 2: Phytochemical composition of T. domingensis, P. australis and T. aphylla in Water and Petroleum ether extracts.

Aquatic plants	*T. domingensis*		*P. australis*		*T. aphylla*	
Phyto-constituents	Water Extract	Petroleum ether Extract	Water Extract	Petroleum ether Extract	Water Extract	Petroleum ether Extract
Alkaloids	±	±	±±	0	±±	±
Amino acids	±±	±±	±±	±	±±	±
Carbohydrates	±±	±	±±	±±	±±	±±
Diterpenes	±	±	±	±±	0	±
Flavonoids	±±	±±	0	±	±	±
Phenolics	±±	±±	±±	±	±±	±±
Proteins	±±	±	±±	±	±±	±
Quinones	±	0	±	0	±	±±
Steroids	0	0	±	±	0	0
Tannins	±±	±±	±±	±±	±	±
Terpenoids	±	0	±±	±±	±	0
Presence of phytochemical = (±±), Absence of phytochemical = (0), Not confirmed = (±)						

organic acids such as Oxalic, malic, malonic, citric and fumaric acids required for the absorption of metals by aquatic plants roots [11]. Organic ligands in the rhizosphere, low molecular weight organic acids and amino acids are known to be metal solubilizes connected to some mineral fractions of soils because they form stable soluble complexes with metal ions [12].

Conclusion

The available data is insufficient for physicochemical and phytochemical analysis of *T. domingensis, P. australis*, and *T. aphylla*. All aquatic plants extensively studied in this research for their character, physicochemical characteristics and active constituents. The information obtained from this analysis revealed the evidence that may useful for correct identification and authentication of selected Aquatic plants and may help to use for phytoextraction of trace and toxic metals from soil.

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Conflict of Interest

All authors have agreed for the submission of current study and have no conflict of interest. It is confirmed that the proposed study in not submitted elsewhere for the publication. On behalf of all authors, the corresponding author states that there is no conflict of interest. Meanwhile, there are no institutional, venders or public financial of non-financial conflict of interest.

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