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Research Article

EFFICACY OF THE SOLVENTS ON THE PHYTOCHEMICAL LOAD AND POLYPHENOL CONTENT OF THE AQUATIC PLANT *AZOLLAMICROPHYLLA*

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ABSTRACT

Objectives: *Azolla microphylla* which is an aquatic macrophyte is used to find out efficacy of the different solvents on phytochemical load and polyphenol content. The whole plant was extracted in the Soxhlet apparatus using different solvents such as aqueous, hydro-ethanol, hydro-methanol, and hydro-acetone.

Methods: The four plant extracts were subjected to phytochemical screening by standard procedure. The polyphenol content was estimated in the four extracts using Folin-Ciocalteu method.

Results: The phytochemicals such as flavonoids, phenols, saponins, diterpenes, and protein were present in all the four solvent extracts. The higher proportions of phytochemicals were seen in hydro-methanol extract. The polyphenol content was also higher in the hydro-methanol extract.

Conclusion: The results showed that the extraction of the phytochemicals varied not only plant wise but also from solvent to solvent. The present study revealed the fact that the combination of water and organic solvents gave a better result than aqueous extract.

Keywords: *Azollamicrophylla*, Phytochemicals, Soxhlet apparatus, Solvent, Aqueous.

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INTRODUCTION

Since ancient period, plants belonging to the division Pteridophyta have been known for its therapeutic ability and medicinal use. *Azolla* is spore, (heterosporous), floats freely in the fresh water, vascular plants that have no seeds and flowers. It has symbiotic relationship with *Anabaena-Azolla*, a blue-green algae. The blue-green algae acts nitrogen-fixers, provides enough nitrogen for both themselves and host *(Azolla*). The aquatic plant contains many useful secondary metabolites such as flavonoids, steroids, alkaloids, phenols, triterpenoid compounds, varieties of amino acids, and fatty acids [1]. These bioactive components contribute to many useful and medicinal properties such as antioxidant, anti-carcinogenic, anti-inflammatory, anti-diabetic, gastro-protective, antiviral, neuro-protective, cardio protective, and anti-hypertensive properties [2]. Previous literature also revealed that the aquatic plant also contains anti-microbial [3], hepato protective [4], anti-oxidant [5,6], and bio-remediation [7].

Extraction procedure is an key process in the phyto-chemistry in which the secondary metabolites are gained from the plant source. The aim of the extraction is to gain maximize yield of the target metabolites and to reach the highest biological activity of the secondary metabolites present in different extracts [8]. The extraction yield and biological activity of the resulting extract not only depends on the technique handled but also on the solvent used [9,10]. Many solvents such as ethanol, methanol, acetone, and water have been used for the extracting of secondary metabolites from the plant source. Due to the various secondary metabolites in the plant with different solubility in different solvents, the ideal solvent for extraction depends on the particular plant source and the metabolites to be isolated. Hence, a recommendation of suitable solvent for each plant is very difficult to understand.

For *Azolla microphylla,* very little research has been focused on the screening and identification of the secondary metabolites. However, no work has been carried out on the effect of solvent on the screening of secondary metabolites and polyphenol content with respect to the soil nature. The present study examined the effect of distilled water and distilled water with organic solvents (liquid-liquid mixture) combination such as hydro-methanol, hydro-ethanol, and hydro-acetone.

METHODS

Chemicals

Every analytical grade chemicals compounds had been utilized for this study has been acquired from Sree Sabari Scientifics, Mayiladuthurai, Nagapattinam (DT), Tamil Nadu, India. Distilled water, methanol, ethanol and acetone, sodium carbonate, Conc. HCL, α-napthol, NaOH, $Fecl_3$, benzene, sodium nitroprusside, pyridine, chloroform, conc. H_2SO_4, acetic anhydride, gelatin, lead acetate, Nacl, conc. HNO_3, and copper acetate.

Plant material

In the study, the whole aquatic plant *Azolla Microphylla (*AM) was collected from the local farm at Nagapattinam district, (TN) during the month of October - January (2018-2019). A voucher was submitted in the herbarium at the PG and Research Department, D. G. Govt Arts College (W), Mayiladuthurai, Tamil Nadu, India, where it was authenticated by a botanist, Dr. K. Sankar Ganesh, Assistant Professor, Department of Botany and the herbarium voucher number DGGACBOT HR-10 was assigned to it. The specimen was kept in the herbarium of the said department for future references.

Systematic position of the plant

Class: Polypodiopsida
Order: Salviniales
Family: Salviniaceae
Genera: Azolla (Fig. 1).

Extraction procedure

The whole plant powders have been extracted with different solvents, that is, distilled water, hydro-ethanol, hydro-methanol, and hydro-acetone in the ratio of 30:70. 20 g whole plant powder was extracted

with 200 ml of the particular solvents by Soxhlet apparatus for 8 h to separate the polar and non-polar compounds. After that, strained using Whatman filter paper and obtained the filtrate, and evaporated to dryness by open-disc evaporation using electrical water bath. The weight of the dried extracts (yield) obtained from different solvents was recorded using electronic balance (Schimantz make). Then the extracts were confronted with phytochemical screening and quantification of polyphenols (Fig. 2) [11].

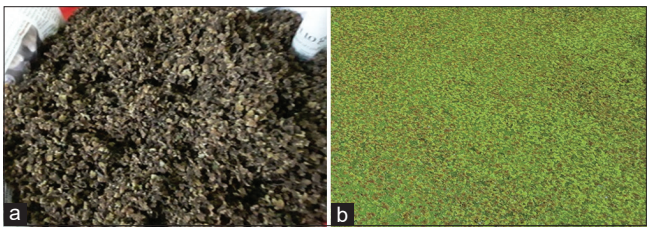

Fig. 1: *Azolla microphylla*. (a) Dried, (b) Fresh

Fig. 2: Extraction using Soxhlet apparatus

Phytochemical screening of the whole plant extract of the *Azolla microphylla* in different solvents

The four different extracts were subjected to phytochemical screening by standard procedure [12].

Quantification of polyphenols

Procedure

10 mg of plant powder and the plant extract were weighed, dissolved in methanol and made up to 10 ml with methanol. 1 ml was pipette out from the extract and 5 ml of Folin-Ciocalteu reagent was added and 4 ml of 7.5% sodium carbonate was added after 5 min. It was stirred and incubated at room temperature for 2 h. After 2 h, the absorbance of the solutions was measured at 750 nnm using a spectrophotometer (UV-VIS spectrophotometer, Model 2230) against the reagent blank. A standard calibration curve was constructed using different concentrations (5, 10, 15, 20, and 25 µg/ml) of catechol and the polyphenolic content was expressed as mg equivalent of catechol/g sample [13].

The total phenolic content was calculated using the formula:
TPC= cV/m
Where, c= Concentration
V=Volume of the extract used
m=mass of the extract used.

RESULTS

Phytochemical screening of the plant extract of the *Azolla microphylla* in different solvents is shown in Tables 1 and 2, the positive signs indicated the intensity of reactions that reflect the amount present in the extract. Flavonoid, phenols, saponins, diterpenes, and protein were present in all the solvent extracts. The alkaloid was present in all the extracts except hydro-acetone. Amino acid and carbohydrate were present in the aqueous extract. Reducing sugar was present in all the extracts except hydro-methanol. Glycoside was present only in the hydro-methanol extract. Cardiac glycoside was present in all the extracts except aqueous extract. Phytosterols were present in hydro-methanol and hydro-acetone extract. Tannins were present in hydro-ethanol and hydro-methanol extract. From the results, it was clear

Phytochemicals	Test	Experiment	Observation
Alkaloids	Mayer's test	Extracts+Mayer's reagent	Yellow-colored precipitate
	Wagner's test	Extracts+Wagner's reagent	Brown/reddish precipitate
	Hager's test	Extracts+Hager's reagent	Yellow-colored precipitate
Amino acids and proteins	Ninhydrin test	Extracts+0.25% w/v Ninhydrin reagent	Blue color
	Xanthoprotein test	Extracts+few a drops of conc. Nitric acid	Yellow color
Carbohydrates	Molisch's test	Extracts+two drops of alcoholic α-naphthol solution	Violet ring at the junction
	Benedict's test	Extracts+Benedict's reagent	Orange-red precipitate
Glycosides	Modified borntrager's test	Extracts+Ferric Chloride solution and immersed in boiling water for about 5 minutes+extracted with equal volumes of benzene+ammonia solution	Rose-pink color
Cardiac glycosides	Legal's test	Extracts+sodium nitroprusside in pyridine and sodium hydroxide	Pink to blood-red color
Saponins	Froth test	Extracts+20ml distilled water shaken for 15 min	1 cm layer of foam
	Foam test	Extracts+2 ml of water shaken well	Foam produced persists for ten minutes
Phytosterols	Salkowski's test	Filtrates+few drops of Conc. Sulfuric acid	Golden yellow color
	Liebermann–Burchard test	Filtrates were treated with a few drops of acetic anhydride, boiled, and cooled. Conc. Sulfuric acid was added	Brown ring at the junction
Phenols	Ferric chloride test	Extract+3-4 drops of ferric chloride solution	Bluish black color
Tannins	Gelatin test	Extract+few drops of gelatin solution containing sodium chloride	White precipitate
Flavonoids	Alkaline reagent test	Extract+sodium hydroxide solution	Intense yellow color; colorless on the addition of dilute acid
	Lead acetate test	Extract+few drops of lead acetate solution	Yellow color precipitate
Diterpenes	Copper acetate test	Extract+3-4 drops of copper acetate solution	Emerald green color

Table 1: Phytochemical screening of the whole plant extract of the *AzollaMicrophylla* in different solvents

Phytochemicals	Name of the test	Aqueous extract Presence/absence	Hydro-ethanol extract Presence/absence	Hydro-methanol extract Presence/absence	Hydro-acetone extract Presence/absence
Alkaloids	Mayer's test	+	+	+	–
	Wagner's test	+	+	+	–
	Hager's test	+	+	+	
Amino acids	Ninhydrin test	+	–	–	–
Proteins	Xanthoprotein test	+	+	+	+
Carbohydrates	Molisch's test	+	–	–	–
Reducing sugar	Benedicts test	+	+	–	+
Glycosides	Borntrager's test	–	–	+	–
Cardiac glycosides	Legal's test	–	+	+	+
Saponins	Froth test	+	+	+	+
	Foam test	+	+	+	+
Phytosterols	Salkowski's test	–	–	+	+
	Liebermann–Burchard test	–	–	+	+
Phenol	Ferric chloride test	+	+	+	+
Tannins	Gelatin test	–	+	+	–
Flavonoids	Alkaline reagent test	+	+	+	+
	Lead acetate test	+	+	+	+
Diterpenes	Copper acetate test	+	+	+	+

+: Presence, –: Absence

Table 2: Polyphenol Content in different extracts of whole plant of *AzollaMicrophylla* by Folin–Ciocalteu Method

S. No.	Extracts	Polyphenolic content Mg/g Mean ± SD
1	Aqueous extract	4.38 ± 0.01
2	Hydro-Ethanol extract	17.86 ± 0.05
3	Hydro-Methanol extract	24.31 ± 0.02
4	Hydro-Acetone extract	18.39 ± 0.01

Mg/g: Milligram/gram, values are expressed in mean ± SD

that the availability of the secondary metabolites was observed in all the four solvent extracts studied. But a most number of secondary metabolites were seen in hydro-methanol extract when compared with other solvent extracts (Table 1).

Polyphenol content in different extracts of the whole plant *Azolla microphylla* was studied by Folin–Ciocalteu Method. The catechol was used for the standard calibration curve. The results revealed that the polyphenol content in the extracts of distilled water, hydro-ethanol, hydro-methanol, and hydro-acetone was shown to be 4.39, 17.89, 24.30, and 18.39 mg/g. From the results, it was seen that the polyphenol content was high in the hydro-methanol extract when compared to other solvent extracts studied (Figs. 3 and 4). The order of degree of the extract was Hydro-Methanol> Hydro-acetone> Hydro-Ethanol >Aqueous.

DISCUSSION

The phytochemicals present in the *Azolla microphylla* have display the presence of various compounds such as flavonoids, phenols, saponins, diterpenes, proteins, alkaloids, and diterpenes. The presence of the compounds differs for the different extracting solvents. This is due to the efficiency of the solvent used in the extraction procedure. The compounds in the aquatic plant are of great importance in area of novel discovery of drugs. The extractive yield of the poly phenolic compounds of the plant extracts depends on the polarity of the solvents used in the extraction process. The extractive yield of the polyphenolic compounds was low in the aqueous extract alone. The amalgamation of the water with different solvents such as methanol, acetone, and ethanol give a better productivity in the extraction of the phenolic compounds. The degree of the extractive yield of the polyphenol compounds was Hydro-Methanol> Hydro-acetone> Hydro-Ethanol >aqueous (Table 2). The results of the degree of the extractive yield were quoted by different authors on different plant-based and food-based extracts [14-18].

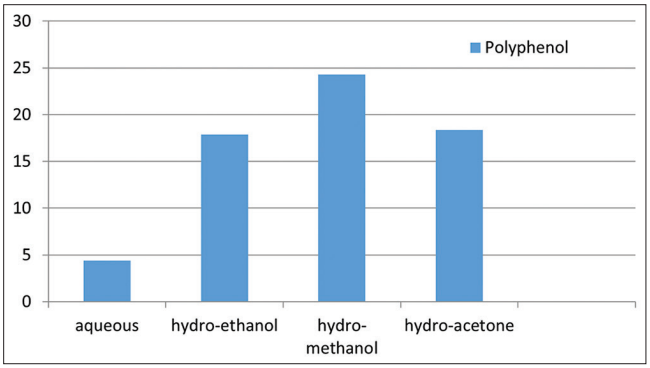

Fig. 3: Polyphenol content in different extracts of whole plant of *AzollaMicrophylla* by Folin–Ciocalteu method

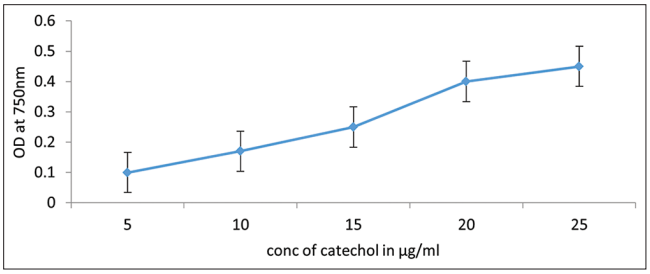

Fig. 4: Standard calibration curve for the polyphenol content using catechol as standard. Conc.: concentration; OD: optical density

The characterization of a good solvent exhibits optimal extraction and its capacity in preserving the stability of the structure of the desired chemical compounds. The different types of solvent used in the extraction and the nature of polarity may have a significant role in the level of extracting polyphenols. The polyphenols having polarity ranging from polar to non-polar. The best extraction of polyphenols is often obtained in the polar solvent due to the interactions of hydrogen bonds between the polar sites of the compounds (polyphenols) and the solvent used. So a better efficiency of solvation was observed in a polar solvent than non-polar one [19].

The disadvantage of the water as the solvent for the extraction of the total phenolic compounds depends on the nature of the polarity. In

nature, the phenolic compounds bind with the other molecules such as protein and carbohydrates [20]. A good solvent is capable of breaking the interactions such as hydrogen bond between the molecules [21]. Distilled water cannot break such bonds due to the polarity. Hence, the polarity of the water was adjusted by some organic solvents such as methanol, acetone, and ethanol. The combination of the water with the solvents acts as a good polarity modifier, thus the yield of the polyphenolic compounds was high (Table 2), because the organic solvents such as methanol, acetone, and ethanol were acts as a good hydrogen acceptors and donors. In the present study, the organic solvents with water (mixture of the solvents) act as a better solvent for the extraction of polyphenolic compound from the aquatic plant, *Azolla microphylla*. A similar result was obtained by earlier studies of [21,22].

CONCLUSION

The results from the present study demonstrated that the plant of *Azolla microphylla* contained the most phytochemicals in the mixture of organic solvent with water, that is, hydro-methanol. The greatest polyphenol content was seen in hydro-methanol. The results suggested that the load of the phytochemicals and the levels of the polyphenol content varied not only from plant to plant but also in the solvent tested. The results indicated that the organic solvents with water gave greatest extraction for this aquatic plant which may be exploited for various medicinal and pharmacological formulations.

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AUTHOR CONTRIBUTIONS

All the authors have made equal contribution.

DECLARATION OF COMPETING INTEREST

No potential conflicts of interest were disclosed.

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