



## Phytochemical Analysis of Extracts of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* Growing in Polluted Water in Sri Lanka

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### Authors' contributions

This work was carried out in collaboration among all authors. Author RGWDBR designed the study, performed the laboratory analysis, and wrote the first draft of the manuscript. Authors BARF and TMABT managed the analyses of the study and wrote the protocol. Author ARNS managed the literature search and finalize the manuscript All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** This study involved four plants (roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis*) for phytochemical analysis, which are growing in the polluted water in Sri Lanka.

**Study Design:** Phytochemical analysis performed according to the standard methods.  
**Place and Duration of Study:** Samples were collected from the wastewater disposal subways near the Colombo area, Sri Lanka in July 2016.  
**Methodology:** The plant extracts were prepared by homogenization of plants in distilled water and subjected to phytochemical analysis.  
**Results:** Results of Phytochemical analysis revealed that the four plant extracts contained flavonoids, proteins, and amino acids. Other than that, *Monochoria vaginalis* contains carbohydrates, phenols, diterpenes and glycosides. *Lasia spinosa* contained carbohydrates and diterpenes in addition to the above-mentioned phytochemicals.  
**Conclusion:** It is concluded that the plants which are growing in polluted water may possess antimicrobial activity and flavonoids, carbohydrates, glycosides, phenols and diterpenes which may contribute to their survival in polluted water.

**Keywords:** Phytochemical analysis; wastewater; semi-aquatic plants.

## 1. INTRODUCTION

Plants are the major source of medicinal compounds that play a dominant role in the maintenance of human health and drug discovery since ancient times. Plant extracts, crude plant materials are used in folk medicine, in traditional and Ayurvedic therapies such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered or their active constituents [1].

According to the World Health Organization (WHO) records, over 50% of all modern clinical drugs are based on natural plant products [1]. As an example mainly the phytochemicals such as carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes, minerals and vitamins (A, C, E and K) are responsible for anti-bacterial and antioxidant activities [2]. The specific function of many phytochemicals is still unclear and under investigation. Nowadays, antimicrobial screening of plant extracts and phytochemicals are represented as a starting point for drug discovery [3].

Plants have been used in treating human ailments for thousands of years in traditional medicine in Sri Lanka as well as around the world [4]. However, some medicinal activity of many plants still unclear and under investigation [5],[4]. *Ipomoea aquatica* is a semi-aquatic, tropical plant grown as a vegetable for its tender shoots and leaves. *Ipomoea aquatica* is a member of the Convolvulaceae family and it has been originated in China and distributed throughout India, Sri Lanka, tropical Asia, Australia and native to Africa. It is called as water spinach, kangkong [6-7]. This plant is traditionally

used as an emetic agent against poisoning arsenic and opium in china [8], [4]. The plant *Lasia spinosa* is a member of the family Araceae, distributed throughout the marshy places in tropical India, Burma, Sri Lanka, Malay Peninsula and China [8]. *Pistia stratiotes* is a member of the family Araceae. *Pistia stratiotes* is called Diya parade in Sri Lanka. This plant is generally found in tropical countries including Sri Lanka, India and Philippine islands and it is native to Sri Lanka [8], [4]. *Monochoria vaginalis* belongs to the family Pontederiaceae. This plant is distributed in South East Asia, Iran, Philippines, Indonesia, China, Korea, Japan and North Australia and Sri Lanka [9]. This plant is called as 'Diya habarala' in Sri Lanka [4].

This study investigates the presence or absence of different phytochemicals in aqueous extracts of roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis*, which are growing in the polluted water in Sri Lanka.

## 2. METHODOLOGY

### 2.1 Plant Collection and Authentication

*Ipomoea aquatica* and *Lasia spinosa* plants were collected from a wastewater subway near the University of vocational technology (6°49'13.0"N 79°53'34.1"E) situated in 7th Lane, Rathmalana, Dehiwala-Mount Lavinia, Colombo, Western Province of Sri Lanka. *Pistia stratiotes* plant was collected from Weras Ganga (6°48'51.6"N 79°54'07.0"E), near the karadiyana garbage dump, Thumbovila north, Boralessgamuwa, situated in Colombo district, Western province of Sri Lanka. *Monochoria vaginalis* plant was collected from a canal of 9th cross street (6°49'02.5"N 79°53'44.2"E), Rathmalana,

Dehiwala-Mount Lavinia, Colombo, Western Province of Sri Lanka. *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* plants were identified and authenticated by National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka.

## 2.2 Preparation of Plant Extracts

The roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* were collected from the above-mentioned locations and followed by immediate washing with running water to remove contaminants and air-dried until a constant weight was obtained. 20 g of plant specimens were cut into small pieces (about 3-6 cm in length) and plant parts were grounded into the fine powder using a mortar and pestle. The powdered plant materials (20g) were resuspended in 20ml of distilled water and followed by filtration using a muslin cloth in order to remove the debris. The filtrate was again filtered by no.01 Whatman filter paper and collected into the bottles until further use [4]. Concentrations of each plant extract considered as 1 g/ml.

## 2.3 Phytochemical Screening

The four aqueous extracts of plants were subjected to phytochemical analysis for check the availability of the following secondary metabolites; alkaloids, saponins, tannins, glycosides, carbohydrates, phytosterols, phenols, protein, amino acids, diterpenes and flavonoids following standard procedures [10].

### 2.3.1 Detection of alkaloids

2 ml of Each plant extracts (1 g/ml) were dissolved in 2 ml of diluted hydrochloric acid (1%) and filtered into a volumetric flask.

Mayer's test: few drops of Mayer's reagent (Potassium Mercuric Iodide) was added into a portion of acidified filtrates of each plant extract. The formation of a yellow coloured precipitate was indicated the presence of alkaloids in the extract.

Wagner's test: few drops of Wagner's (Iodine in Potassium Iodide) reagent was added into a portion of acidified filtrate of each plant extract. The formation of brown/reddish precipitate was indicated the presence of alkaloids.

Dragendroff's test: a portion of filtrates of each plant extracts were treated with few drops of

Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of red precipitate was indicated the presence of alkaloids [10].

### 2.3.2 Detection of carbohydrates

Each plant extracts (1 g/ml) were diluted using 5 ml of distilled water and filtered through the filter paper. Each filtrate was collected and used to test for the presence of carbohydrates.

Benedict's test: Each filtrate was treated with Benedict's reagent and heated gently in a boiling tube. The formation of Orange-red precipitate was indicated the presence of reducing sugars [10].

### 2.3.3 Detection of glycosides

Each plant extract was hydrolyzed with the diluted hydrochloric acid (1%), and extracts were subjected to test for glycosides.

Four plant extracts were treated separately with sodium nitroprusside in pyridine and sodium hydroxide. The formation of pink to the blood-red colour was indicated the presence of cardiac glycosides in the extracts [10].

### 2.3.4 Detection of saponins

Froth test: Each plant extracts were diluted with distilled water and each extract was shaken in a 100 ml graduated cylinder for 15 minutes. The formation of a 1 cm layer of foam was indicated the presence of saponins.

Foam test: 0.5ml each plant extracts were shaken with 2ml of water. The produced foam is persists for ten minutes was indicated the presence of saponins [10].

### 2.3.5 Detection of phytosterols

Salkowski's test: Each plant extracts were treated with chloroform and filtered. Few drops of concentrated Sulphuric acid each filtrate. The mixture was shaken and allowed to stand. The appearance of the golden yellow colour was indicated the presence of phytosterols [10].

### 2.3.6 Detection of phenols

LiebermannBurchard's test: Each plant extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish-black colour was indicated the presence of phenols [10].

### 2.3.7 Detection of tannins

Braymer's test: 1ml of each plant extracts were treated with 10% alcoholic ferric chloride solution. The formation of the blue or greenish-grey colour solution was indicated the presence of tannins [10].

### 2.3.8 Detection of flavonoids

Alkaline reagent test: Few drops of sodium hydroxide solution was added to each plant extracts. The formation of intense yellow colour, which becomes colourless with the addition of dilute acid, was indicated the presence of flavonoids [10].

### 2.3.9 Detection of proteins and amino acids

Xanthoproteic test: each plant extracts were treated with few drops of concentrated nitric acid. The formation of yellow colour was indicated the presence of proteins.

Ninhydrin test: 0.25% w/v ninhydrin reagent was added into each plant extracts and boiled for a few minutes. The formation of blue colour was indicated the presence of amino acid [10].

### 2.3.10 Detection of diterpenes

Copper acetate Test: Each four plant extracts were dissolved in distilled water and treated with 3-4 drops of copper acetate solution. The formation of emerald green colour was indicated the presence of diterpenes [10].

## 3. RESULTS AND DISCUSSION

Results of the phytochemical profile of aqueous extracts of roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* are expressed in Table 1.

Phytochemical analysis revealed that the presence of phytochemicals considered active medicinal chemical constituents. Important phytochemicals such as carbohydrates, phytosterols, Flavonoids, Diterpenes, Proteins and amino acids. This study investigated phytochemical constituents of aqueous extracts of four medicinal plants, which are growing in the wastewater near Rathmalana area of Sri Lanka, namely roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria*

*vaginalis*. These plants are growing in wastewater, but they survive in it as they have resistance for bacterial pathogenesis. All the plants have their own immune system called the innate immune system [11]. Which may consist of physical barriers and chemical barriers against the bacterial pathogens [12].

The phytochemical profile of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* extracts showed the presence of flavonoids, proteins, carbohydrates and amino acids. Other than that, the *Lasia spinosa* contained phytosterols and diterpenes. Furthermore, the *Monochoria vaginalis* extracts contained phytosterols, phenols, glycosides and diterpenes. Generally, the flavonoids and phenolic have been shown to mediate antibacterial activity via interactions with proteins on the bacterial cell wall and thereby disrupting its functional and structural integrity [13].

Sewage water containing a high amount of Nitrogen and Phosphorous. The plants growing in wastewater absorb the high levels of phosphorus and nitrogen from the sewage water [14]. Usually, phosphorus occurs in the form of phosphates. The level of phosphate can be an effect on the production of secondary metabolites. It's reported that higher levels of phosphates enhance cell growth and negative impact on secondary metabolites production and accumulation [15].

According to the literature, Alkaloids isolated from plants are shown to possess antibacterial action which is mediated via interactions with bacterial DNA and inhibit dihydrofolate reductase [16-17]. Saponins are also shown to induce antibacterial actions through a membranolytic action by increasing permeability of bacterial cell wall by intact with Lipopolysaccharides [18-19]. Such a mode of actions is possible in this study as well. Here, the phytochemical profile of *Monochoria vaginalis* revealed that it has contained carbohydrate, glycoside, phytosterols, phenols and diterpenes. *Lasia spinosa* contained carbohydrate, glycoside and phytosterols. Due to this reason, there is a possibility of glycosides and diterpenes also contribute to the antibacterial activity. The findings of this study obtained different results from the previously available research studies relevant to these plants possibly due to the adaptation to withstand harsh conditions of polluted water [20-21].

Table 1. Phytochemical screening of four plants

Phytochemical	Test	Results			
		<i>Ipomoea aquatica</i>	<i>Lasia spinosa</i>	<i>Pistia stratiotes</i>	<i>Monochoria vaginalis</i>
Alkaloids	Wagner's test	-	-	-	-
	Mayer's test	-	-	-	-
	Dragendroff's Test	-	-	-	-
Carbohydrates	Benedict's test	+	+	-	+
Glycosides	Legal's Test	-	-	-	+
Saponins	Froth Test	-	-	-	-
	Foam Test	-	-	-	-
Phytosterols	Salkowski's Test	-	+	+	+
Phenols	Libermann	-	-	-	+
	Burchard's test	-	-	-	-
Tannins	Braymer's test	-	-	-	-
Flavonoids	Alkaline Reagent Test	+	+	+	+
Proteins and amino acids	Xanthoproteic Test	-	+	-	+
	Ninhydrin Test	+	+	+	+
Diterpenes	Copper acetate Test	-	+	-	+

#### 4. CONCLUSION

In conclusion, this study demonstrates, an aqueous extract of roots of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* phytochemical profile. Results of this phytochemical study further provide information regarding the possibility of having an antibacterial, antioxidant and anti-inflammatory activity of these four plant extracts based on the secondary metabolites present in the plants. Moreover, these results implicated that the survival of *Ipomoea aquatica*, *Lasia spinosa*, *Pistia stratiotes* and *Monochoria vaginalis* plants against the bacteria found in sewage water due to the presence of secondary metabolites of the plants found in sewage water.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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