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Phytochemical analysis of some selected aquatic macrophytes of Holalkere, Chitradurga District, Karnataka

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Abstract

The current study was carried out to analyze the phytochemical components of selected aquatic macrophytes. Phytochemicals are secondary metabolites produced by all plants which have medicinal uses. The analysis of whole plant extracts on aqueous, petroleum ether, chloroform, and methanol extracts of aquatic macrophyte plants of *Ipomoea fistulosa, Cyperus longus, Polygonum glabrum* and *Hydrilla verticillata* was investigated. The preliminary qualitative phytochemical analysis was done for the presence of bioactive constituents such as Alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, amino acids, proteins, saponins, sterols, tannins, terpenoids, quinones, oxalates, fats and oils in the solvent extract by using standard methods. Phytochemical screening not only helps reveal the constituents of the plant extracts and the ones that predominate over the others but also help search for bioactive agents that can be used to synthesize useful drugs. This review aims to provide a thorough review of traditional uses, phytoconstituents, and pharmacological effects.

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Introduction

Phytochemicals are isolated from plants, which are useful and effective for us in this era. Phytochemicals generally originating from the plant source are nothing but bioactive compounds also known as secondary metabolites. There are two types of metabolites produced in plants viz. Primary metabolites and Secondary metabolites. Primary metabolites are important for the plant's regular metabolism such as growth and development. Secondary metabolites produced by plants may have little need for them. These are synthesized in almost all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. During the past several years, phytochemicals have been used worldwide as traditional herbal medicine. Because of these pharmaceutical industries as well as researchers put a greater emphasis on phytochemical studies. Also, these phytochemicals present in the different plant parts are used up by the local people for the healing of certain disorders (Ugochukwu *et al.*, 2013).

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body; bark leaves stem, root, flower, fruits, seeds, etc. i.e., any part of the plant body may contain active components (Hussain *et al.*, 2012). These are also widely used in the field of agriculture. Secondary metabolites are economically important in the production of drugs, flavors and fragrances, dyes and pigments, pesticides, and food additives. Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of these naturally obtained substances (Tiwari *et al.,* 2011). In India, the treatment of microbial diseases, fungal diseases, and deficiency diseases was treated with the assistance of plant crude extract but now this idea has been spread everywhere in the world. Ayurveda is also a traditional strength in India and many research scholars now endorse natural remedies for some diseases that were already completely treated with the help of phytochemical components. India is a medicinal plant varietal emporium and one of the world's richest countries in terms of medicinal plant genetic resources. It also has a varied topography and weather conditions that are demonstrated in the plants and floristic morphology. Besides that, the agro-climatic conditions are conducive to the initiation and cultivation of new exotic plant varieties (Parekh *et al.*, 2007).

Phytochemicals are generally used to describe plant compounds that are under research with unestablished effects on health and are not scientifically defined as essential nutrients. Analysis of the phytochemical properties of the medicinal plants used to show and isolate the drug, lead compounds, and components from the parts of the plant. The unique biological activity of the plants can be identified by their phytochemical properties.

Phytochemical metabolites are certainly found in therapeutic plant parts like roots, leaves, bark, vegetables, and fruits that have an express mechanism and protect from innumerable diseases. Phytochemical studies have extended a lot of interest among researchers due to the amplification of newer technology and advanced outcome. These practices play a substantial role in finding important material for the pharmaceutical industry (Alston and Turner, 1963). Plants have constituents that persuade a great interest due to their multipurpose applications (Baris *et al.*, 2006). Predictably, 14-18% of the higher plants are used remedially, and related 74% of the pharmacologically vigorous plants are revealed after following upon ethnomedicinal practice of the plants (Ncube *et al.,* 2008). Plants are able with several phytochemical compounds such as Alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, amino acids, proteins, saponins, sterols, tannins, terpenoids, quinones, oxalates, fats, oils, and other constituents which are the rich cause of free radical scavengers (Gracelin *et al.,* 2012 and Chaithanya *et al.,* 2008). Such preliminary phytochemical screening of plants is the need of the hour to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world (Raphael, 2012 and Dasgupta *et al.,* 2013).

Plant cells' primary and secondary metabolites are the final recipients of biological information flow. These metabolites' qualitative and quantitative measurements reflect the cellular state under defined conditions, providing critical insights into the cellular mechanisms that regulate the biochemical phenotype of the cell, tissue, or entire organism. In several fundamental ways, metabolomics differs from the previous targeted phytochemical analysis (Tugizimana *et al.,* 2013). The phytochemical analysis is essential for identifying bioactive constituents in plants to develop new therapies and treatments.

Material and methods

Study Area

Holalkere taluk, Chitradurga District, Karnataka state, covering an area of 1108 sq km. It has an average elevation of 711 meters (2332 feet) and is located at longitudes 14.0541° N and 76.1958° E. It is bounded by Channagiri taluk towards west, Davanagere taluk towards the north, Chitradurga taluk towards the east, and Hosadurga taluk towards the south. The Location map of the taluk is in Fig. 1.

Collection of Plant Material

The required plants were collected from the region of Holalkere, Chitradurga District. After the plants are collected from the region and then identified, those plants were cleaned, and washing and drying are to remove the water content from plants so that the plants can be stored. The hole plants taken to do phytochemical analysis in this process for drying can be done by a natural process, like air-dried in sheds, but this may take a few weeks for complete drying. The time depends upon the temperature and humidity. After the complete drying of plants, they have to be powdered well for further analysis.

Preparation of Extraction

Soxhlet Extraction

The powdered samples were subjected to petroleum ether, chloroform, and methanol. 50g of powdered sample was filled in a Whatmann filter paper and placed inside the thimble. 200mL of the solvent was added in a thimble. The thimble was fit into a round bottom flask containing 700mL of the solvent and run for 24 hours at the temperature based on the boiling point of the respective solvent using the Soxhlet apparatus. Later the extract was subjected to distillation for 2-3 hours. These extracts were kept in the water bath at 40°C for drying. The dried extracts thus obtained were used for various analyses.

Preliminary phytochemical screening

Test for Alkaloids (Wagner's reagent)

A fraction of the extract was treated with 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of a reddish-brown precipitate (or coloration).

Test for Carbohydrates (Molisch's test)

Few drops of Molisch's reagent were added to the 2ml portion of the various extracts. This was followed by the addition of 2 ml of concentrated H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. The formation of a red or dull violet color at the interphase of the two layers was a positive test.

Test for Cardiac glycosides (Keller Kelliani's test)

5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underplayed with 1ml H_2SO_4. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the ring while a greenish ring may form in the acetic acid layer.

Test for Flavonoids (Alkaline reagent test)

2ml of extracts was treated with a few drops of 20% sodium hydroxide solution. Formation of intense yellow color. Which becomes colorless with the addition of dilute hydrochloric acid, indicating the presence of flavonoids.

Test for Phenols (Ferric chloride test)

A fraction of the extracts were treated with aqueous 5% ferric chloride and observed for the formation of deep blue or black color.

Test for Phlobatannins (Precipitate test)

Deposition of a red precipitate when 2mls of the extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Amino acids and Proteins (1% ninhydrin solution in acetone)

2ml of the filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Test for Amino acids and Proteins (1% ninhydrin solution in acetone)

2ml of the filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Test for Saponins (Foam test)

To 2mls of the extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Sterols (Liebermann-Burchard test)

1ml of the extract was treated with drops of chloroform, acetic anhydride, and concentrated H_2SO_4 and observed for the formation of dark pink or red color.

Test for Tannins (Braymer's test)

2mls of the extract was treated with 10% alcoholic ferric chloride solution and observed for the formation of a blue or greenish color solution.

Test for Terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formationof a yellow precipitate (or coloration).

Test for Oxalate

To a 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish-black coloration indicates the presence of oxalates.

Test for Fats and Oils

To 5 drops of sample, 1mL of 1% copper sulphate solution was added, and a few drops of 10% sodium hydroxide. Observed for the formation of a clear blue solution.

Result and discussion

The standard preliminary phytochemical qualitative analysis of the extract was carried out for the various plant constituents and screened for the presence or absence of biologically active compounds or secondary metabolites using standard procedures. As a result, the major phytochemical constituents identified were Alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, amino acids, proteins, saponins, sterols, tannins, terpenoids, quinones, oxalates, fats, and oils in 80% petroleum ether, chloroform and methanol extracts of each plant using modified standard procedures (Habtamu *et al*., 2017). The results revealed the presence of medicinally important constituents in the plants studied. Much evidence was gathered in earlier studies confirming the identified phytochemicals as bioactive. Several studies confirmed the presence of these phytochemicals contributes medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source of useful drugs. In the present studies phytochemical screening of four plants (Aquatic Macrophytes).

Table 1. Botanical Information of Selected Plants.

SL	Botanical Name	Common Name	Family	Part Used
1	*Ipomoea fistulosa*	Pink Morning Glory	Convolvulaceae	Whole Plant
2	*Hydrilla verticillata*	Water Thyme	Hydrocharitaceae	Whole Plant
3	*Cyperus longus*	Sweet Cyperus	Cyperaceae	Whole Plant
4	*Polygonum glabrum*	Marsh Buckwheat	Polygonaceae	Whole Plant

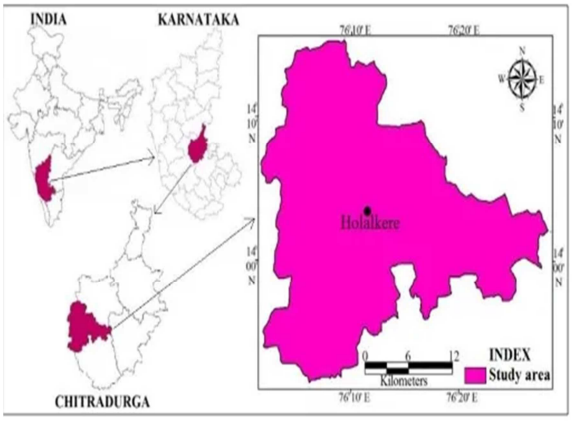

Fig. 1. Location map of Holalkere Region.

Fig. 2. Selected Aquatic Macrophytes.

Hydrilla verticillata, Ipomoea fistulosa, Cyperus longus, and Polygonum glabrum are subjected to phytochemical screening of various plant constituents. The selected aquatic macrophyte plants are shown in Table 1 and Fig. 2.

It was found that all the plants have a considerable proportion of important phytochemicals that are easily detected by qualitative tests. In our analysis it was clear that the *Ipomoea fistulosa* is rich in Carbohydrates, Cardiac glycosides, and Tannins, *Cyperus longus* is rich in Carbohydrates, Cardiac glycosides, and Quinones, *Hydrilla verticillata* is rich in Carbohydrates, Cardiac glycosides, and Tannins and *Polygonum glabrum* is rich in Carbohydrates, Cardiac glycosides, and Tannins. The important thing is that all plant samples contain three common and abundant secondary metabolites. The data are shown in (Table 2,3,4, and 5) screening of aqueous extracts of different parts of four plants. The results revealed the presence of important constituents in the plants studied.

Table 2. Phytochemical analysis of *Ipomoea fistulosa.*

Tests	*Ipomoea fistulosa*		
	Petroleum Ether	Chloroform	Methanol
Alkaloids	-	+	+
Carbohydrates	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	-
Phenols	-	+	+
Phlobatannins	-	-	-
Aminoacidsand Proteins	-	-	-
Saponins	-	-	-
Sterols	-	-	-
Tannins	+	+	+
Terpenoids	-	-	-
Quinones	+	-	+
Oxalates	-	+	-
Fats and Oils	-	-	-

Table 3. Phytochemical analysis of *Hydrilla verticillata.*

Tests	*Hydrilla verticillata*		
	Petroleum Ether	Chloroform	Methanol
Alkaloids	-	+	+
Carbohydrates	+	+	+
Cardiac glycosdes	+	+	+
Flavonoids	+	+	-
Phenols	-	-	+
Phlobatannins	-	-	-
Aminoacidsand Proteins	-	-	-
Saponins	-	-	-
Sterols	-	-	-
Tannins	+	+	+
Terpenoids	-	-	-
Quinones	-	-	-
Oxalates	-	+	+
Fats and Oils	+	-	-

Table 4. Phytochemical analysis of *Cyperus longus.*

Tests	*Cyperus longus*		
	Petroleum Ether	Chloroform	Methanol
Alkaloids	-	-	+
Carbohydrates	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	-	-	+
Phenols	-	-	+
Phlobatannins	-	-	-
Amino acids and Proteins	-	-	-
Saponins	-	-	-
Sterols	-	-	-
Tannins	-	+	+
Terpenoids	-	-	-
Quinones	+	+	+
Oxalates	-	-	-
Fats and Oils	+	-	-

Table 5. Phytochemical analysis of *Polygonum glabrum*.

Tests	*Polygonum glabrum*		
	Petroleum Ether	Chloroform	Methanol
Alkaloids	-	-	-
Carbohydrates	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	-	-
Phenols	-	+	+
Phlobatannins	-	-	-
Amino acids and Proteins	-	-	-
Saponins	-	-	+
Sterols	-	-	+
Tannins	+	+	+
Terpenoids	-	-	-
Quinones	+	-	-
Oxalates	-	+	-
Fats and Oils	-	-	-

The inhabitance's wide range of phytochemical components indicates that the plant could be used in the aggregation of ways that may be beneficiary to the population. A predominant part of natural products from plants, biomolecules, and secondary metabolites habitually manifests some kind of biological activity. They are extensively used in human hungry, veterinary, agricultural, and science research and multitudinous in other areas.

Conclusion

Phytochemical screening played an important role in identifying various phytoconstituents present in plant extracts. From the overall scenario, it is concluded that as the plants studied, out of all secondary metabolites, Carbohydrates, Cardiac glycosides, Tannins, and Quinones are found to be abundant in all plant material is mainly dependent on the type of solvent used. Similarly, the test applied for phytochemical analysis determines the presence or absence of a phytochemical in the sample. The study would be more beneficial if the detection, analysis, and separation of the phytoconstituents could be done.

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Declarations

Ethics approval is not applicable. Consent to participate is Not applicable. Consent for publication is not applicable.

Conflict of interest

The authors declare no competing interests.

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