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PRELIMINARY PHYTOCHEMICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *IPOMOEA AQUATICA*FORSSK.FROM ALIYAR RIVERINEIN SOUTH INDIA

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ABSTRACT

Phytoconstituents of the plants are essential source for herbal drug development. Hence, the study on detection about those is playing majorly in the pharmaceutical, botanical, agricultural and other life sciences fields. These chemicals are divided into two categories depend on its metabolism such as primary and secondary metabolites. Among this, the later one acts as ailments in treating many diseases in human and are known as traditional medicine. The aim was to distinguish the components present in the hydroalcoholic extract of the water spinach qualitatively using preliminary phytochemical tests. The experimental procedures were followed for identifying carbohydrates, proteins, aminoacids, alkaloids, glycosides, flavonoids, tannins, steroids &triterpenoids and saponins. From the analysis, primary metabolites such ascarbohydrates, proteins & aminoacids and secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, steroids &triterpenoids were found positive for the tests carried out. The presence of greater number of secondary metabolites in the extract of *Ipomoea aquatica*leads the detection, quantification, isolation and characterization of these constituents by means of modernized, simple & sensitive analytical techniques.

KEYWORDS:*Phytochemical, Screening, Hydroalcoholic, Secondary metabolites, Ipomoea aquatica.*

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INTRODUCTION

Plants are composed entirely of chemicals which are produced through primary or secondary metabolism. They generally have biological activity in the host and play a role in plant growth or defense against challengers, pathogens or carnivores.[1-4] They are regarded as research compounds rather than fundamental nutrients.[4-10] The actions of phyto constituents are antioxidants, hormonal actions, stimulation of enzymes, interference with DNA replication, anti-bacterial effect and Physical action.[11-18]Foods containing phytochemicals are already part of our daily diet. Literally, most foods contain these except for some refined foods such as sugar or alcohol. Fruits and vegetables are also rich in minerals, vitamins and fibers and low in saturated fat. *The analysis* is devoted to the development, improvement, validation and extension of usage of analytical methodology in the plant sciences. The spectrum of coverage is broad, encompassing methods and techniques relevant to the detection (including bio-screening), extraction, separation, purification, identification and quantification of compounds in plant biochemistry, plant cellular and molecular biology, plant biotechnology, the food sciences, agriculture and horticulture.[19-26]Ipomoea aquatica (Family: Convolvulaceae) is a semiaquatic, tropical plant grown as a vegetable for its tender shoots and leaves. It is found throughout the tropical and subtropical regions of the world, although it is not known where it originated. This plant is known in English as water spinach, river spinach, water morning glory, water convolvulus, or by the more ambiguous names Chinese spinach, Chinese Watercress, Chinese convolvulus, swampcabbage or kangkong in Southeast Asia. In South India, the leaves are finely chopped and mixed with grated coconut to prepare thoran, a dish in Kerala. The same dish in Tamil Nadu is prepared as thuvaiyal or kootu.[27-34] To achieve a suitable concentration of the active ingredients contained in the plants and that their action can be more effective, it is necessary to perform several procedures through which are extracted the compounds with the adequate solvents, chosen according to the solubility and stability of the beneficial substances. The main extraction methods are maceration, percolation, digestion, infusion and decoction.[35-46] To reveal the phytoconstituents being part of the selected herb through appropriate chemical tests.

MATERIALS AND METHODS

Materials

The aimed plant contents are given in the table 1. The instruments, chemicals/reagents and glass wares/apparatus effective for the study are illustrated in the table 2, table 3 and table 4 subsequently.

Table 1
Plant details

S. No.	Parameters	Subject
1.	Plant Name	Water Spinach
2.	Botanical Name	*Ipomoea aquatica* FORSSK.
3.	Family	Convolvulaceae
4.	Location	Parambikulam – Aliyar Riverine, Pollachi
5.	Part of the plant	Whole plant
6.	Authentication No.	BSI/SRC/5/23/2017/Tech./3269
7.	Place of Authentication	BSI, Coimbatore-641003, Tamil Nadu

Table 2
Instruments used

S. No.	Name of the Instrument	Model Name
1.	Precision Balance	Wensar
2.	Hot plate	Cintex
3.	Ultra Sonicator	Labman
4.	Electrical Water bath	Technico

Table 3
Chemicals/Reagents used

S.No.	Name of the Reagent	Company	Location
1.	Petroleum Benzine boiling range 60.0^0C-80.0^0C GR (Petroleum ether)	Merck Specialities Private Limited	Mumbai – 400 018
2.	Pyridine GR		
3.	Trichloro acetic acid		
4.	Sodium nitro prusside dehydrate purified		
5.	o-toluidine	Nice Chemicals	Kochi – 682 024
6.	Barfoed's reagent	Oxford Laboratory	Mumbai – 400 002
7.	Ethanol AR 99.9%	Jiangsu Huaxi International Trade Co., Ltd.	China
8.	Zinc (metal) Powder	Laboratory Rasayan	
9.	Gelatin, Extra pure	Hi Media Laboratories Private Limited	Mumbai – 400 086
10	Potassium dichromate	Qualigens Fine Chemicals	Mumbai – 400 030
11	Sulphur powder		

12	Distilled water		
13	Seliwanoff's reagent LR	Reachem Laboratory chemicals Private Limited	Chennai – 600 098
14	Molisch's reagent LR		
15	1-naphthol LR		
16	Dimethyl sulphoxide LR		
17	Toluene (Sulphur free)		
18	Formic acid LR		
19	Phenyl hydrazine hydrochloride LR		
20	Sodium oxalate LR		
21	Dimethyl formamide LR		
22	Ammonia solution LR		
23	Benedict's quantitative reagent LR		
24	Barium chloride LR		
25	Fehling Solution A LR	Chemspure	Chennai – 600 098
26	Acetic acid Glacial LR		
27	Acetone LR		
28	Biuret reagent	LobaChemie Private Limited	Mumbai – 400 005
29	Mayer's solution		
30	Fehling Solution No.2		
31	Bial's reagent		
32	Picric acid		
33	Ferric chloride		
34	Ammonium oxalate LR		
35	Methanol LR	S d Fine Chemicals Limited	Mumbai – 400 030
36	Potassium iodide		
37	Oxalic acid		
38	Sodium hydroxide pellets purified		

Table 4
Glasswares/Apparatus used

S. No.	Name of the Glassware	Capacity	Brand Name
1.	Round bottomed flask	1000.0ml	Riviera
2.	Funnel	Medium Size	Sh Borosilicate Glass
3.	Beaker	1000.0ml	Borosilicate Glass
4.	Measuring cylinder	10.0ml	Riviera
5.	Measuring cylinder	50.0ml	Sh Borosilicate Glass
6.	China dish	Big & Small size	Chinese Porcelain
7.	Stirrer	Small size	Sh Borosilicate Glass
8.	Conical flask	250.0ml	Borosilicate Glass
9.	Test tubes	10.0ml	Borosilicate Glass
10.	Pipettes	5.0ml	Borosilicate Glass

Miscellaneous
Aluminium foil, Muslin cloth, Filter paper, Tripod stand, Test tube holders and test tube stands and butter paper.

METHODS

Plant Collection, drying and powdering
The plant was collected from Parambikulam – Aliyar Riverine in Pollachi, Tamil Nadu. The collected portions of the plant were washed with distilled water three times. They were allowed to dry under shade kept over the news paper. Then the half dried portions were cut into small pieces using stainless steel knife and kept under shade only for drying completely. It took 22 days for complete drying. The dried material was pulverized into coarse powder by means of manual blender. The powdered plant material was stored in air tight containers at 4.0^0C for further use. The weight of powder obtained was 450.0g.[47-52]

Maceration
350.0g of coarse powder of drug was weighed and was taken in a 5000.0ml Round bottomed flask. Petroleum ether was added to remove the fatty matters associated with the powder. The solvent retained was evaporated at room temperature after rinsing for few minutes. Then the dried defatted powder was immersed in 2000.0ml of solvents which comprises 1000.0ml of distilled water and 1000.0ml of ethanol. The content was closed with aluminium foil and kept for cold maceration at ambient temperature with occasional shaking to bring about rapid equilibrium between intra and extra cellular fluids thereby bringing fresh menstrum to the particle surface for further extraction. The contents would take 3 days for imbibitions and another 4 days for extraction. Wherefore, totally the content was kept for 7 days of maceration. The volume of menstrum retained after extraction was 1700.0ml.After the mentioned period, the content of extraction was strained through a muslin cloth. The marc was separated from the menstrum. The extract was divided into two portions in two separate 1000.0ml beakers and kept at 40.0^0C for concentration. After 4 days, the concentrated extract was poured into a previously weighed china dish of big size for evaporation at the same temperature. After 3 days, the completely dried extract was cooled to room temperature and weighed. The percentage yield was calculated.[53-54]

Preliminary phytochemicals Evaluation
Tests for Primary metabolites
Tests for Carbohydrates
Molisch's Test
1.0ml of reagent was combined in 2.0ml of test solution and 1.0ml concentrated sulphuric acid was added. Appearance of red to violet ring at the junction of the two

liquids depending on the amount of sugar designated the occupancy of carbohydrates.

Iodine test
0.5ml of iodine solution was assorted with 1.0ml of the solution. Formation of deep blue colour pointed out the being of starch.

Fehling's test
1.0ml of Fehling solution A and 1.0ml of Fehling solution B was mixed and added to 1.0ml of test solution. It was kept for boiling. Formation of yellow to red precipitate marked the existence of carbohydrates.

Benedict's test
2.0ml of Benedict's was compounded with 2.0ml of test solution and boiled in a water bath. Formation of red, yellow or green colour/precipitate depending on the sugar concentration illustrated the subsistence of carbohydrates.

Barfoed's test
2.0ml of reagent was embodied with 1.0ml of the test solution. Boiled and waited for few minutes. Appearance of brick-red precipitate proved the presence of monosaccharide.

Seliwanoff's test
2.0ml of reagent was mixed with 1.0ml of test solution and boiled. Formation of deep red colour was due to the existence of ketoses.

Bial's Test for pentoses
5.0ml of reagent was combined with 1.0ml of test solution and warm slowly. Formation of green colour or precipitate signified the closeness of pentoses.

Osazone test
0.5g of phenyl hydrazine hydrochloride and 0.1g of sodium acetate were added in 5.0ml test solution. 5.0ml glacial acetic acid was added to that. Boiled in a water bath and cooled slowly.Needle – shaped yellow osazone crystals: Glucose, Fructose and Mannose.Mushroom shaped crystals: Lactose.Flower – shaped crystals: Maltose.

Tests for Proteins
Biuret test
2.0ml of test solution was united with 2.0ml of reagent. Formation of violet to pink colour showed the presence of proteins.

Millon's test
2.0ml of test solution was combined with 2.0ml of reagent and boiled. Formation of red colour made the commorance of proteins.

Xanthoprotein test
2.0ml of test solution was mixed with 2.0ml of concentrated sulphuric acid. Formation of white precipitate symbolized the impression of proteins.

Precipitation test
2.0ml of test solution was assorted with 2.0ml of 5% mercurous chloride or 5% ammonium sulphate solution. Formation of white precipitate specified the rise of proteins.

Lead acetate test
2.0ml of test solution was added with 2.0ml of 40% sodium hydroxide solution and 0.5ml of lead acetate solution and boiled. Formation of black to brown precipitate recorded the carriage of proteins.

Test with Trichloroacetic acid
To the test solution, trichloroacetic acid was added. Formation of precipitate denoted the outward aspect of proteins.

Hydrolysis test
The solution was hydrolyzed with hydrochloric acid or sulphuric acid. Then the Ninhydrine test was carried out for amino acids.

Tests for Amino acids
Ninhydrine test
To 2.0ml of test solution, 1.0ml of 5% Ninhydrine solution was added and boiled for 5 minutes in a water bath. Formation of blue-purple colour evidenced the characteristic of amino acids.

Tyrosine test
To 2.0ml of test solution, 1.0ml of Millon's reagent was combined and boiled. Formation of dark red colour implied the presence of amino acids.

Tryptophan test
To 2.0ml of test solution, 1.0ml of glyoxalic acid and concentrated sulphuric acid were added. Appearance of red violet ring at the junction of two layers represented the location of amino acids.

Cysteine test
3.0ml of test solution was mixed with 0.5ml of 40% sodium hydroxide solution and 0.5ml of 10% lead acetate solution and boiled. Formation of black precipitate expressed the address of amino acids.

Tests for Secondary metabolites
Tests for Alkaloids
Dragendorff's test
To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was mixed. Formation of reddish brown precipitate connoted the occupancy of alkaloids.

Hager's test
To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was assorted. Formation of yellow colour evinced the attendance of alkaloids.

Mayer's test
To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was added. Formation of reddish brown precipitate suggested the residence of alkaloids.

Wagner's test
To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was poured. Formation of reddish brown precipitate revealed the existence of alkaloids.

Tannic acid test
To 2.0ml filtrate of plant drug extract, 2.0ml of tannic acid solution was mixed. Formation of buff colour precipitate mean the appearance of alkaloids.

Tests for Glycosides
General test
Solution A
To the hydroalcoholic extract, Fehling solution was added.

Solution B
To the hydroalcoholic extract, Sulphuric acid and Fehling solution were added. If solution B had darker colour than solution A or if sugar content was high in solution B than solution A indicated the presence of alkaloids. Here, acid hydrolyzes glycone – aglycone moiety and thus content was increased in solution B.

Test for Cardiac glycosides
Legal's test
1.0ml of test solution was mixed with 2.0ml of pyridine and sodium nitroprusside solution. Formation of pink or red colour imported the occurance of cardiac glycosides.

Test for anthraquinone glycosides
Borntrager's test
A little quantity of aqueous solution of sample was taken. Sulphuric acid was added to it and then carbon tetrachloride or ether was added. Separate the organic layer and shake with dilute ammonia. Formation of rose pink colour of ammonia layer read the omnipresence of anthraquinone glycosides.

Test for coumarin glycosides
Alkali test
The test solution was mixed with alkali (40% potassium hydroxide or sodium hydroxide solution). Observation of blue green fluorescence confessed the observance of coumarin glycosides.

Tests for Flavonoids
Shinoda test
Magnesium powder and a few drops of concentrated hydrochloric acid or sulphuric acid were added to 2.0ml of sample solution. Formation of orange, pink, red or purple colour indicated the presence of flavones, flavonols and xanthones. Formation of weak pink to magenta colour/no colour acknowledged the ubiquity of flavonones and flavonols.

Sulphuric acid test
Sulphuric acid was added to the sample solution. Formation of deep yellow colour indicated the presence of flavones and flavonols. Formation of red or red-bluish colour indicated the presence of chalcones and aurones. Formation of orange to red colour confirmed the composure of flavonones.

Lead acetate test
The test solution was mixed with lead acetate solution. Formation of yellow precipitate admitted the ghost of flavonoids.

Alkali test
The test solution was treated with increasing amount of sodium hydroxide solution. Yellow colouration which was decolourized after addition of acid.

Zinc hydrochloride test
To the test solution, a mixture of zinc dust and concentrated hydrochloric acid was added. It should give red colour after few minutes.

Tests for tannins
Ferric chloride test
To 2.0ml of test solution, 5.0% ferric chloride solution was added. Appearance of blue colour indicated the presence of hydrolysable tannins and of green colour disclosed the inhabitance of condensed tannins.

Gelatin – Salt test
Three test tubes of extract solution were prepared. To the first, 1.0% sodium chloride solution was added. To the second, 1.0% sodium chloride solution and 5% gelatin solution were added. To the third, ferric chloride solution was added. Formation of a precipitate in the second treatment suggests the presence of tannins and a positive response after addition of ferric chloride to the third portion supported this inference.

Lead acetate test
The test solution was mixed with lead acetate solution. Formation of white precipitate proclaimed the ubiety of tannins.

Bromine water test
The test solution was mixed with bromine water. Discoloration of original solution narrated the presence of tannins.

Dilute iodine test
The test solution was mixed with dilute iodine solution. Formation of red colour exposed the residence of tannins.

Potassium dichromate test
The test solution was mixed with potassium dichromatesolution. Formation of red precipitate enumerated the potentiality of tannins.

Dilute nitric acid test
The test solution was mixed with dilute nitric acidsolution. Formation of red to yellow colour divulged the latency of tannins.

Tests for Steroids and Triterpenoids

Salkowski's test
1-2mg of the sample dissolved in 1.0ml of chloroform and 1.0ml of concentrated sulphuric acid. Formation of red colour at lower layer indicated the presence of steroiods and formation of yellow colour at lower layer predicted the demeanor of triterpenoids.

Sulfur powder test
Small amount of sulphur powder to the test solution was added and it had sink at the bottom.

Tests for Saponins
Foam test

Aqueous solution of a saponin containing sample producing foam was shaked which should be stable for 15 seconds or more.

Haemolytic test

Red blood sample with sufficient quantity of extract solution was shaked and observed. Formation of clear red solution revealed the vicinity of saponins.[55-59]

RESULTS AND DISCUSSION

The evaluation patterns are depicted in the Figure 1, Figure 2 and Table 5.

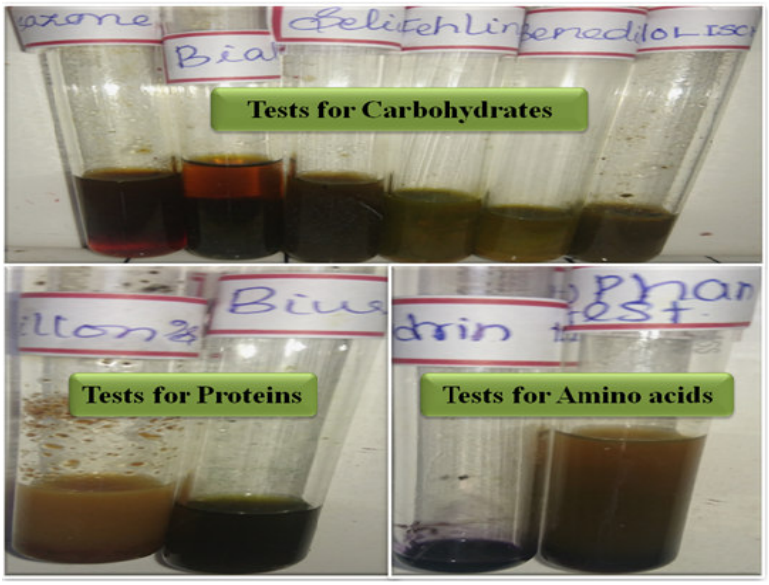

Figure 1
Tests for Primary metabolites

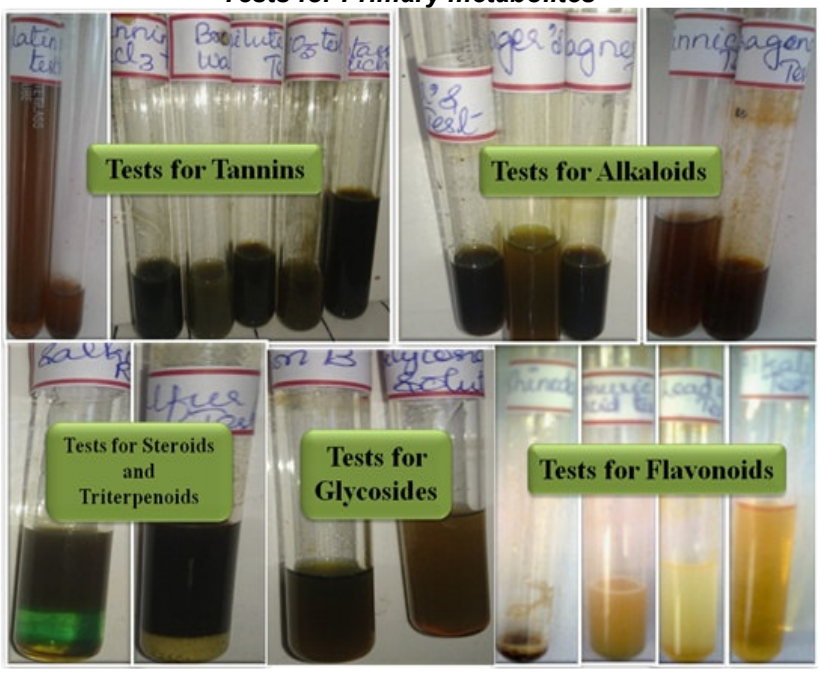

Figure 2
Tests for Secondary metabolites

Table 5
Preliminary Phytochemical evaluation

S. No.	Metabolites	Phyto-constituents	Chemical test	Observation	Inference
1.	Primary metabolites	Carbohydrates	Molisch's Test	Reddish violet ring	+
2.			Iodine test	No blue colour	-
3.			Fehling's test	Red precipitate	+
4.			Benedict's test	Red precipitate	+ (Reducing sugar)
5.			Barfoed's test	No brick red precipitate	-
6.			Seliwanoff's test	No deep red precipitate	- (Ketoses)
7.			Bial's Test	Green precipitate	+ (Pentoses)
8.			Osazone test	Needle – shaped crystals	+ (Glucose and Mannose)
9.		Proteins	Biuret test	Green colour	+
10			Millon's test	Red colour	+
11			Xanthoprotein test	No white precipitate	-
12			Precipitation test	No white precipitate	-
13			Lead acetate test	No black to brown colour	-
14			Test with Trichloroacetic acid	No precipitate	-
15			Hydrolysis test	No characteristic change	-
16		Amino acids	Ninhydrine test	Purple colour	+
17			Tyrosine test	Dark red colour	+
18			Tryptophan test	Red – violet ring	+
19			Cysteine test	No black precipitate	-
20	Secondary metabolites	Alkaloids	Dragendorff's test	Reddish brown precipitate	+
21			Hager's test	Yellow colour	+
22			Mayer's test	Reddish brown precipitate	+
23			Wagner's test	Reddish brown precipitate	+
24			Tannic acid test	Buff coloured precipitate	+
25		Glycosides	General test	Solution B was darker colour than solution A	+
26			Legal's test	No pink or red colour	- (Cardiac glycosides)
27			Borntrager's test	No rose pink at the ammoniacal layer	- (Anthra-quinone glycosides)
28			Alkali test	No blue – green fluorescence	- (Coumarin glycosides)
29		Flavonoids	Shinoda test	Red colour	+
30			Sulphuric acid test	Yellow colour	+
31			Lead acetate test	Yellow precipitate	+
32			Alkali test	Dark yellow colouration but no decolourization when acid was added	+
33			Zinc hydrochloride test	No red colour on standing	-
34		Tannins	Ferric chloride test	Blue colour	+ (hydrolysable tannins)
35			Gelatin – Salt test	Precipitate formation in the second solution	+
36			Lead acetate test	No white precipitate	-
37			Bromine water test	Discolouration of original solution	+
38			Dilute iodine test	Red colour	+
39			Potassium dichromate test	Red precipitate	+
40			Dilute nitric acid test	Yellow colour	+
41		Steroids and Triterpenoids test	Salkowski's test	Yellow colour at lower layer	+ (Tri-terpenoids)
42			Sulphur powder	Sulphur sinks at the bottom of the solution	+
43		Saponins	Foam test	No foam	-
44			Haemolytic test	No clear red solution	-

Note: '+' indicates the presence of the test and '–' indicates the absence of the test

From the chemical tests, it was found that the primary metabolites such as carbohydrates, proteins and amino acids and the secondary metabolites like alkaloids, glycosides, flavonoids, tannins, triterpenoids and saponins were exhibited in the hydroalcoholic plant extract. These secondary metabolites could be isolated and characterized using various analytical techniques.

CONCLUSION

The existence of maximum secondary metabolites in the hydroalcoholic extract of *Ipomoea aquatica*leads the detection, quantification, isolation and characterization of these constituents by means of rejuvenated, simple & sensitive analytical techniques.This shows the way to develop herbal formulations containing the constituents/plant in treating many life threatening diseases.

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CONFLICT OF INTEREST

Conflict of interest declarednone.

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