



## PRELIMINARY PHYTOCHEMICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *IPOMOEA AQUATICA* FORSSK. FROM ALIYAR RIVERINE IN 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 as carbohydrates, 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.<sup>1-4</sup> They are regarded as research compounds rather than fundamental nutrients.<sup>4-10</sup> The actions of phytoconstituents are antioxidants, hormonal actions, stimulation of enzymes, interference with DNA replication, anti-bacterial effect and Physical action.<sup>11-18</sup> 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.<sup>19-26</sup> *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.<sup>27-34</sup> 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.<sup>35-46</sup> 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	<i>Ipomoea aquatica</i> 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°C-80.0°C 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		
14	Molisch's reagent LR		
15	1-naphthol LR		
16	Dimethyl sulphoxide LR		
17	Toluene (Sulphur free)		
18	Formic acid LR	Reachem Laboratory chemicals Private Limited	Chennai – 600 098
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		
26	Acetic acid Glacial LR	Chemspure	Chennai – 600 098
27	Acetone LR		
28	Biuret reagent		
29	Mayer's solution		
30	Fehling Solution No.2		
31	Bial's reagent	LobaChemie Private Limited	Mumbai – 400 005
32	Picric acid		
33	Ferric chloride		
34	Ammonium oxalate LR		
35	Methanol LR		
36	Potassium iodide	S d Fine Chemicals Limited	Mumbai – 400 030
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°C for further use. The weight of powder obtained was 450.0g.<sup>47-52</sup>

### 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°C 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.<sup>53-54</sup>

### 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 occurrence 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 xanthenes. 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 inhabitation 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 dichromate solution. Formation of red precipitate enumerated the potentiality of tannins.

**Dilute nitric acid test**

The test solution was mixed with dilute nitric acid solution. 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 steroids 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**

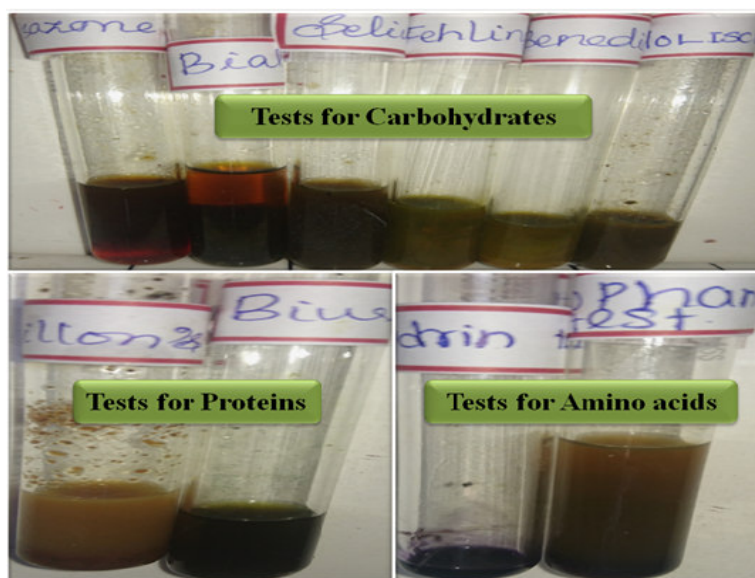
Aqueous solution of a saponin containing sample producing foam was shaken which should be stable for 15 seconds or more.

**Haemolytic test**

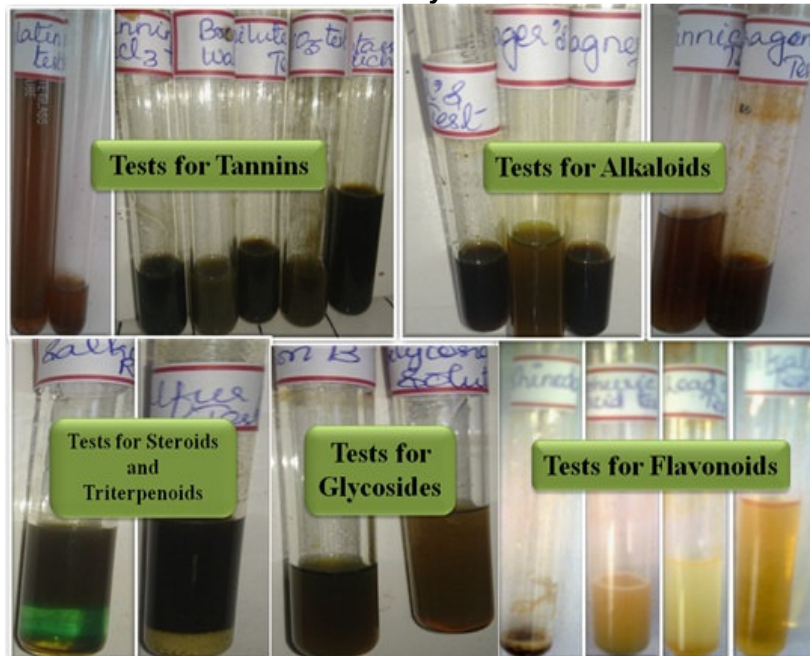
Red blood sample with sufficient quantity of extract solution was shaken and observed. Formation of clear red solution revealed the vicinity of saponins.<sup>55-59</sup>

**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	+
5.			Barfoed's test	No brick red precipitate	-
6.			Seliwanoff's test	No deep red precipitate	-
7.			Bial's Test	Green precipitate	+
8.			Osazone test	Needle – shaped crystals	+
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.			Ninhydrine test	Purple colour	+
17.		Amino acids	Tyrosine test	Dark red colour	+
18.			Tryptophan test	Red – violet ring	+
19.			Cysteine test	No black precipitate	-
20.			Dragendorff's test	Reddish brown precipitate	+
21.		Alkaloids	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.	General test		Solution B was darker colour than solution A	+	
26.	Legal's test		No pink or red colour	-	
27.	Glycosides	Borntrager's test	No rose pink at the ammoniacal layer	-	
28.		Alkali test	No blue – green fluorescence	-	
29.	Secondary metabolites	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.		Tannins	Zinc hydrochloride test	No red colour on standing	-
34.			Ferric chloride test	Blue colour	+
35.			Gelatin – Salt test	Precipitate formation in the second solution	+
36.			Lead acetate test	No white precipitate	-
37.	Steroids and Triterpenoids	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.	Saponins	Salkowski's test	Yellow colour at lower layer	+	
42.		Sulphur powder	Sulphur sinks at the bottom of the solution	+	
43.	Haemolytic test	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

## REFERENCES

- Molyneux, RJ, Lee, ST, Gardner, DR, Panter, KE, James, LF. "Phytochemicals: the good, the bad and the ugly?". *Phytochemistry*. 2007; 68(22-24):2973-85. doi:10.1016/j.phytochem.2007.09.004. PMID 17950388.
- Chitrajit Malakar and Pinak Pani Nath Choudhury. Pharmacological Potentiality and Medicinal Uses of *Ipomoea Aquatica* Forsk: A Review. *Asian J Pharm Clin Res*. 2015; 8(2):60-3.
- Manjulika Yadav, Sanjukta Chatterji, Sharad Kumar Gupta and Geeta Watal. Preliminary Phytochemical Screening of Six Medicinal Plants used in Traditional Medicine. *Int J Pharm Pharm Sci*. 2014;6(5):539-42.
- Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh and Abhishek Gupta. *Phytochemistry of Medicinal Plants*. *J Pharmacogn Phytochem*. 2013;1(6):168-82.
- Selvaraj K, Ranjana Chowdhury and Chiranjib Bhattacharjee. Isolation and structural elucidation of flavonoids from aquatic fern *Azolla microphylla* and evaluation of free radical scavenging activity. *Int J Pharm Pharm Sci*. 2013;5(3):743-49.
- Salna KP, Sreejith K, Uthiralingam M, Mithu A Prince, John Milton MC and Albin T Fleming. A Comparative Study of Phytochemicals Investigation of *Andrographis paniculata* and *Murrayakoenigii*. *Int J Pharm Pharm Sci*. 2011;3(3):291-92.
- Khadabadi SS, Deore SL and Baviskar BA. *Experimental Phytopharmacognosy – A Comprehensive guide*. Nirali Prakashan, Pune, Maharashtra. Second edition, 2013:p.3.1-3.13, A 1.1-2.8.
- Ranjith Kumar D, Mohan S., Vanathi P., Prakash G., Sasikala M., Karpakavalli M. Development and Validation of UV Spectroscopic Method for Estimation of Milrinone in Formulation. *World J Pharm Res*. 2016,5(4):1501-08.
- Sasikala M., Karpakavalli M., Ranjithkumar D., Prakash G., Vanathi P., Mohan S. In-vitro free radical scavenging activity studies of extracts and constituents/plant in treating many life threatening diseases.
- isolated compounds of *Eugenia jambolana* lam. Seeds. *World J Pharm Res*. 2016;5(6): 2021-30.
- Sasikala M., Karpakavalli M., Vanathi P., Ranjithkumar D., Prakash G., Mohan S. Validated UV spectrophotometric method development for evaluation and stability analysis of Tolvaptan in bulk drug and its formulation. *World J Pharm Pharm Sci* 2016;5(6):2048-63.
- Karpakavalli M, Sangilimuthu AY, Sasikala M, Komala M, Ranjithkumar D and Mohan S. A Predominant Evaluation of Viable Templates for Asthma Therapy Exerting an In silico Analog Method, Virtual Library Screening. *J Chem Pharm Res*. 2017; 9(1):43-53.
- Sasikala M, Sundara Ganapathy R, Karpakavalli M and Mohan S. Chemotaxonomical and pharmacological review on medicinal plants in temperate region. *Int J Pharm Technol*. 2017;9(1): 29165-72.
- Wallis TE. *Text Book of Pharmacognosy*. 3rd edn. CBS Publishers and Distributors, Delhi. 1985.
- Evans WC. *Trease and Evan's Pharmacognosy*. 14th Edition. W. B. Saunders Company Limited, London. 1998: p. 119.
- Sambamurthy K. *Pharmaceutical Engineering*. Reprint, New Age International (P.) Ltd., New Delhi. 2002:p. 173-94.
- Khandelwal K R. *Practical Pharmacognosy – Techniques and Experiments*. 9th Edn. Nirali Prakashan, Pune. 2002:82.
- Anonymous, the Ayurvedic Pharmacopoeia of India, Vol-I, the Controller of Publication, New Delhi, 2001, 321-22.
- Anonymous, *The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products*. Vol-2 (B). CSIR, New Delhi. 1998:49-52.
- Kokate CK, Purohit AP and Gokhale SB. *Pharmacognosy*, 51<sup>st</sup> edition, Nirali Prakashan, Pune, Maharashtra. 2015: p. A.23-A.27.
- Junli Hu, Fuyong Wu, Shengchun Wu, Cheung Lung Lam, Xiangui Lin and Ming Hung Wong. Biochar and Glomus caledonum Influence Cd Accumulation of Upland Kangkong (*Ipomoea aquatica* Forsk.) Intercropped with Alfred Stonecrop (*Sedum alfredii* Hance). *Scientific Reports*. 2014;4: 4671. DOI: 10.1038/srep04671.



21. DhoreMukundManohar and LachurePareshShyaam. Survey of Aquatic Macrophyte diversity in Yavatmal District, Maharashtra, India. Int. J. of Life Sciences. 2014;2(3):273-75.
22. Parimala K and Sukumar D. Phytochemical Studies on the Flowers of *Ipomoea Aquatica*. International J Sci Res. 2013;2(6):53-5.
23. ElijaKhatiwora, Vaishali B. Adsul, ManikKulkarni, DeshpandeNRandKashalkar RV. Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. J Pharm Res. 2012;5(1):150-52.
24. ShirinAkhtar, ChandanKarak, PriyankaBiswas, Arup Chattopadhyay and PranabHazra. Indigenous Leafy Vegetables: A Potential Source of  $\beta$ -Carotene and Ascorbic Acid. International Veg Sci. 2012;18:370-75.
25. ElijaKhatiwora, Vaishali B. Adsula , Anjali D. Ruikar, TusharGadkari, DeshpandeN Rand Kashalkar RV. Isolation and Characterization of Dibutyl phthalate from *Ipomoea carnea* stem and its Quantification by HPTLC. J Pharm Res. 2011;4(10):3264-65.
26. Hongfei Fu, BijunXie, Shaojun Ma, Xinrong Zhu, Gang Fan and Siyi Pan. Evaluation of antioxidant activities of principal carotenoids available in water spinach (*Ipomoea aquatica*). J. Food Compos. Anal. 2011;24:288-97.
27. KanchanLataVishwakarma and VeenapaniDubey. Nutritional analysis of indigenous wild edible herbs used in eastern Chhattisgarh, India. Emir. J. Food Agric. 2011;23(6):554-60.
28. Mital N. Manvar. Pharmacognostical Investigations on *Ipomoea Aquatica* Forsk. Int J Pharm Sci Res. 2011;2(11):2812-15.
29. Ratnatilaka A, Yakandawala D, Rupasinghe N and Ratnayake K; Poisoning of 'binthamburu' (*Ipomoea asarifolia*) due to misidentification as 'kankun' (*Ipomoea aquatica*); Ceylon Med J .2010;55(2):54-6.
30. Maria Greger and BeataDabrowska. Influence of nutrient level on methyl mercury content in water spinach. Environ Toxicolchem. 2010;29(8):1735-39.
31. Vimal Kumar, GogoiBJ, MeghvansiMK, Lokendra Singh, SrivastavaRB, and Deka DC Determining The Antioxidant Activity of Certain Medicinal Plants of Sonitpur, (Assam), India Using DPPH Assay. J. phytol.. 2009;1(1):49-56.
32. ShantaIS, Anisuzzaman, Mohanta UK, Farjana T and Mondal MMH. Efficacy of some Indigenous Plants in Controlling Vector Snails of Trematode Parasites of Medical and Veterinary Importance. Bangladesh J Vet Med . 2008;6(1):107-14.
33. AruniWeerasinghe, SanduniAriyawansa and RohanWeerasooriya. Phyto-remediation potential of *Ipomoea aquatica* for Cr(VI) mitigation. Chemosphere. 2008;70(3):521-24.
34. Wang BL and Xie ZM. Effects of phosphorus application on translocation of lead, zinc and cadmium in the soil-plant system. Huan Jing keXue= HuanjingKexue; 2008;29(11):3225-29.
35. Balasuriya BMGK and Dharmaratne HRW. Cytotoxicity and antioxidant activity of green leafy vegetables consumed in Sri Lanka. J NATL SCI FOUND. 2007;35(4):255-58.
36. Daniel F. Austin. Water Spinach (*Ipomoea aquatica*, Convolvulaceae) A food gone wild. Ethnobot Res Appl. 2007;5:123-46.
37. Miao Li, Yue-Jin Wu, Zeng-Liang Yu, Guo-Ping Sheng and Han-Qing Yu. Nitrogen removal from eutrophic water by floating-bed-grown water spinach (*Ipomoea aquatica* Forsk.) with ion implantation. Water Research. 2007;41(14):3152-58.
38. Yan L , Wang SH , Luo WG , Huang J and Zhong QS. Study on the oxygen condition in subsurface flow wetlands in operation. Huan Jing keXue= HuanjingKexue. 2006;27(10):2009-13.
39. Zhou X, Wang J, Xue L, Xu X and Yang L. N and P removal characters of eutrophic water body under planted float. The J Appl Eco. 2005;16(11):2199-03.
40. Lu Jindeng, ShuaiFangmin, Zhao Liya, Kang Qun and Li Zhaohua. Choosing plants for treating eutrophic water by artificial floating rafts. J Hubei Univ (Natural Science). 2005;27(4):402-04.
41. Bhat SC, Goswami S and Ghosh UC. Removal of trace chromium (VI) from contaminated water: bio-sorption by *Ipomeeaquatic*. J Environ SciEng. 2005;47(4):316-21.
42. Li Xiuling, Li Wei, Li Yeguang, GuiJianping, GengYahong and Hu Hongjun. A primary study on the removal of eutrophication and restraint of algae by some plants. WuhanBotanical Research. 2005;23(1):53-7.
43. Sun Zhaohai, ZhengChunrong, Zhou Dongmei, Yang Linzhang and Chen Huaiman. Effects of soil Pb pollution on growth of *Brassica chinensis* and *Ipomoea aquatica* and urease activities. Rural Eco-Environment. 2005;21(1):38-43.
44. Qin Houguo , Ye Zhengxiang , Huang Shuijin, Ding Jian and LuoRenhua. The correlation of the different host plants with preference level, life duration and survival rate of *SpodopteralituraFabricius*. Chinese Journal of Eco-Agriculture. 2004;12(2):40-2.
45. Cai Q, Mo C, Zeng Q, Li Y, Xiao K, Li H, XuG, Wang B and Wu Q. Accumulation of di-(2-ethylhexyl) phthalate in various genotype *Ipomoea aquatica*-paddy soil system. The Journal of Applied Ecology. 2004;15(8):1455-58.
46. Lin Dongjiao, Tang Shujun, He Jiawen, LuoJian, Li Yan, Zhang Jiean and Liu Shizhe. Purification of piggery wastewater by *Ipomoea aquatica* and *Eichhorniacrassipes* in a floating culture system. Journal of South China Agricultural University. 2004;25(3):14-7.
47. Cai Q, Mo C, Zhu X, Wu Q, Wang B, Jiang C and Li H. Effect of municipal sludge and chemical fertilizers on phthalic acid esters (PAEs) contents in *Ipomoea aquatica* grown on paddy soils. The Journal of Applied Ecology. 2003;14(11):2001-05.
48. Zhao Xiaoying, Liu Mingyue, Xiao Jie, Song Yong and Liu Xuanming. Effects of Nitrogen,

- Kalium, Molybdenum on the Nitrate Accumulation and Nitrate Reductase Activity of Water Spinach. Journal of Hunan Agricultural University. 2003;29(3):239-42.
49. AgnetaGothberg, Maria Greger and Bengt-Erik Bengtsson. Accumulation of heavy metals in water spinach (*Ipomoea aquatica*) cultivated in the Bangkok region, Thailand. Environmental Toxicology and Chemistry. 2002;21(9):1934-39.
  50. Hu Shiyi and Yuan Zongfei. Distribution of the cytoplasmic DNA in male cells of the Convolvulaceae and its systematic significance. ActaPhytotaxonomicaSinica. 2002;40(6):511-16.
  51. Koo HuiMiean and Suhaila Mohamed. Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. J. Agricultural and Food Chem. 2001;49(6):3106–12.
  52. Kanungo VK, Sinha S and Naik ML. Net primary productivity of some aquatic macrophytes in sewage-sullage mixture. Journal of Environmental Biology. 2001;22(3):219-23.
  53. SugandhikaMalalavidhane T, NalinieWickramasinghe S M D and Jansz E R. Oral hypoglycaemic activity of *Ipomoea aquatica*; Journal of Ethnopharmacology. 2000;72:293 – 98.
  54. En-Jang Sun and Fen-Yi Wu. Wu-Nickel indicator symptoms on water spinach along-vein necrosis as indicator symptom on water spinach caused by nickel in water culture. Bot. Bull. Acad. Sin. 1998;39:255-59.
  55. Zhenbo Yang. The potential of using *Eichhorniacrassipes* and *Ipomoea aquatica* as biomonitors in assessing heavy metal pollution in the Mae Kha Canal and the Mae Ping river in Chiang Mai, Northern Thailand. Journal of Social Sciences, Thailand. 1997;23:371-80.
  56. Will RBHandAzhariRangga. Determination of carotenoids in Chinese Vegetables. Food Chemistry. 1996;56(4):451-5.
  57. Tang Shaohu , Sun Min and Li Kunpei. Studies on artificial seed of *Ipomoea aquatica*Forsk. ActaHorticulturaeSinica. 1994;21(1):71-5.
  58. Chen BH, Chuang JR, Lin JH and Chiu CP. Quantification of Provitamin A Compounds in Chinese Vegetables by High-Performance Liquid Chromatography. Journal of Food Protection. 1993;56(1):51-4.
  59. Chen BH. Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. Food Chemistry. 1992;45(2):129-34.

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