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Original Article

PRELIMINARY PHYTOCHEMICAL AND STANDARDIZATION PARAMETERS OF *IPOMOEA QUAMOCLIT LINN* WHOLE PLANT- AN ETHNOMEDICINALLY IMPORTANT PLANT

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ABSTRACT

Objective: Preliminary phytochemical studies and proximate analysis of *Ipomoea quamoclit Linn* whole plant.

Methods: The whole plant was collected, shade dried and made into powder. The powdered plant material was studied for its proximate values which include ash values, extractive values, fluorescence analysis and moisture content by standard methods. The powdered plant material was subjected to successive solvent extraction by maceration using petroleum ether, chloroform, ethyl acetate, methanol, water and hydroalcoholic mixture as solvents. All the extracts were subjected to a preliminary phytochemical screening in which chemical tests were carried out for the detection of various phytoconstituents.

Results: Proximate analysis revealed that the dry plant powder has 9.68% total ash, 3.57% acid insoluble ash, 2.95% water soluble ash, 3.49% sulphated ash, 12.92% alcohol soluble extractive value, 9.45% water soluble extractive values, 5.41% ether soluble extractive value and 5.25% moisture content. The whole plant powder found to possess phytoconstituents like alkaloids, carbohydrates, saponins, phenolic compounds, tannins, phytosterols, amino acids, proteins and flavonoids. Fluorescence analysis revealed the behavior of the plant powder when treated with different chemical reagents and observation under UV light at 365 nm.

Conclusion: The present study reveals the preliminary phytochemical and proximate analysis of *Ipomoea quamoclit* whole plant.

Keywords: *Ipomoea quamoclit,* Phytochemical screening, Proximate values, Fluorescence analysis.

INTRODUCTION

Plants were an important source for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants. During the twentieth century, the emphasis gradually shifted from extracting medicinal compounds from plants to making these compounds or their analogues synthetically. Natural products were widely viewed as templates for structure optimization programs designed to make perfect new drugs, referred to by industry as new chemical entities (NCEs) [1]. Today, nearly 80% of the global population turns to plant derived medicines as their first line of defense for maintaining health and combating diseases [2]. *Ipomoea quamoclit* also called as *Quamoclit pinnata* belongs to *Convolvulaceae* family is one of the most commonly seen plant in and around of the living area. In English it is called as Cypress Vine, Indian Pink and Cupid's Flower [3]. In Philippines, the leaves are used as poultices for bleeding haemorrhoids.

The crushed leaves are used for carbuncles. The seeds are used as a laxative. In India, the powdered roots were given as sternutatory (substance that tends to cause sneezing) in Spain, the crushed leaves are used for ulcers and chest pain [4] where as in Siddha Medicine, decoction of leaves and stems is used to treat fever, is also used in diabetes [5] and in Thailand, is used for snake bites and as snuff, as a laxative and for haemorrhoids and in bloody cough [6]. Leaves and stems contain small amounts of alkaloids and cyanogenetic glycosides. Seeds have been reported to contain the resin glycosides, quamoclins I-IV and Jalapin. Pyrrolizidine alkaloids like mono and diesters of platynecine and minalobines like minalobine O and R, ipangulines like ipangualine B_2 and D_{11} [7, 8] and ergoline alkaloids [9] and anthocyanins [10] were identified from the plant. Total alkaloid in seeds was found 0.012% [11].

After conduction a thorough literature review, in the present study an attempt was made to reveal the preliminary phytochemical and proximate analysis of *Ipomoea quamoclit Linn* in a systematic way.

MATERIALS AND METHODS

Collection of plant material

For the present study, *Ipomoea quamoclit* whole plant was collected from the forest area near to the Madanapalli of Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati, A.P., India and a voucher specimen (RIPER/ASK/002) was preserved in Division of Pharmacognosy, RIPER, Anantapuramu for further reference.

Proximate analysis

Proximate analysis includes determination of physical characteristics like ash value, extractive values, moisture content, fluorescence analysis etc. The powder of *Ipomoea quamoclit* was evaluated in terms of ash values, extractive values, and moisture content.

Determination of Ash values

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts naturally occurring in drug or adhering to it. In the present investigation, total ash, acid insoluble ash, water soluble ash and sulphated ash were determined for *Ipomoea quamoclit* plant powder. [12]

Total Ash

2g of *Ipomoea quamoclit* whole plant powder was taken in a tarred silica crucible. The powdered drug was incinerated at a temperature not exceeding 450^0 C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air dried drug. [12]

Acid-Insoluble Ash

Boiled the ash obtained from total ash for 5 minutes with 25 ml of dilute hydrochloric acid, the insoluble matter was collected in a silica

crucible or on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried drug. [12]

Water-Soluble Ash

Boiled the ash for 5 minutes with 25 ml of water, insoluble matter was collected in a tarred silica crucible or on an ash less filter paper, wash with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug. [12]

Sulphated ash

A silica or platinum crucible was heated to redness for 30 min, allowed to cool in desiccator and weighed. 1 gm of powdered *Ipomoea quamoclit* was placed in the crucible and added 2 ml of dilute sulphuric acid (9.8 % w/v). Heated first on a water bath, then cautiously over a flame finally progressively to about 600° C. The incineration was continues until all black particles have disappeared and allowed the crucible to cool. Added few drops of dil. sulphuric acid (9.8 % w/v), heated and incinerated as before and allowed to cool. Added a few drops of a 15.8 % w/v ammonium carbonate solution. Evaporated and incinerated carefully, allowed to cool, weighed and repeated the ignition for 15 min to constant mass. [12]

$$\text{Percentage of Sulphated Ash value} = \frac{\text{Weight of sulphated ash}}{\text{Weight of sample}} \times 100$$

Determination of Extractive Values

Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. Various solvents ranging from polar to non polar are used for determination of extractive values. The solvents used for extraction is in a position to dissolve appreciable quantity of substance desired. Various parameters used to find out the extractive values include *alcohol-soluble extractive value, water soluble extractive value* and *ether soluble extractive value.*

Alcohol-Soluble Extractive value

Macerated 5 g of the coarsely powdered air dried drug with 100 ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105° C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug. [13]

Water-Soluble Extractive value

Water soluble extractive value was determined as directed for the determination of alcohol-soluble extractive, by using 5gm of crude drug and 100ml of chloroform water I.P. as solvent.

Ether soluble extractive value

Macerated 5 g of the coarsely powdered air dried drug with 100 ml of petroleum ether in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105° C to constant weight and weighed. The percentage of ether-soluble extractive was calculated with reference to the air-dried drug. [13]

Moisture content

Moisture content was determined by loss on drying method. Accurately weighed 1.5 gm of powdered *Ipomoea quamoclit* was transferred into a weighed flat and thin porcelain dish. It was dried in an oven at 100°C and then it was cooled in desiccator and weighed. Pre and post heating weight difference was calculated and expressed as percent moisture content. [13]

Fluorescence analysis

The powder was treated with different reagents like and observed under visible, UV light so as to analysis the fluorescence character of the power sample under study. [13]

Extraction

For the present study, successive solvent extraction was carried out using petroleum ether, chloroform, ethyl acetate, methanol, water and hydroalcoholic mixture at a ratio of 3:2 as solvents. The maceration was continued for 72 hours after which, the contents were filtered and concentrated by rota evaporator. Colour, nature and percentage yield were calculated and the extracts were stored in desiccator till further study. [14]

Phytochemical Screening [14, 15]

Detection of alkaloids: Extract was dissolved individually in dilute hydrochloric acid and filtered. The filtrate was further tested with following reagents for the presence of alkaloids.

Dragendroff's Test: Filtrate was treated with potassium bismuth iodide solution (Dragendroff's reagent). Formation of orange red precipitate indicated the presence of alkaloids.

Hager's Test: Filtrate was treated with saturated aqueous solution of picric acid (Hager's reagent). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

Mayer's Test: Filtrate was treated with potassium mercuric iodide solution (Mayer's reagent). Formation of a whitish yellow or cream coloured precipitate indicated the presence of alkaloids.

Wagner's Test: Filtrate was treated with iodine in potassium iodide solution (Wagner's reagent). Formation of reddish brown precipitate indicated the presence of alkaloids.

Detection of carbohydrates: Extract was dissolved in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube, shaken and conc. sulphuric acid added from the side of the test tube. Development of a violet ring at the junction of two liquid confirmed the presence of carbohydrates.

Detection of reducing sugars:

Benedict's test: Filtrate was treated with Benedict's reagent and boiled in a thermostatic water bath for 5 minutes. Formation of an orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrate was acidified with dil. Hydrochloric acid, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Detection of saponins

Foam Test: Small quantity of the extract was shaken with 2 ml of water. Persistence of foam produced for ten minutes indicated the presence of saponins.

Detection of phytosterols: Small quantity of extract dissolved in 5 ml of chloroform was subjected to Salkowski's and Liebermann Burchard's tests for detection of phytosterols.

Salkowski's Test: On adding a few drops of conc. Sulphuric acid and allowing the solution to stand, formation of brown ring indicated the presence of phytosterols in hydroalcoholic extract.

Liebermann Burchard's test: The extract was treated with few drops of acetic anhydride, boiled and cooled. On adding conc. sulphuric acid, formation of a bluish green colour solution confirmed the presence of phytosterols.

Detection of phenolic compounds:

Ferric Chloride Test: The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Lead Acetate Test: The extract was treated with 3 ml of 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds.

Detection of tannins: About 0.5 g of the dried powdered plant material was boiled in 20 ml of water in a test tube and then filtered. On adding a few drops of 0.1% ferric chloride, development of a brownish green or a blue-black colouration indicated the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: The extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on further addition of dilute acid, indicated the presence of flavonoids.

Lead acetate Test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids.

Ferric chloride Test: Addition of a few drops of ferric chloride solution to the extract solution resulted in the development of intense green colour.

Detection of proteins and amino acids: The extract solution (100 mg in 10 ml of distilled water) was filtered through Whatman No.1 filter paper. The filtrate is tested for the presence of protein and amino acids.

Millon's Test: The test solution is treated with few drops of Millon's reagents. A white precipitate is formed which when warmed changes to a brick red or disappears.

Biuret's Test: The test solution when treated with few drops of 2% of copper sulphate solution and added 1ml of ethanol followed by excess of potassium hydroxide pellets led to the formation of pink colour in the ethanolic layer.

Ninhydrin Test: Ninhydrin reagent was added to the test solution and boiled for few minutes. Formation of blue colour indicated the presence of amino acids.

Detection of terpenoids

Salkowski's test: The extract was mixed with 2 ml of chloroform, and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Detection of cardiac glycosides

Keller-Killani test: The extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. On addition of 1 ml of concentrated sulphuric acid, appearance of brown ring at the interface indicates a deoxy sugar characteristic of cardenolides. Appearance of a violet ring below the brown ring and a greenish ring in the acetic acid layer confirmed the results.

Test for fixed oils and fats

Spot Test: Small quantity of extract is placed between two filter papers. Oil stain produced with any extract showed the presence of fixed oils and fats in the extracts.

Saponification test: A few drops of 0.5N alcoholic potassium hydroxide added to the extract with few drops of phenolphthalein solution and heated on a water bath for 1-2 hours. Formation of soap indicated the presence of fixed oils and fats in the extracts.

Test for gums and mucilages

Small quantity of the extract is diluted with water and to it ruthenium red solution was added. A pink colour production showed the presence of gums and mucilages.

RESULTS

Proximate analysis

The powdered *Ipomoea quamoclit* whole plant was subjected to proximate analysis in which it was studied for determination of ash values, extractive values, moisture content and fluorescence analysis and the results were tabulated in the following table number 1 and 2.

Table 1: Proximate analysis of *Ipomoea quamoclit* whole plant

S. No.	Proximate value	Content (w/w)
01	Total ash	9.68±0.19
02	Acid insoluble ash	3.57±0.15
03	Water soluble ash	2.95±0.08
04	Sulphated ash	3.49±0.06
05	Alcohol soluble extractive value	12.92±0.13
06	Water soluble extractive value	9.45±0.13
07	Ether soluble extractive value	5.41±0.17
08	Moisture content	5.25±0.10

All the values were expressed as Mean+SD and n=3.

Table 2: Fluorescence analysis of *Ipomoea quamoclit* Whole plant

S. No.	Powdered drug	Daylight	UV light (365nm)
01	Power as such	Green	Dark brown
02	Power + $Fecl_3$	Greenish yellow	Green
03	Power + conc. HCl	Green	Dark green
04	Power + 10% HNO_3	Brown	Greenish black
05	Power + 10% $K_2Cr_2O_7$	Brown	Blackish brown
06	Power + 1M NaOH	Chocolate brown	Green
07	Power + $AgNO_3$	Green	Green
08	Power + conc. HNO_3	Bright yellow	Greyish green
09	Power + conc.H_2SO_4	Yellow	Yellowish green
10	Power + Br_2 water	Light brown	Light green
11	Power + 5% H_2O_2	Coffee brown	Light green
12	Power + CCl_4	Yellow	Dark brown
13	Power + Methanol	Dark brown	Yellowish green
14	Power + Acetic acid	Yellowish brown	Light brown
15	Power + Xylene	Reddish brown	Light brown
16	Power + Ammonia	Orange	Brownish yellow
17	Power + Iodine solution	Dark brown	Blackish brown

Extraction

Whole plant powder of *Ipomoea quamoclit* was subjected to successive solvent extraction using different solvents and the percentage yield, colour and nature were tabulated in table number 3.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of whole plant of *Ipomoea quamoclit* was carried out and it showed the presence of alkaloids, carbohydrates, saponins, tannins, phytosterols, amino acids, proteins and flavonoids. The results were shown in table number 4.

Table 3: Nature of *Ipomoea quamoclit* whole plant extract

S. No.	Extract	Physical Form	Color	Yield (%w/w)
1	Petroleum ether	Resinous	Green	0.89
2	Chloroform	Semisolid	Black	1.59
3	Ethyl acetate	Semisolid	Green	1.46
4	Methanol	Semisolid	Green	2.98
5	Water	Solid	Brownish red	3.01
6	Hydroalcoholic (3:2)	Semisolid	Dark green	3.64

Table 4: Preliminary phytochemical screening of *Ipomoea quamoclit* whole plant

S. No.	Tests	PEIQ	CEIQ	EAIQ	MEIQ	AEIQ	HAIQ
01	Alkaloids	-ve	-ve	+ve	+ve	-ve	+ve
02	Carbohydrates	+ve	+ve	-ve	-ve	+ve	+ve
03	Saponins	+ve	-ve	-ve	+ve	-ve	+ve
04	Phytosterols	-ve	-ve	+ve	+ve	-ve	+ve
05	Phenolic compounds	+ve	-ve	-ve	+ve	-ve	+ve
06	Tannins	-ve	-ve	-ve	-ve	-ve	+ve
07	Flavonoids	+ve	-ve	-ve	+ve	+ve	+ve
08	Proteins and amino acids	+ve	+ve	+ve	-ve	+ve	+ve
09	Terpenoids	-ve	+ve	+ve	-ve	+ve	+ve
10	Cardiac glycosides	-ve	-ve	-ve	-ve	+ve	-ve
11	Fixed oils and fat	-ve	+ve	-ve	-ve	+ve	- ve
12	Gum and mucilages	-ve	-ve	+ve	-ve	-ve	+ve

CONCLUSION

From the above observation it can be concluded that the various proximate and preliminary phytochemical aspects of an important ethnomedicinally important plant, *Ipomoea quamoclit* were calculated experimentally and reported.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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