



Phytochemical analysis and antimicrobial activity of leaves of *Ipomoea aquatica* forssk

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Abstract

Ipomoea aquatica forssk is commonly found in Asia, Africa, and Australia. The plant is commonly used as vegetable having nutraceutical applications as it is a good source of vitamins, minerals, proteins, fibers etc. The aim of the current study was to analyze the photochemical and antimicrobial activity of plant leaves of the *Ipomoea aquatica* Forssk. For this purpose, first ethanol extract and aqueous extract of leaves were prepared. The antimicrobial activity of the plant extract was examined against common bacteria such as *Escheriachia Coli*, *Staphylococcus aureus* using agar well diffusion method. Screening for Various petrochemicals such as flavonoid, tannins, glycosides and saponins were also carried out using standard methods. For GC-MS analysis, methanol extract of leaves was prepared. Aqueous extract of *Ipomoea aquatica* Forssk did not show any antimicrobial activity, but ethanol extract of leaves showed moderate antibacterial activity against *E. coli* and *Staphylococcus aureus*. The presence of tannin, Flavonoide, Glycosides, Saponins were confirmed in the leaf extract while the phytochemical terpenoids and Coumarinst are absent. Out of 7 compounds detected in GC-MS, 6 compounds are reported by various researchers for biological activity. The reported six compounds possessing biological activity like antimicrobial, antibacterial, antifungal, hemolytic, antioxidant, antileukemic, antidiabetic and anti convulsant property. The findings of the study will generate baseline data on *Ipomoea aquatica* Forssk., which will be of immense commercial interest for research institute and pharmaceuticals companies.

Keywords: phytochemical, antimicrobial, *Ipomoea aquatica* forssk., flavonoid, terpenoid

Introduction

Herbal medicine is also called as botanical medicine or phytomedicine is refers to the using of plant parts such as flowers, seeds, roots and leaves for medical purposes. Herbalism has a long tradition and has been used along with standard medicines. Clinical research has showed the value of herbal medicine in treating and preventing various diseases. The plant *Ipomoea aquatica* Forssk. is a semi-aquatic, tropical plant which is used as a vegetable for the tender leaves and shoots. The plant *Ipomoea aquatica* Forssk. belongs to the family Convolvulaceae. This perennial plant is commonly known as water spinach, water morning glory, Chinese water spinach, kangkong, swamp cabbage, swamp morning glory, water convolvulus, (English) ^[1-6], Kolamani (Manipuri), Kalmisak (Hindi and Bengali), Kolmou xak (Assamese) ^[7]. Traditionally the leaves of the plant are used for treat cough, liver disease ^[8]; shoots are mildly laxative and are used by diabetic patients. ^[9, 10], constipation ^[11], nose bleeds and high blood pressure ^[12, 13]; anthelmintic ^[14] central nervous system depression depressant, anti-epileptic agent, hypolipidemic effects ^[15]. The plant is considered to have a wide distribution and grows in moist soils as well as in fresh water, ditches, lakes, ponds, marshes and wet rice fields but marshy lands and waterlogged soils are ideal for its growth. The plant is grown in the wild and usually grows in all-seasons of the year as well as cultivated throughout South East Asia. It is generally consumed as a vegetable in different regions of the world. In part of the tropical Asia. In the rural area of

Assam, it is generally consumed as green leafy vegetable ^[16]. Phytochemicals are formed in the primary and secondary metabolism of the plant. The metabolic products are extracted into acceptable solvents in which these are soluble. It has been observed that most therapeutically active phytochemicals of the plant will come into polar solvents like alcohol, water, etc. The extraction process thus is a key component it recognizing the phytochemical potential of a natural source like the studied plant ^[17].

Materials and Method

Collection of plant materials

Fresh leaves of *Ipomoea aquatica* Forssk. were collected from rice field and pond of Nalanipam village situated in the Dhemaji district of Assam. These plant samples were washed with tap water in order to remove the dust. Collected plant material were air dried followed by thermostatic oven drying at a considerably low temperature not exceeding 30^oc for 24 hours. The plant material become well dried for grinding. After grinding the plant material were transferred into air tight containers with proper labeling for future use. 50 grams of powder extracted from leaves of *Ipomoea aquatica* Forssk. was taken and extracted with adequate amount of ethanol (4:1) using soxhlet apparatus. The liquid part is stored at 4^o c in separate container.

Chemicals and Reagents

Distilled water, methanol, Di-ethyl ether hexane, Sodium phosphate buffer, DNS, Starch, DPPH, Ethanol, MH agar, Sodium acetate, Sodium hydroxide, Hydrogen peroxide,

95% ethanol, 0.1% Lead acetate, Hydro chloric acid, Sulphuric acid, Sodium carbonate, Chloroform.

Glass wares and plastic wares

Beaker, conical flask, test tube, measuring cylinder, Pipette, Petri dish, test tube stands, plastic tray, 2 ml Syringe for filtration, glass vial etc.

Equipment

Some of the equipments utilized for the study include Electronic Balance (ANAMED), spectro-photometer, pipette, soxhlet apparatus, micro pipettes, centrifuge, Hot plate, water bath, Laminar air flow, -70°C Temperature Freezer, Rotary evaporator and GCMS Instrument (Perkin Elmer, USA) etc.

Water extract

The water extract was prepared using classical method, where 5gm of plant mass were weighted using an electronic balance and was crushed in 100ml of sterile water. Then the mixture was boiled at 50-60°C for 30 minutes on water bath and it was filtered through Whatman No.1 filter paper. Then the filtrate was centrifuged at 2500 rpm for 15 minutes. The extract was then collected, labeled and stored in sterile bottles at 4°C for further different experimental use.

Ethanol extract

Grinded samples (5gm) were mixed with 100 ml of 95% ethanol and kept in water bath at 70°C for 2 hours. The extracted samples were centrifuged and the supernatant was transferred into 50 ml volumetric flask and adjust the volume to 50 ml with 95% ethanol. The sample were stored at -4°C.

Qualitative analysis of phytochemicals

Chemical tests were carried out by using aqueous as well as ethanol extract to identify various phytochemicals, using standard methods [18-21]. The extracts were subjected to qualitative analysis for presence of chemicals constituents by performing various chemical tests such as Terpenoids (Salkowski test), Flavonoids (Alkaline reagent test, Sulfuric acid test, Lead acetate test), Tannins (Lead acetate test), Glycosides (killer kiliani test), Saponin (Forthing test) and coumarins (Sodium chloride test).

Test for the antimicrobial activity

For antimicrobial test *Escherichia coli* and *Staphylococcus aureus* were inoculate in already prepared 20 ml of sterilized Muller Hinton agar plates and incubate the plates at 37°C for 24 hours. A heat sterilized 10 mm cork borer was then used to make wells in the already inoculated medium and the plant extracts to be tested against each test organism were placed.

Gas chromatography-Mass spectrometry analysis

The phytochemical composition of was analyzed by GC-MS system (Perkin Elmer, USA) make GCMS instrument,

Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler). For GCMS analysis, 0.1 ml of each concentrated extracts (4mg/ml) was diluted to 1ml with solvent (Methanol) and transferred to standard GCMS sample. The Software used in the system was TurboMass

Ver. 5.4.2. The capillary column used was 'Elite-5MS' having dimensions-length-60 m, ID-0.25 mm, and film thickness-0.25 µm, and the stationary phase is 5% diphenyl 95% dimethylpolysilox-ane. Helium (99.99%) was used as carrier gas (i.e., mobile phase) at a flow rate of 1 ml/min. An injection volume of 2 µl was employed in splitless mode. Injector and ion-source temperatures were 280 °C and 180 °C, respectively. The oven temperature was programmed at 60 °C (for 1 min), with an increasing rate of 7 °C/min to 200 °C (hold for 3 min) then again increased at rate of 10 °C/min to 300 °C (hold for 5 min). The total run time was of 60 min. The solvent delay was kept for 8 min. MS Protocol Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e., m/z range is 50-600 amu.

Identification of peaks

Interpretation of the peaks that appeared in the GC Chromatogram were done by library search of the mass spectrum of the corresponding peaks using the database software of National Institute Standard and Technology-2008 (NIST-2008).

Results

Phytochemicals analysis

Preliminary qualitative phytochemicals screening of the extract of *Ipomoea aquatica* Forssk. leaves confirmed the presence of tannin, Flavonoide, Glycosides, Saponins while the phytochemical terpenoids and Coumarins are absent. Summarized result tabulated in table 1

Table 1: Summarized result of phytochemical analysis of *Ipomoea aquatica* forssk. plants

Phytochemicals	Name of the test	Ethanol extracts	Aqueous extract
Terpenoids	Salkowski test	-ve	-ve
Flavonoids	Alkaline reagent test	+ve	+ve
	H ₂ SO ₄ test	-ve	-ve
	Lead acetate test	+ve	+ve
Tannins	Lead acetate test	+ve	+ve
Glycosides	Killer kiliani test	+ve	+ve
Saponins	Forthing test	+ve	+ve
Coumarins	Sodium chloride test	-ve	-ve

Antimicrobial activity of *Ipomoea aquatic forssk*

Aqueous extract of leaves of *Ipomoea aquatic* Forssk. did not show any antimicrobial activity as shown in Table 2, but ethanol extract of leaves of *I. aquatic* Forssk showed moderate antibacterial activity against *E. coli* and *Staphylococcus aureus*.

Table 2: Result of antimicrobial activity.

Bateria	Aqueous extracts	Ethanol extracts
<i>Escheriachia coli</i>	nil	30mm
<i>Staphylococcus aureus</i>	nil	34mm

GC-MS analysis of *Ipomoea aquatica* leaves

GC-MS study of the methanolic leaves extract of *Ipomoea aquatica* revealed the presence of some important phytochemicals in the chromatogram (Figure 1). The compounds with their retention time (RT), molecular formula, molecular weight (MW) peak area percentage are listed in Table 3.

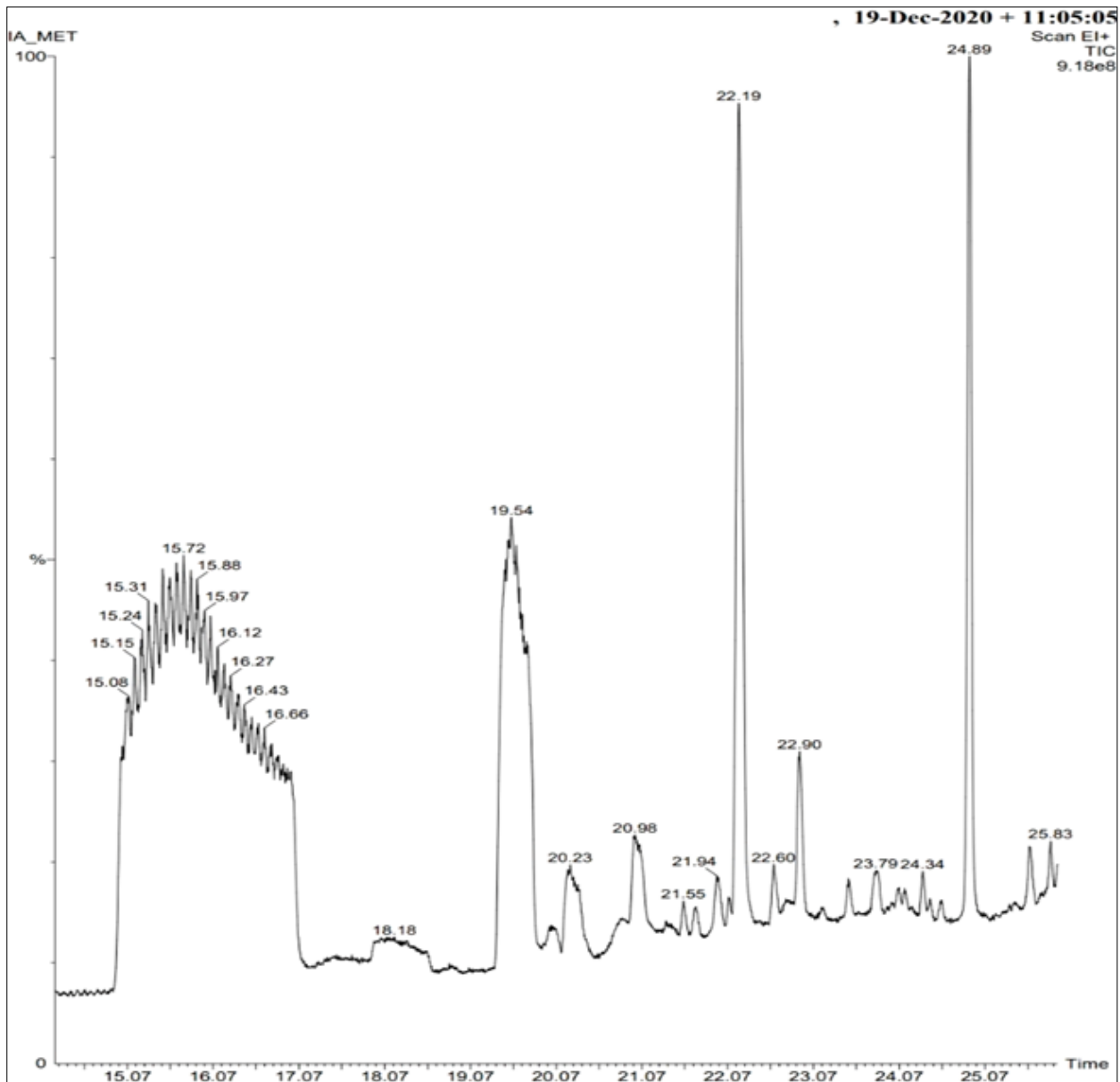


Fig 1: GC-MS chromatogram of methanolic extract *Ipomoea Aquatica*

Table 3: Phyto-compounds detected in the combined fraction from column chromatography of the methanolic extract of *Ipomoea aquatica* leaves.

Sl No.	RT	Name of the compounds	Molecular formula	Molecular weight	Peak area %
1	20.228	Decamethyl-5 (trimethylsiloxy) Hexasiloxane	C ₁₃ H ₄₂ O ₆ Si ₇	490	0.721
2	20.984	3ethoxy1, 1, 1, 7, 7, 7 hexamethyl 3,5,5 tris tetrasiloxan	C ₁₇ H ₅₀ O ₇ Si ₇	562	0.752
3	22.194	Cyclononasiloxane octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	3.255
4	22.904	Octasiloxane1, 1, 3,3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 Hexadecamethyl	C ₁₆ H ₅₀ O ₇ Si ₈	578	0.545
5	27.206	Cyclo octasiloxane, Hexadecamethyl	C ₁₆ H ₄₈ O ₈ Si ₈	592	1.950
6	29.792	N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.066
7	29.967	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312	0.721

The compound confirmed in the spectrum profile of GC-MS were 3 Ethoxy 1, 1, 1, 7, 7, 7 hexamethyl 3, 5, 5 tris tetrasiloxan, cyclononasiloxane octadecamethyl, cyclooctasiloxane ‘hexadecamethyl, N-Hexadecanoic acid, Octasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 hexadecamethyl, eicosanoic acid, 1, 1, 1, 3, 5, 7, 9, 11, 11, 11 decamethyl-5 (trimethylsiloxy) hexasiloxane.

The medicinal and biological activities of the phyto compounds identified in the leaves of *Ipomoea aquatica* are 1, 1, 1, 3, 5, 7, 9, 11, 11-Decamethyl-5(Trimethylsiloxy) Hexasiloxane (0.721%) have anti convulsant property, 3Ethoxy 1, 1, 1, 7, 7, 7 hexamethyl 3, 5, 5 tris tetrasiloxan

(0.752%) have no active action reported, Cyclononasiloxane Octadecamethyl (3.255%) have antibacterial and antifungal activity, Octasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 Hexadecamethyl (0.545%) have great antimicrobial activity, Cyclo octasiloxane, Hexadecamethyl (1.950%) have hemolytic activity, antioxidant, antibacterial and antifungal activity, N-Hexadecanoic acid (1.066%) demonstrated anti flammatory activity by competitively inhibiting phospholipase A2. Furthermore, hexadecanoic acid methyl ester (palmitic acid methyl ester) is demonstrated to be a novel neuroprotective compound in

cerebral ischemia, Eicosanoic acid (0.721%) have antileukemic and anti-diabetic property.

Discussion

By observing Phytochemicals analysis of the leaf extract of *Ipomoea aquatica* Forssk., we have found positive results for Flavonoids, tannins, glycosides, saponins. Flavonoids are antioxidant in nature and protect the body against allergies, inflammation, microbes, viruses etc. These bioactive components are used in different ways such as syrups, decoctions, essential oil [22] etc. The flavonoids also can protect low density lipoproteins from being oxidized. It has ability to relieve hay fever, eczema and asthma. Water extracts of *Ipomoea aquatica* Forssk. did not show antimicrobial activity against *E. coli* and *Staphylococcus aureus*. Selected medicinal plants like *Lagerstroemia indica* and *Annona reticulata* leaf are potentially good sources of antibacterial against the pathogens like *K. pneumoniae*, *S. aureus*, *S. typhi*, *P. vulgaris* and *P. aeruginosa* [23, 24]. In ethanol extract of *Ipomoea aquatica* to show positive activity against *E. coli* (30mm) and *Staphylococcus aureus* (34mm). The plant extract also present antimicrobial activity against the microbes such as *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* [25]. GC-MS analysis of *Dillenia scabrella* leaves also find Octadecanoic acid to antimicrobial activity [26]. Present study of GC-MS analysis of *Ipomoea aquatica* in methanolic extract is also found of N-hexadecanoic acid but was reported earlier [27]. Previous study reported that in *Oldenlandia sp* strong antibacterial activity against *K. pneumoniae* with zone of inhibition of 16mm disc diffusion method [28]. In our study the compound show strong antibacterial activity against *Staphylococcus aureus* in ethanol extract with zone of inhibition of 34 mm in disc diffusion method. Previous GC-MS study reported that the N-Hexadecanoic acid is palmitic acid in nature and antioxidant, nematocidal, pesticidal, 5-alpha reductase inhibitor [29]. In our study the compound n-hexadecanoic acid also anti inflammatory activity. 6 compounds out of 7 compounds detected in GC-MS, have biological activity like antimicrobial, antibacterial, antifungal, hemolytic activity, antioxidant, antileukemic, antidiabetic and anti convulsant property.

Conclusion

The current study confirms the presence of the following phytochemicals namely flavonoids, tannins, glycosides, saponins. However, the study fails to detect certain phytochemicals such as terpenoids and coumarins in the studied plant. Aqueous extract of leaves of *Ipomoea aquatica* did not show any antimicrobial activity, but ethanol extract of leaves of *Ipomoea aquatica* showed moderate antibacterial activity against *E. coli* (30mm) and *Staphylococcus aureus* (34mm). The findings of the study will generate baseline data on *Ipomoea aquatica* Forssk which will be of immense commercial interest for research institute and pharmaceuticals companies. The study also proposes this plant as a potential candidate for developing drugs for antioxidant activity, against allergies, liver diseases, diabetes, blood pressure, hypolipidemic effects etc. Medicinal plant is used for discovering and screening of the phytochemicals constituents which are very helpful for manufacturing of new drugs. The phytochemicals analysis of the medicinal plants is important and have commercial

interest in both research institute and pharmaceutical companies for the manufacturing of the new drugs treatment of various diseases. Thus we hope, that importance of phytochemicals properties identified in the current study in the local plant of our states.

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Conflicts of Interest

There is no conflict of interest.

Financial Disclosure

None

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