Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (4), 2022; 896-904

Phytochemistry, antioxidant activity and traditional uses of *Ipomoea aquatica* Forssk among the people of Lower Assam, India

Research Article

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Abstract

Naturopathy has gained popularity in recent years for the treatment of various health diseases, due to the numerous adverse side effects of synthetic medications. Assam in North-Eastern part of India is a great treasure of bio-resources, particularly for plant diversity and animal diversity, and has become a source of hundreds of medicinal herbs. Different tribal and non-tribal inhabitants of this area have knowledge about using of various plants as well as their products to heal a variety of health illnesses. Among these, *Ipomoea aquatica* Forssk is widely used among the Lower Assam ethnic groups. A survey study was conducted to validate the traditional therapeutic uses of *Ipomoea aquatica* Forssk in treating various health related disorders among the native people of Lower Assam. Further, an investigation in the laboratory was performed to examine the phytochemical components and the antioxidant activity of *Ipomoea aquatica* Forssk using standard methods. As the methanolic extract showed the best results, a GC-MS analysis was also performed in attempt to identify the bioactive component of the methanolic extract. This confirms their traditional use as food and medicinal.

Key Words: Therapeutic, Antioxidant, Bioactive component, Health ailments, Phytochemical, Plant extract.

Introduction

For hundreds of years, people have passed on their knowledge of herbs from one generation to the next. The rise of interest in natural medications began in recent decade partly because of the common idea that green medication is healthier than synthetic products. In recent years, there has been a significant increase in the use of health products derived from plants in both developing nations and developed nations, which has led to an exponential boom in the market for herbal items across the globe. According to World Health Organization estimation, up to eighty percent of people continue to primarily depend on traditional treatments such as herbs for their medical needs (1). In this age of drug research and the identification of novel therapeutic molecules, the traditional uses of a great number of plant products are taken into consideration throughout the evaluation process. In this context, *Ipomoea aquatica* Forssk (IA) is one of several plants that are being researched to determine the extent of their potential as medicinal agents. IA is a green leafy vegetable that is extensively produced in India, Ceylon, Tropical Asia, Africa, and Australia. It belongs to the Convolvulaceae family. This plant is cultivated as a semi-aquatic crop, and it can be seen growing in large quantities in marshy locations (2). It is a type of plant that can grow as an annual or a biennial; its long, thick, hollow, trailing, or floating stems are rooted at the nodes (3). In different countries, this plant is known by several names, Kangkong, or water spinach, is the most common name (4). IA has been shown to have several health advantages due to its high content of vitamins, minerals, proteins, fibres, carotenes, and flavonoids (5). It has traditionally been used as a carminative and also been proven to reduce inflammation, making it effective in the treatment of fever, jaundice, biliousness, bronchitis, and liver problems, among other things. In Southeast Asia, it is believed that various parts of the IA plant can be used efficiently in the treatment of high blood pressure, as well as in the treatment of opium and arsenic poisoning (6). Since Assam is home to various aboriginal tribes and is part of the Indo-Burma hotspot variety, it has a lot of potential for ethnobotanical research. It has a diverse flora as well as a huge number of commercial and therapeutic plants that are an important element of the ethnic communities (7). One of such important indigenously used plant is IA which is commonly and locally known as “Kolmou” in Assamese. Therefore, in the present study, the emphasis has been placed on the plant IA and its parts that can be of great use in various health care practices as well as for food and nutritional aspects prevalent among the people of the districts of lower Assam through survey work via personal interaction. Further phytochemical

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screening, antioxidant activity and GC-MS analysis of the plant has been done in laboratory.

Materials and methods

Study area and data collection

A survey work was done among the residents of the districts of Lower Assam, viz Barpeta, Bajali, Bogaigaon, Dhubri, Goalpara, Nalbari, and Kamrup (Figure 1). In the study, 15-20 villages from each district were chosen at random. There were 461 people in all, each of whom participated as a family representative while using IA for a wide variety of physiological abnormalities. Also, information has been gathered from the bej, the village head, and the old women. During the period of March 2021 to April 2022, the survey was conducted in the form of a bilingual standard questionnaire, which was then followed by personal interaction and telephonic conversation in case of closed proximate people. People who have freely participated in this programme were given a thorough explanation of the specifics of the questionnaire as well as the purpose of the research prior to conducting the inquiry. The questionnaire that was used in this research project included a series of questions that emphasis about the section of the plant that was used, the mode of consumption, and the traditional uses of IA for treating a variety of health disorder.

Chemicals and Reagents

Distilled water, ethanol, methanol, 1- butanol (n-butyl alcohol), acetone, chloroform, petroleum ether, sodium hydroxide and concentrated sulphuric acid were purchased from Northeast chemicals, Guwahati, Assam. Glacial acetic acid, ferric chloride, potassium ferric cyanide, hydrochloric acid, Molisch's reagent and Wagner reagent (Potassium iodide, Iodine) were purchased were purchased from Merck Private Limited. Benedict's reagent, Fehling A and B solutions, hydrochloric acid, potassium mercuric iodide, aluminum chloride, potassium acetate, sodium carbonate, Folin-Ciocalteu's reagent, gallic acid were purchased from Fisher Scientific India Private Limited.

Plant material, extraction and sample preparation

IA was gathered in the Nalbari district of Assam in the month of January-February and June-July 2022. The leaves of the plant was given a thorough washing with water, and were allowed to dry in the shade for three weeks before being dried in an incubator at a temperature not exceeding 37 °C for 2 days. By using a manual blender, the dry material was reduced to a powdery consistency after being crushed. For later usage, the powdered plant material was kept at 4°C in airtight containers. The powder collected weighed 500 gram. Soxhlation was used to extract 40g of powdered IA using 400 ml of methanol, ethanol and double distilled water until the solvent became colorless in the main chamber of the Soxhlet extractor. The extracts were dried out by evaporating, and then crude extracts were produced. The phytochemical contents of the crude extracts were tested.

Figure 1: Geographical map of study area [A] India; [B] Assam; [C] Studied districts of lower Assam.

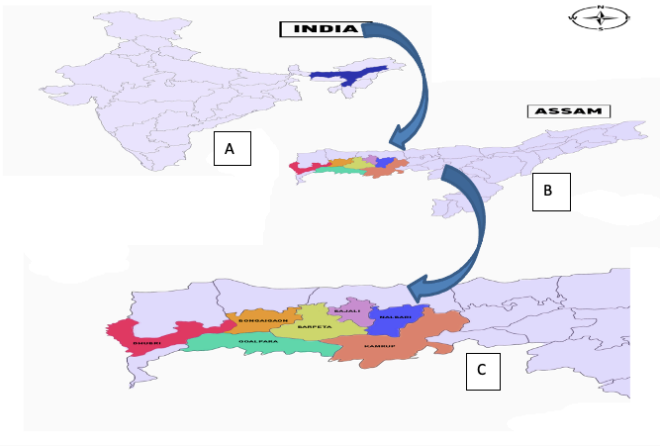


Figure 2: The morphology of IA. [A] The plant creeping on moist soil; [B] The flower of IA; [C] The plant floating on water; [D] Leaves arise from nodes; [E] Flowering twig with leaves

Phytochemical screening

The methanolic, ethanolic and aqueous extracts of IA were put through preliminary phytochemical screening to determine whether or not they included any active ingredients. This screening was carried out with the use of standard techniques of analysis.

Test for Carbohydrates

The dried extract was mixed with 5 ml of distilled water and filtered afterward. The presence of carbohydrates was determined using filtrate.

Benedict's test: Benedict's reagent was used to treat the filtrate, which was then gently boiled on a water bath. An orange-red precipitate indicates the presence of reducing sugar.

Fehling's test: To hydrolyze the solution, diluted HCL was added to the filter. The filtrate was then alkali-neutralized and heated with Fehling A and B solutions.

When red precipitate forms, it shows that there are reducing sugars in the solution.

Test for Alkaloids

Few drops of HCL were added to a small amount of solvent-free extract, which was then filtered and treated with Mayer's reagent (potassium mercuric iodide). The presence of cream precipitate indicates the presence of alkaloids.

Test for Flavonoids

Extracts were treated with a few drops of NaOH solution. The presence of flavonoids is indicated by a bright yellow tint that disappears when acid is added in diluted form.

Test for Saponins

In a test tube, crude extract and 5 mL of distilled water were mixed and vigorously agitated. A few drops of olive oil are added to the mixture. The formation of stable foam revealed the presence of saponins.

Test for Tannins

In a test tube, 1 ml of the sample and 1 ml of 0.008 M potassium ferric cyanide were added. When 0.1 N HCl was added to 1 ml of 0.02 M Ferric chloride, a blue-black colour was seen.

Test for Phytosterols

The extract was blended with chloroform, and then it was strained. After a few drops of concentrated sulphuric acid were added to the filtrates, they were stirred and left alone. Triterpene is present when there is a golden yellow colour.

Test for Glycoside

Glacial acetic acid, ferric chloride, and concentrated sulphuric acid are used to dissolve the extract in water. At the intersection, they display a brown indication.

Test for Proteins

A few drops of strong Nitric acid were added to the extract. The yellow colour of the precipitate indicates the presence of proteins.

Estimation of total phenolic content

The phenolic content of methanolic, ethanolic and aqueous extracts was estimated by Folin-Ciocalteu reagent method (8) with modification by using gallic acid as standard.

Estimation of total flavonoid content

The quantitative estimation of flavonoids was performed using the Aluminum chloride method (9) with modification by using quercetin as standard.

Antioxidant activity

Determination of total antioxidant activity

The total antioxidant activity of the methanolic, ethanolic and aqueous extracts was determined using the phosphomolybdenum technique **(**10).

Determination of free radicle scavenging activity

The antioxidant activity of the methanolic, ethanolic and aqueous extract was determined using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging assay (11).

GC-MS analysis

Gas chromatographic mass spectrometry was used to perform quantitative study of chemical components in methanolic plant extract. A GC-MS system (Model-Perkin Elmer Clarus 680 GC/600C MS) was used to investigate the phytochemistry of methanolic extract. TR 5- MS capillary standard non polar columns with a 30 Mts dimension was employed for the analysis. The mobile phase flow rate was fixed at 1.0 ml/min. In the GC portion, the temperature was increased at the rate of 5°C/min from 40° to 250° C, and the injection volume was 1 µl. Chloroform was used to dissolve the samples, and the Wiley Spectral Library software was used to do the analysis on the data.

Results

A total of 461 families were investigated throughout the research period, with 345 families belonging to tribes and 116 families belonging to non-tribes, as indicated in Table 1. According to the poll, IA is a favorite among tribal populations and is often eaten as a green leafy vegetable. According to the results of the field research, the indigenous people were quite familiar with the medicinal properties of the plant. The results that emerged from the interaction suggest that IA is routinely utilized by the people living in rural areas of Lower Assam, particularly in areas inhabited by tribal groups to treat a number of health conditions, including fever, liver problems, stomach disorders, high blood pressure, piles, worm infection, jaundice skin diseases like itching, rashes, and other similar conditions. IA is particularly beneficial in treating feminine health associated features, such as raising breast milk supply after child delivery, treating irregular menstrual cycle, monthly menstrual cramps, and reducing heavy bleeding, as was documented by the female participants (Table 2). According to the findings of the study, the tribal people have indigenous knowledge regarding the application of IA for the treatment of a variety of health problems. The consumption of IA varies greatly across tribes and non-tribes. IA is widely consumed by ethnic tribes as a green leafy vegetable.

Table 1: Showing distribution of total number and percentage of studied cases of both tribal and non-tribal people

Place where survey conducted	Total number of studied cases of people	Percentage of studied cases of people	Total number of studied cases of tribal people	Percentage of studied cases of tribal people	Total number of studied cases of non-tribal	Percentage of studied cases of non-tribal
Barpeta	86	18.65	65	75.58	21	24.42
Bajali	56	12.15	36	64.29	20	35.71
Bogaigaon	55	11.93	45	81.82	10	18.18
Dhubri	35	7.60	23	65.71	12	34.29
Goalpara	89	19.30	70	78.65	19	21.35
Nalbari	90	19.52	65	72.22	25	27.78
Kamrup	50	10.85	41	82	9	18

Table 2: Showing the plant parts, mode of consumption and usages of IA in different health disorders among tribes and non-tribes of Lower Assam districts

Serial number	Plant parts	Mode of consumption	Tribe/non-tribe(district)	Medicinal uses
1	Aerial parts	Raw parts are consumed as salad	Koch Rajbangsi (Bongaigaon)	Jaundice
2	Leaves	Leaves are cooked by boiling	Bodo (Kamrup)	Piles
3	Aerial parts	Consumed as leafy vegetable	Plain tribe (Kamrup)	High blood pressure
4	Flower	Fresh flowers are boiled in water and consumed once in a day	Rabha (Bajali)	Treatment of diabetes, irregular menstrual ramps
5	Whole plant	Whole plant is cooked with oil and consumed as a leafy vegetable	Plain tribe (Nalbari)	Liver complaints
6	Leaf	Decoction of leaves is trickle in each nostril	Garo (Bongaigaon)	Against nose bleed
7	Leaf	Consumed as a leafy vegetable	Kaibarta (Kamrup)	Fever
8	Whole plant	Whole plant parts are crushed and applied	Bodo (Goalpara)	Eye disease
9	Whole plant	Whole plant is cooked with vegetables	Non-tribe (Dhubri)	Constipation
10	Whole plant	Paste is applied directly on skin	Plain tribe (Barpeta)	Cure itching
11	Leaf	Leaves are prepared along with vegetables	Koch Rajbanshi (Nalbari)	Promote lactation
12	Whole plant	Whole plant is cooked with oil and consumed as leafy vegetable	Kaibarta (Kamrup)	Stomach disorders
13	Root	Paste is given orally	Rabha (Goalpara)	Malaria fever
14	Leaf	Paste is given orally	Garo (Bongaigaon)	Worm infections
15	Whole plant	Consumed as leafy vegetable	Plain tribe (Dhubri)	Liver tonic
16	Leaf	Leaves are cooked by boiling	Non-tribe (Nalbari)	Irregular menstrual cycle
17	Whole plant	Consumed as leafy vegetable	Non-tribe (Kamrup)	Reduce heavy bleeding
18	Whole plant	Consumed as leafy vegetable	Bodo (Kamrup)	Heavy bleeding
19	Leaf	Paste is given orally	Non-tribe (Bongaigaon)	Stomach disorder
20	Leaf	Paste is directly applied on skin	Plain tribe (Goalpara)	Skin rashes

The preliminary phytochemical screening of the crude aqueous, methanolic and ethanolic extracts of IA is showed in Table 2. This shows the presence of most of the phytochemicals in methanolic crude extract of IA followed by aqueous and ethanolic extract. Polysterols and glycoside both are absence in ethanolic and aqueous extract. Presence of saponin was noted in aqueous extract while absence in ethanolic extract.

Table 2: Phytochemical constituents present in aqueous, methanolic and ethanolic crude extracts of IA.

Phytochemical constituents	Test performed	IA aerial parts		
		Aqueous	Methanolic	Ethanolic
Carbohydrates	Benedict's test and Fehling's test	+	+	+
Alkaloids	Mayer's test	+	+	+
Flavonoids	Alkaline reagent test	+	+	+
Saponins	Foam test	+	+	-
Tannins	Ferric chloride test	+	+	+
Polysterols	Salkowski test	-	+	-
Glycoside	Kellar-killani's test	-	+	-
Protein	Xanthoproteic test	+	+	+

+ =Presence and - = Absence

Flavonoids and phenolic compounds play significant role in antioxidant system of plants. In the present study, quantitative analysis for these two specific groups of phytochemicals was carried out by using standard methods. Amount of flavonoids were expressed as quercetin equivalents in mg/g of plant material and phenols were expressed as mg/g of gallic acid equivalent of dried plant extract. The highest amount of flavonoids and phenolic content found in the methanolic extract followed by ethanolic and aqueous extract. Again, the flavonoid and phenolic content were observed to be higher in June-July season as compared to January-February (Table 3).

Table 3: Quantitative estimation of total Flavonoids and Phenol content in methanolic, ethanolic and aqueous extract of IA collected in January-February and June-July

	Total Flavonoids (mg/g of Quercetin equivalent)		Total Phenol (mg/g of Gallic acid equivalent)	
	January-February	June-July	January-February	June-July
Methanolic extract of IA	4.57±0.12	4.87±0.23	2.87±1.34	3.35±1.34
Ethanolic extract of IA	2.34±1.12	2.22±0.09	2.45±2.50	2.87±0.05
Aqueous extract of IA	2.15±0.05	3.15±0.13	1.15±0.09	2.23±0.01

(Values are expressed in Mean ±S.E.)

Total antioxidant activity recorded by various concentrations (20-100 µg/ml) of aqueous, methanolic and ethanolic crude extracts of IA are shown in Table 3. At the highest concentration 100 µg/ml, the total antioxidant activity of methanolic extract of IA was 30.2 µg AAE/ml which is maximum while that of ethanolic extract (12.7 µg AAE/ml) and aqueous extract (9.4 µg AAE/ml).

Table 4: Total antioxidant activity of IA

Concentration (µg/ml)	Total antioxidant activity in µg/ml of AAE (Ascorbic acid equivalent) extract		
	Aqueous	Methanol	Ethanol
20	3.5±0.03	15.2±0.02	7.5±0.04
40	4.8±0.01	17.4±0.01	8.1±0.02
60	6.2±0.05	21.2±0.05	9.5±0.05
80	7.1±0.02	25.7±0.02	10.3±0.02
100	9.4±0.03	30.2±0.01	12.7±0.01

Values are expressed as mean±SD

The similar results observed in DPPH scavenging activity where methanolic extract showed highest activity. The capacity of diverse samples to scavenge free radicals has been extensively tested using the free radical compound DPPH (12). The ability of IA aqueous extract, methanolic extract, and ethanolic extract to scavenge DPPH was investigated, and the results are displayed as relative activity in comparison to the control (Figure 4). Ascorbic acid had the most activity, followed by IA extracts. The activity of the methanolic extract was marginally greater than that of the ethanolic and aqueous extracts. All IA extracts had dose-dependent activity.

GC-MS is the most effective way to find the bioactive components of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid nitro compounds, and so on. GC-MS analysis was used to find the bioactive parts in the methanolic extract, since the methanolic extract was showing better results. By using Gas Chromatography-Mass Spectrometry (GC-MS), 50 chemicals were detected from a methanolic extract of the edible portions of IA (Figure 5). Among the 50 compounds, 11 were identified as significant compounds, namely: 3, 4-Altrosan, Sucrose, Neophytadiene, 3, 7, 11, 15-Terramethyl-2-Hexadecen-1-ol, 1-Heptacosanol, Tridecanal, Fumaric acid,dodecyl tetradec-3-enyl-ester, Methyl-19-methyl-eicosanoate, Phytol, Stigmasterol. The identified compounds and their retention time, molecular weight, molecular formula, and activities related to medicinal uses are given in Table 4. The structure of the significant compunds is shown in Figure 6.

Figure 4: DPPH scavenging activity shown by Ascorbic acid and crude extracts (Aqueous, methanolic, ethanolic) of IA.

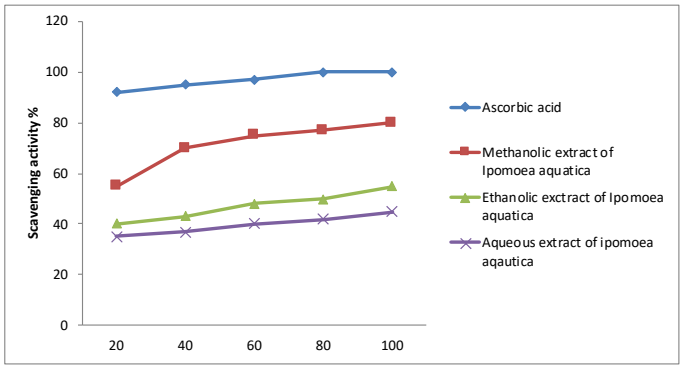


Figure 5: Gas Chromatography-Mass Spectrometry of methanolic extract of IA

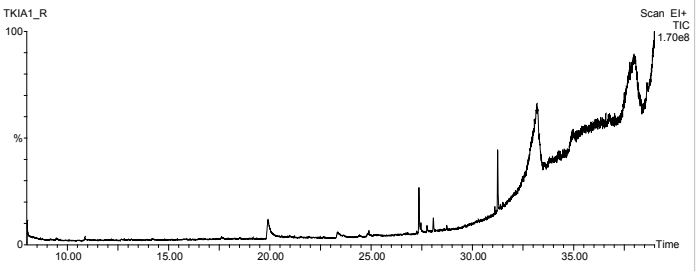
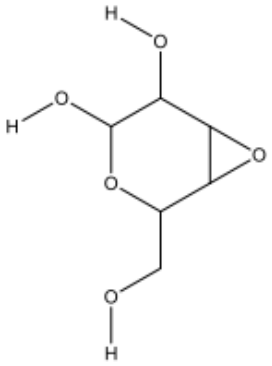
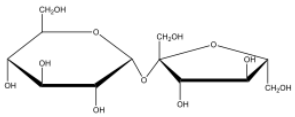
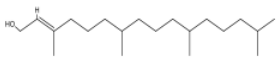
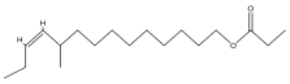
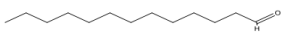
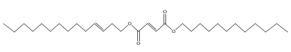
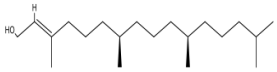
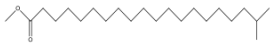
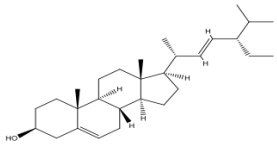


Table 4: Significant compounds identified in the methanolic extract of IA

SL No.	Name of compound	Structure	RT	Molecular weight	Molecular formula	Medicinal value
1	3,4-Altrosan		19.609	162	C6H10O5	Bacteriostatic, fungicide (13)
2	Sucrose		19.644	342	C12H22O11	Antioxidant activity (14)
3	Neophytadiene		27.866	278	C20H38	Antiulcerative, antiprotozoal and antiparasitic (15)
4	3,7,11,15-Terramethyl-2-Hexadecen-1-ol		27.827	296	C20H40O	Antimicrobial and anti-inflammatory (16,17)
5	Z-10-Methyl-11-Teradecen-1-ol-propionate		27.779	282	C18H34O2	5-alpha reductase inhibitor, antipsychotic (18)
6	1-Heptacosanol		27.759	396	C27H56O	Antimicrobial and antioxidant (19)
7	Tridecanal		28.745	198	C13H26O	Antimicrobial (20)
8	Fumaric acid,dodecyl tetradec-3-enyl-ester		28.746	478	C30H54O4	Huntington's disease and neurodegenerative diseases (21)
9	Methyl-19-methyl-eicosanoate		28.743	340	C22H44O2	Antifungal and antigenotoxic (22)

10	Phytol		31.799	296	C20H40O	Antimicrobial, anti-inflammatory, anticancer (23, 24, 25)
11	Stigmasterol		33.702	412	C29H48O	Anti-hypercholestrolemic activity, anti-tumor, Anti-osteoarthritic, antioxidant, anti-mutagenic, anti-inflammatory (26, 27, 28, 29, 30, 31)

RT= Retention time

Discussion

Health is one of the most important human needs in both traditional and contemporary civilizations, where there are a variety of methods for overcoming bodily condition or illness. In contrast to contemporary civilizations, the people of indigenous nations have their own healing practices that are important to their society's healthcare system. The main sources of health concerns in contemporary times include exposure to xenobiotics via different means, such as eating processed foods, altered lifestyle. This then results in a variety of physiological conditions and physical illnesses, such as cancer, obesity, hypertension, heart conditions, gastrointestinal issues, diabetes, and other gynecological conditions. Although receiving medical care and using prescribed medications may provide instant comfort, they do not constitute a trustworthy long-term fix since they have certain negative effects when used repeatedly. In this case, phytotherapy may provide a different or efficient means of treating various bodily afflictions. Researchers' focus is shifting to contemporary, affordable drug isolation due to ethno-traditional uses of several medicinal plants. Because of cultural acceptance and less negative effects, certain medicinal plants are increasingly recognized and utilized for traditional health care methods. Some therapeutic herbs are used as food by native cultures. IA is one the important medicinal herbs consumed as a leafy vegetable by the inhabitants Assam. In the current research, it was investigated how various ethnic tribes and non-tribes living in several districts of lower Assam use various sections of the IA plant as part of their healthcare practices. According to the findings of the survey research, this plant is a significant component of a variety of traditional remedies used by non-tribal people as well as members of various tribes to treat a broad variety of diseases of the body. Study showed that the leaves of IA commonly consumed by various tribes of Dhemaji district of Assam for urinary disorders (32). The Mishing community of North-East India consumed aerial parts of IA as a vegetable for the improvement of eye sight (33). Villagers in Nalbari district, Assam, cure their diabetes with a mixture of dried IA leaf powder and *Piper nigrum* (34). Later it was found that IA show oral hypoglycemic activity due to the presence of terpenoids and flavonoids (35). Another survey revealed that the Moran community of Tinisukia district of Assam used the shoot parts of IA as vegetable to enhance the lactation in nursing mothers (36).

Alkaloids, flavonoids, tannins, and steroids are among the phyto-constituents found in plants that are opening new pathways for the development of current treatments for a wide range of illnesses. In the present preliminary phytochemical screening, presence of carbohydrates, flavonoids, saponin, tannins and protein in the aqueous and methanolic crude extract of IA was also reported in earlier study (37).The presence of alkaloids in both methanolic and aqueous crude extract also reported (38). Studies on different plant extracts have shown that saponins, tannins, steroids, flavonoids, and alkaloids may be responsible for the antidiarrheal effects (39). Alkaloids have been utilized therapeutically for a very long time, and one of the biological properties that they share in common is their cytotoxicity (40). Numerous studies have shown that glycosides can lower blood pressure (41). The antioxidant activity of methanolic extract of IA has showed highest antioxidant activity, similar results showed by previous study where methanol extract showed highest antioxidant activity followed by aqueous extract (38). The antioxidant activity of several IA extracts was evaluated using the DPPH test. The DPPH assay used in this study revealed a dose-dependent pattern, similar to the case also reported by (42).The methanolic extract of IA showed highest DPPH free radicles, similar result shown earlier work (43). According to the findings of the DPPH free radical scavenging assay, the entire plant of IA has a powerful antioxidant property. This could be because the extracts have different phytochemicals in them.

Conclusion

According to the findings of the study, the plant is used to treat a variety of common ailments, and as a result, it has a folkloric medicinal reputation. This plant has the potential to be used all over the world as a source of herbal medication as well as traditional medicine. IA has a wide variety of phytoconstituents, including proteins, carbohydrates, alkaloids, and tannins. Flavonoids are also present. The plant's methanol extract displayed a significant level of antioxidant activity and showed the highest levels of DPPH free radicals. By using GC-MS, a total of 11 main bioactive compounds and their functional groups that may be in charge of antioxidant activity have been

discovered. Based on the results of the research, it is strongly suggested that more work be done to isolate bioactive chemicals from the plant and figure out their structures and how they might work against different biological actions.

Acknowledgement

Authors wish to express sincere thanks to the local people of the study area for their cooperation. Again want to express heartfelt gratitude to the village heads.

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