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A comparative study on ethno medicinal and nutraceutical potentials of the leaves of three species of genus Ipomea from Tripura, NE-India

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Abstract
Herbal phytoresource have been recognized as a potential source of traditional therapeutics and nutrients. The genus Ipomea with 500-600 species comprises the largest number of species within the convolvulaceae. Since ancient time, this genus has been in continuous use for different purposes, such as- nutritional, medicinal, ritual and agricultural purposes. These rich sources of ethno medicinal and nutritional information demand successful chemical studies for effective selection of plants. With the aim in view, a field survey was conducted on 192 respondents of the Mog, Reang and Uchai of South Tripura. Using semi structured interviews and discussions on medicinal uses of plants, the investigator gathered information from the respondents in addition to some traditional health prescriptioners; viz; Ujha, Baidya, Sadhan, Kabiraj. A rigorous qualitative and quantitative analysis of collected data on 73 plants with their uses in curing 69 ailments, three species of Ipomea such as- *I.aquatica* Forsk., *I. batatus* Linn. and *I. carnea* Linn. were found as potential source of trado-medicines. In the present study, the leaves of these plants were targeted to analyze by standard chemical techniques to evaluate their primary and secondary metabolites. The analysis of the results provided evidences that leaf extracts of these tested plants contain moderate amount of moisture (12.27-22.73%), solid content (77.27-87.72%), total ash (4.85-12.25g%),carbohydrate (7.57-17.71g%), crude proteins (25.35-27.25 g%), alkaloids (17.66-19.26 mg g^{-1}), phenolics (15.33-20.25 mg g^{-1}), flavonoids (11.41-12.71 mg g^{-1}), carotenoids (0.55-0.64 mg g^{-1}), ascorbic acid (32-146 mg/100g) that justifies their use in the traditional medicines for the treatment of various common diseases. The outcome of the study also suggest that these three tested species of genus Ipomea have very good medicinal potentials, meet the standard requirements for drug formulation and serve as good sources of nutrients predominant in the leaf.

Keywords: phytoresource, ipomea, traditional medicines, nutrients, chemical studies

1. Introduction
Herbal phytoresource of India and the knowledge of their nutritional and therapeutic properties have a long tradition, as referred in Rig Veda and other ancient literature [1]. Earlier survey by WHO reported that about 80% world's population use herbal drugs and in contrast 10% of the world's plants has been explored for their medicinal values [2]. In Asia where traditional medicine has utilized in herbal preparations for thousands of years and India is the birth place of renewed system of indigenous medicines such as Siddha, Ayurveda and Unani [3]. The value of medicinal plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of discovering modern drugs [4-6].

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases [7].The ethnomedicinal significance of herbal plants lies in the type and quantity of phytochemical they contain. Plant cells synthesize metabolites in the form of phytochemical for two types of essential functions, viz., primary and secondary. Primary metabolites are directly involved in growth and development of plants. Primary metabolites perform the key role as building blocks of cellular machinery and they are also utilized as fuel for energy supply. They are like chlorophyll, carotenoids, carbohydrates, protein; amino acids have a key role in metabolic process. They are used as industrial raw material and food additives [2, 3, 8].

The plant secondary metabolites are synthesized from primary metabolites or they are synthesized during secondary metabolism of plants [2, 3]. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism [9]. On the contrary, they have been worked as biocatalysts and their common roles are attraction of pollinator or defense against herbivores, pests and pathogens [2, 10].

They are the basic source for the establishment of several pharmaceutical industries since they have enormous medicinal properties [11]. All secondary metabolites have specific function as like alkaloids may be useful against HIV function[12], protect against chronic diseases [13]; phenolic compounds are able to break the cycle of generating new free radicals [14] and flavonoids have been reported to posses many useful properties including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antiviral [15, 16, 17], cytotoxic, anti-tumours, treatment of neurodegenerative diseases, anti-cancer, vasodilatory action [9, 18, 19, 20].

It has become very necessary to recover the ancient traditional use of plants from the older and rapidly-declining generation so as to maintain and preserve the uses of the plants and to form a basis for scientific research into the relevant phytochemical principles with the view of designing pharmaceutical research and production [21]. The effectiveness of these plant products from traditional claims must be proved to help developed novel drugs acting against some common disorders in our day to day life. Human has benefited from the presence of these chemicals by exploiting the plant products as sources of sustenance in a variety of ways. For example, many drugs today are of plant origin. Pharmacological history is replete with examples such as quinine, aspirin, picrotoxin, reserpine etc., while many of the synthetic drugs are fashioned after natural plant products [22]. The genus Ipomea Linn. (Family:-Convolvulaceae) a class of medicinally important plant species with approximately 500-600 species distributed worldwide [23] and 60 species reported from India [24]. Out of 60 species *Ipomea quamoclit* Linn., *Ipomea hederifolia* Linn., *Ipomea alba* Linn., *Ipomea aquatica* Forsk., *Ipomea kingie* Diels., *Ipomea batatus* Linn., *Ipomea purpurea* L.(Roth)., *Ipomea carnea* Jacq. And *Ipomea pestigridis* Linn. reported from Tripura [25]. The traditional use of Ipomea plant increasing and getting popularity throughout the developed and developing world. Furthermore, Elujoba (1997) [26] noted that a plant become a medicinal plant only when its biological activity has been ethno botanically reported or scientifically established.

In Tripura, leaves of Ipomea *aquatica*, *Ipomea batatus* and *Ipomea carnea* were widely use by Mog, Reang and Uchai tribes for curing their general ailments. The active principles differ from plant to plant due to their biodiversity and they produce a definite physiological action on the human body [27]. Due to expanding focus on the use of traditional medicine, it has become necessary to document the trado-medicinal uses of three selected Ipomea plants, as well as expand our knowledge of the possible active principles involved in the acclaimed efficacies of plants used in this system. With this aim in view, the present report is an attempt to select tradomedicinally important herbal plants from field survey and find out the rationality in curing different ailments by evaluating the amount of primary and secondary metabolites present in them.

Systematic classification of three Ipomea plants

Kingdom- Plantae	Kingdom- Plantae	Kingdom- Plantae
Phylum-Mangoliophyta	Phylum-Mangoliophyta	Phylum-Mangoliophyta
Classs-Tracheobionta	Class-Tracheobionta	Class-Tracheobionta
Order-Solanales	Order-Solanales	Order- Solanales
Family-Convolvulaceae	Family-Convolvulaceae	Family-Convolvulaceae
Genus-Ipomea (L.)	Genus- Ipomea (L.)	Genus-Ipomea (L.)
Species-*I. aquatica* (Forsk.)	Species- *I. batatus* (L.)	Species- *I. carnea* (L.)

A. Ipomea aquatica, B. Ipomea batatus C. Ipomea carnea

Fig 1: Figure A, B and C show different three species of Ipomea.

Materials and Methods

Survey on the use of medicinal plants

A self-administered questionnaire was prepared following the method of Martin, 1995 [31]. The percentage of informants claiming the use of a certain plant for the same major purpose was estimated using the fidelity level index with the following formula:

$$FL = Ip/Iu \times 100$$

Where, Ip is number of informants who indicate use of a species for the same major ailment,

Iu is the total number of informants who mentioned the plant for any other use [29, 40].

The percentage of respondents who have knowledge (PRK) regarding the use of a species (frequency of citation) in the treatment of diseases was estimated using the formula:

PRK= (Number of people interviewed citing species/ the total number of people interviewed) X 100 [29].

Plant materials

Leaves of three species of *Ipomea aquatica* Forsk., *Ipomea batatas* Linn. and *Ipomea carnea* Jacq. found in wild state were collected from Unakoti Tripura and grown in the experimental garden of Tripura University for experimental purposes. Their local names, place of collection, Latitude and altitude and herbarium no. were given in the table 1.

Table 1: Different species of *Ipomea* collected from Unakoti District of Tripura

Scientific Name	Herbarium no.	Availability status	Vernacular name (English-E, Bengali-B, Mog-M, Reang-R, Uchai-U)	Place of collection	Latitude and altitude
Ipomea aquatica Forsk.	TUH-529	Wild	Water spinach(E), Kalmishak(B), Reegrahmah (M), Athuoyakong (R), Twi-moi-moshu (U)	TilaBazar, Kailashahar, Unakoti Tripura	$24^0 21' 49.6'' N$ $91^0 59' 33.8'' E$
Ipomea batatas Linn.	TUH-530	Cultivated as well as wild	Sweet potato(E), Mistialoo (B), Maror(M), Denga-thak-Tai (U)		
Ipomea carnea Linn.	TUH-528	Wild	Pink morning glory(E), Dholkalmi (B), Letok (M), Kuthikathangbwphang (R), Alaangmiyaphang(U)		

Estimation of moisture content

The moisture content of the fresh leaves collected after every two intervals of internodes from the shoot apex was estimated by the method developed by AOAC, 1980 [30]. The sample and the asbestos were moistened thoroughly with a few ml of hot water. The sample and the asbestos together were mixed with a spatula. The spatula was washed with hot water to remove the sample residues from it, letting the residues and the water fall into the dish.

The open dish was heated on a boiling-water bath (Model: Bain-Marie) to evaporate the water to dryness. Then the dish was placed, with the lid alongside it into the oven and continued drying for six hours at 70°C under a pressure not exceeding 100 mm Hg, during which time the oven was never opened. A slow current of air (about 2 bubbles per second) was admitted to the oven during drying, the air having been dried by passing through H_2SO_4. The metal dish was placed in direct contact with the metal shelf of the oven. After drying, the dish was removed; it was covered immediately with its lid and was placed in the desiccators. After cooling to ambient temperature, the covered dish was weighed to the nearest 0.01 g.

The moisture content of the samples were calculated using the following formula

$$\text{The moisture content} = \frac{\text{M1\&M2}}{\text{M1\&M}_0} \times 100$$

Where:

Mo is the mass of the empty dish with its lid and containing the asbestos, g.

M1 is the mass of the dish with its lid, asbestos and test portion before drying, g.

M2 is the mass of the dish with its lid after drying, g.

The results were expressed to one decimal place. Duplicate determinations agreed to 0.2 per cent moisture.

Preparation of air dried powder of selected medicinal plants

For this study leaves parts were cleaned with water, wiped out the external moisture with dry cloth, dried to a constant weight in the shade at room temperature to prevent the decomposition, dissected into small pieces. The dried materials were stored in dark until analysis. The mixture was first pulverized by grinding in a blender so as to enhance effective contact of solvent with sites on the plant material. The powdered material is dried in thermostatically controlled hot air oven below 45°Cuntil they attained a constant weight and passed through a 40 unit mesh sieve [32].

Total ash

2 g of the dry leaves powder was accurately weighed in a previously ignited and tarred crucible (usually of platinum or silica). The material was spread in an even layer and ignite it by gradually increasing the heat to 500–600 °C until it is white, indicating the absence of carbon. It was cooled in desiccators and weighed. The content of total ash in mg per g of dried material was calculated [33].

Estimation of chlorophyll and carotenoids

The contents of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were estimated in dry leaves following the method of Arnon [34]. For this, 100 mg dried powder of leaves was weighed accurately (Electronic balance, Model No-M300-DR) and quickly crushed in 10 ml of 90% ethanol (v/v) using mortar and pestle. The homogenate was centrifuged at 5000 rpm for 5 min in ordinary centrifuge (Model No-B8C/GMLC-5316). The volume was raised to 10 ml with 90% ethanol. If the color intensity was very dark the supernatant was diluted to a desirable transparency with 90% ethanol. The absorbance of sample was further taken at four wave-lengths, *viz.*, 450, 645, 652 and 663 nm. The absorbance values were incorporated in the Arnon's formula to calculate mg of different fractions of chlorophyll and **c**arotenoids in terms of mg per gram of leaf dry weight.

$$\text{Mg Chl-a per g leaf tissue dry weight} = \{I12.7(D663) - 2.69(D645)\} \times \frac{V}{1000 \times W}$$

$$\text{Mg Chl-b per g leaf tissue dry weight} = \{I22.9(D645) - 4.68(D663)\} \times \frac{V}{1000 \times W}$$

$$\text{Mg total Chl per g leaf tissue dry weight} = \{I20.2(D645) + 8.02(D663)\} \times \frac{V}{1000 \times W}$$

Or

$$\text{Mg total Chl per g leaf tissue dry weight} = D652 \times \frac{1000}{34.5} \times \frac{V}{1000 \times W}$$

$$\text{Mg total carotenoids per g leaf tissue dry weight} = \{(D450) \times \left(V \times \frac{10}{2500}\right) \times \frac{1}{W(g)}\}$$

Estimation of ascorbic acid [35].

A. Extraction of ascorbic acid

500 mg of dry leaves powder of each Ipomea plant was macerated into a mortar with a pastel in 10 ml of 5 % Trichloroacetic acid. The slurry was centrifugated at 3000 rpm for 5 minutes. The supernatant was transferred into a 10 ml measuring cylinder and the volume was made up to the marked with 5% TCA.

B. Estimation of ascorbic acid

The ascorbic acid content was estimated by the method of dinitrophenylhydrogine (2, 4-DNPH) using bromine as an oxidizing agent. In this method ascorbic acid is converted to dihydro ascorbic acid which undergoes spontaneous transformation to dikitogluconic acid in acidic medium. Dihydroascorbate and dihydrogluconate react with 2, 4-DNPH in strong acidic medium forming a radish brown colored complex derivatives. The intensity of the color is measured at 540 nm with a spectrophotometer setting the instrument 'Zero' by the reagent blank that contains no source of ascorbic acid instead contains 5% TCA and other reagent as usual.

5 ml of the extract was taken in a test tube a drop of bromine was added. The solution was mixed thoroughly and waits for 5 minutes. Then air was passed through the solution to remove excess bromine. 1 ml of this solution was taken into another test tube and 3 ml of 5 % TCA and 1 ml of 2, 4-DNPH were added. Then the test tube was kept in a water bath at 37^{0}C for 3 hours. Removing the test tubes from the water bath brought to room temperature. After that, 5 ml of 85% ice cold $H_2SO_4$was added and the test tube was vortexed bringing it to room temperature. The experiment was performed in triplicate for each plant species.

A standard curve (R^2= 0.979) was prepared taking four different concentrations of standard ascorbic acid such as 20 µ/ml, 40µ/2 ml, 60µ/ 3 ml and 80 µ/4 ml. The absorbance was read at 620 nm on a Spectrophotometer (Systronics UV-VIS-119, Model no-Dynamica HALO BD-20). Each absorbance value was divided by its respective ascorbic acid content to get the factor of change. The average of four factors thus obtained from four different contents was determined and considered as the final multiplying factor.

Calculation

A common multiplying factor was determined as done earlier. In both the cases, the amount of ascorbic acid was calculated by using the following formula:

Mg/g amount of ascorbic acid in the sample

$$Common\ factor \times \frac{1}{gm\ weight\ of sample\ taken} \times \frac{Total\ volume\ of extract\ prepared}{Volume\ of extract\ taken\ for\ colour\ production}\ \text{mg/g}$$ DW ascorbic acid.

Estimation of soluble, insoluble and total carbohydrate

A. Extraction of soluble sugar

The soluble sugar was extracted using 50 mg of finely powdered oven-dried samples of leaves in 5 ml of 50% aqueous ethanol followed by centrifugation at 5000 rpm for 5 min in an ordinary centrifuge. The supernatant was transferred into another dry clean centrifuge tube and the volume was raised to 5 ml.

B. Estimation of soluble sugar

The total soluble sugar was estimated by anthrone reagent method [36]. In this method, three clean oven dried test tubes were taken and to each of the test tubes 0.5 ml of supernatant was slowly mixed with 2 ml anthrone reagent and the content was kept on water bath at 75 $\pm$ 2ºC exactly for 8 min followed by cooling at room temperature on running water. The absorbance was read at 620 nm on a Spectrophotometer (Systronics UV-VIS-119, Model No-Dynamica HALO BD-20) and the total soluble sugar was calculated with a standard curve (R^2= 1.00) prepared from glucose by factorial analysis.

C. Extraction of insoluble sugar

The pallet obtained from the extraction of soluble sugar by 50% aqueous ethanol (v/v) was transferred into a boiling tube with several washings by 5 ml of 25% sulphuric acid (H_2SO_4). It was then digested by placing into a boiling water bath for 30 minutes where all the complex insoluble carbohydrate were digested to their respective monosaccharide units, mostly (90%) in the form of glucose. It was then taken out and brought at room temperature. The content was shaked well and centrifuged at 500 rpm for 10 min. The supernatant was transferred into a 100 ml volumetric flux and the volume was made up to the mark with distilled water.

D. Estimation of insoluble sugar

It was also estimated by the same method as mentioned above.

Calculation

A common multiplying factor was determined as done earlier. In both the cases, the amount of soluble/insoluble sugar was calculated by using the following formula:

Mg/g amount of soluble/insoluble sugar in the sample

$$Common\ factor \times \frac{1}{gm\ weight\ of sample\ taken} \times \frac{Total\ volume\ of extract\ prepared}{Volume\ of extract\ taken\ for\ colour\ production}\ \text{mg/g}$$

DW glucose.

A. Calculation of total carbohydrate

The values of soluble and insoluble sugar estimated by the above procedure were summed together to get total carbohydrate in the leaves.

Estimation of soluble, insoluble and total protein

A. Extraction of buffer soluble protein

The phosphate buffer soluble protein was estimated in the dried powdered leaves by the procedure of Lowry *et al.* (1951) [37]. For this, 200 mg of sample was crushed in 5 ml cold potassium phosphate buffer solution (pH 7.5; 0.1 M) on ice-bath using pestle and mortar. The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 12000 rpm for 25 min in a refrigerated centrifuge. The supernatant was taken in a 10 ml measuring cylinder and its volume was raised to 10 ml with same buffer. The pellet was kept in a refrigerator for estimation of insoluble protein. This supernatant contains soluble protein. To1ml of supernatant 1 ml of Trichloro Acetic Acid (TCA) solution (10%, w/v) was added in a centrifuge tube. Immediately a whitish or cream colored precipitate appeared. The precipitate was centrifuged at 1000 rpm for 2 min and after centrifugation the supernatant was discarded and the pellet contained precipitate of soluble protein. To this pellet 2 ml of ethyl alcohol (95%) was added and stirred the precipitate so as to remove the TCA sticking on the surface of the protein as well as to remove pigments, if any. The alcohol reacts with TCA and forms ester. The tube containing the precipitate was re-centrifuged (1000 rpm for 5 min) and the supernatant was discarded. The pellet was used for estimation of soluble protein.

B. Estimation of buffer soluble protein

Crude buffer soluble protein was estimated using reagents prescribed in the Lowry method and the absorbance of navy blue coloured complex was read at 690 nm in a Spectrophotometer (Systronics Model UV-VIS 117). A standard curve (R^2= 0.999) was prepared taking five different concentrations of bovine serum albumin (BSA) (200 µg, 400µg, 600µg, 800 µg and 1mg). A common multiplying factor was determined as done earlier.

C. Extraction of insoluble protein

To determine insoluble protein or complex protein modified procedure of Lowry *et al.*, (1951) was used[37]. For this, the pellet obtained earlier after centrifugation of the crude extract of leaves in the phosphate buffer during extraction of soluble protein was digested (keeping in boiling water bath for 1 hr with 2 ml of 1N NaOH). It was brought to room temperature. 8.0 ml distilled water was added to it and mixed thoroughly followed by centrifugation at 5000 rpm for 5 minutes. The pellet was discarded and supernatant was taken in a 10 ml measuring cylinder and its volume was raised to 10 ml with distilled water.

D. Estimation of insoluble protein

From the supernatant 0.1 ml was taken in a test tube and reagents of Lowry method were added. The remaining procedure was same as described for estimation of phosphate buffer soluble protein.

Calculation

A common multiplying factor was determined as done earlier. In both cases, the protein content of the sample was calculated by the following formula:

Mg/g amount of protein in the sample=

$$Common\ factor \times \frac{1}{gm\ weight\ of\ sample\ taken} \times \frac{total\ volume\ of extract prepared}{volume\ of\ extract\ taken\ for\ colour\ production}\ \text{mg/g}$$

DW BSA

E. Calculation of total crude proteins in leaves

The amount of soluble and insoluble proteins estimated by the above procedure were summed together to get total protein in the leaves.

F. Estimation of total free amino acids

The total free-amino acids were estimated by using the method of Yemn and cocking (1955) [38]. In this, 50 mg dried leaves were homogenized in 10 ml of 50% ethanol (1:1 with water) and ethyl alcohol), with a pinch of activated charcoal. The contents were centrifuged at 1000 rpm for 10 min and free amino acids were collected in the form of clear supernatant which was used for estimation later on. To 1ml of the supernatant 2.5 ml of 2.0% ninhydrin (w/v in isopropyl alcohol) and 2.5 ml of acetate buffer (0.1 M, pH 5.5) were added. The mixture was then heated on a boiling water bath for 30 min; and after cooling, aqueous isopropyl alcohol (1:1) was added to make up the volume to 10 ml. the colour intensity of the violet complex was measured at 570 nm as absorbance. The amount of total free-amino acids was calculated with the help of a calibration curve (R^2= 0.999) prepared from five different concentrations (10 µg, 20µg, 30µg, 40 µg and 50 µg) of alanine and was expressed as mg amino acids produced per gram dry weight of the sample.

Calculation

A common multiplying factor was determined as done earlier**.** The total free amino acids content of the sample was calculated by the following formula:

Mg/g amount of amino acids in the sample=

$$Common\ factor \times \frac{1}{gm\ weight\ of\ sample\ taken} \times \frac{total\ volume\ of\ extract\ prepared}{volume\ of\ extract\ taken\ for\ colour\ production} \text{mg/g}$$

DW alanine.

Estimation of secondary metabolites

Screening and estimation of alkaloids

A. Preparation of plant extract for alkaloids

5 g of air dried powdered plant part was weighed by a regularly calibrated electronic balance (Model No. M-300 DR), crushed in n-butanol as little amount as possible and taken into a reagent bottle with several washings. The volume of n-butanol was taken as 10 ml, the slurry was kept overnight at room temperature, then centrifuged at 6000 rpm for 10 min by an ordinary centrifuge (Model No. B8C/GMLC-5316) and the supernatant was made up to 10 ml with n-butanol.

B. Testing of alkaloids

Crude extract was mixed with 2ml of Wagner's reagent (prepared by dissolving 1.27g iodine and 2g potassium iodide in 100 ml of distilled water). Reddish brown colour precipitate indicates presence of alkaloids [39].

C. Determination of total alkaloids by titrimetric methods [40, 41].

10 ml of the supernatant was taken into a separating funnel; 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 min. The bottom layer inside the separating funnel contains 0.1 (N) HCl neutralized with alkaloids and the upper layer contains n-butanol. The nozzle of the separating funnel was opened slowly to release the HCl portion. 10 ml HCl portion was kept in a 100 ml beaker and 2-3 drops methyl red was added to it, a slightly reddish colour appeared. The contents of beaker were titrated against 0.1 (N) NaOH, until colour changed from red to pale yellow. The procedure was repeated thrice and the neutralization point was determined.

The total amount of alkaloids was calculated following the result of titration: 10ml of 0.1 Normal HCl portion containing dissolved alkaloids is neutralized with less than 10ml of 0.1(N) NaOH. As a small amount was required to neutralize the alkaloids present in 10ml HCl, so, it can be calculated as- 1ml 0.1(N) HCl≅0.0162 g alkaloids.

Screening and estimation of phenolics

A. Preparation of plant extract for phenolics

250mg of dried and powdered plant part was weighed accurately by a regularly calibrated electronic balance (Model No. M-300 DR) and crushed in 5ml of chilled 80% ethanol. The slurry was taken in a centrifuged tube with several washing and then centrifuged at 6000 rpm for 10 min by an ordinary centrifuge (Model No. B8C/GMLC-5316). It was taken out and supernatant transferred to a 10 ml measuring cylinder and the volume of the extract was made up to 5ml with chilled 80% ethanol.

B. Testing of phenolics

2ml of crude extract was mixed with 1ml of 10% ferric chloride (w/v) and formation of precipitates or/and any colour change was observed. A bluish-black or brownish green precipitate indicates presence of phenolics [42].

C. Determination of total phenolics by Folin and Ciocalteau's phenol reagent method

The total phenolics in plant extract were determined by using Folin-Ciocalteau's colorimetric method based of oxidation-reduction reaction [43]. For determining the total phenolics, a standard stock solution of catechol (1mg/ml) was prepared in 80% chilled ethanol. It was diluted ten times and this was considered as the working standard solution. From this stock, 0.1, 0.2, 0.3, 0.4, 0.5 ml sample were taken into separate test tubes. Then 0.5 ml of Folin-Ciocalteau's phenol reagent and 1ml of saturated sodium bicarbonate was added to each of the test tube. The volume of each of the test tube was made up to 5ml with distilled water. After proper mixing the test tubes were incubated in a boiling water bath exactly for 2 min, then subsequently cooled at room temperature and the absorbance was measured at 560 nm by a UV-VIS 190 Spectrophotometer (Model No. Dynamica HALO DB-20).

For plant sample 0.4 ml of prepared extract was taken in triplicate and the colour was generated as usual. The amount of phenolics was calculated based on the values of standard curve. A common multiplying factor was determined as done earlier. The amount of phenolics was calculated as:-

$$Commonfactor X \frac{1}{Amount\ of\ plant\ material\ taken\ (g)} X \frac{Total\ amount\ of\ extract\ prepared\ (ml)}{Amount\ of\ extract\ taken\ (ml)} \text{ mg/g DW catechol.}$$

Screening and estimation of flavonoids

A. Extraction of flavonoids

600 mg of sample is crushed in 5 ml of 80% methanol and centrifuged at 5000 rpm for 5 minutes in an ordinary centrifuge. The pellet was discarded and supernatant was taken in a 10 ml measuring cylinder and its volume was raised to 5 ml with 80% methanol.

B. Testing of flavonoids

2 ml of methanolic extract was taken into a dry and cleaned test tube; a piece of magnesium ribbon was added followed by 1ml of concentrated HCl was added by the side of the test tube. Pink-red/red colouration indicated the presence of flavonoids [44].

C. Determination of total flavonoids

Total flavonoids content was determined according to the procedure by aluminium chloride colorimetric method [45] extract solution (1ml, 0.1mg/ml) was placed in a 10ml-volumetric flask, and then 5 ml of DW was added followed by $NaNO_2$ solution (0.3 ml, 5%). After 5 min, $AlCl_3$ solution (0.6 ml, 10%) was added. After another 5 min, NaOH solution (2 ml, 1M) was added and the volume was made up with distilled water. The solution was mixed thoroughly and absorbance was measured at 510 nm. A standard curve was prepared using quarcetin as standard flavonoids from its five different concentrations (0.4 mg, 0.8mg, 1.2 mg, 1.6 mg and 2 mg) Total flavonoids amount was expressed as mg of quarcetin equivalent per mg of dry weight. All tests were performed in triplicate.

Calculation

A common multiplying factor was determined as done earlier. The amount of flavonoids was calculated as:-

$$Common factor X \frac{1}{Amount\ of\ plant\ material\ taken\ (g)} X \frac{Total\ amount\ of\ extract\ prepared\ (ml)}{Amount\ of\ extract\ taken\ (ml)}$$ mg/g DW quarcetin.

Statistical analysis

All the analyses were performed in triplicate and the results were statistically analyzed (Microsoft office Excel Software 2007) and expressed as mean (n = 3) ± standard error of mean.

3. Results and Discussion

The present report is a part of an elaborate work initiated with the field survey of medicinal plants used by three ethnic tribes, viz., Mog, Reang and Uchai of South Tripura. The first part of this series has been published in The Journal of Medicinal Plants Studies in its 4(5): 122-137; 2016 publication. In the present report we have tried to estimate the amount of primary and secondary metabolites present in the leaves of the three active plants as reported by the respondents and the analysis of data collected from field survey.

Table 2: Ethno medicinal uses of Ipomea plants as reported by three ethnic tribes and their potentiality in curing different ailments.

Plant material	Parts used	Dosage and administration	Fidelity Level (FL %)	PRK (n=192)
TUH 529	Leaves	i) Leaves juice is taken with water regular or pain less menstrual cycle in women.	51.02	25.52
		ii) Young leaves are mixed with paste of unboiled rice (Atap Chal) and made into large sized pills, fried and taken orally or with meals to prevent constipation and hypertension.	48.97	
TUH 530	Leaves	i) 10-20 leaves are paste and taken orally twice a day in case of diarrhea and dysentery.	58.97	26.56
		ii) Leaf decoction (1-2 cup) with black pepper is given as medicine for stomach pain and for the treatment of intestinal worms and abdominal pain.	54.90	
TUH 528	Leaves	i) Leaves are crushed and its juice is consumed 2 teaspoonfuls twice per day against the remedy of diabetes and rheumatism.	60	23.43
		ii) Leaves decoction is prescribed in stomach complaints.	40	

According to the survey and people information on some medicinal plants and also from field guides, it was found that 73 medicinal plants with their uses in curing 69 ailments, three species of Ipomea such as- *I. aquatica* Forsk., *I. batatus* Linn. and *I. carnea* Linn.were found to be potential source of trado-medicines used by Mog, Reang and Uchai tribes of south Tripura district. Herbarium number, used parts, mode of preparation and administration, fidelity level and PRK value of these plants were described above. From this survey it was noticed that leaves of *Ipomea aquatica* Forsk. is used for regular or painless menstrual cycle in women (FL% = 51.02) and prevent constipation and hypertension (FL% = 48.97) with PRK value 25.52%. Leaves of *Ipomea batatus* Linn. is used to mitigate disorders like diarrhea and dysentery (FL% =58.97%); intestinal worm and abdominal pain (FL% = 54.90) with PRK value 26.56%. Leaves of *Ipomea carnea* Linn. are used against diabetes and rheumatism (FL% =60%); stomach complaints (FL% = 40) with PRK value 23.43%.

Available information in literature in this field revealed that a plant species with high PRK value could be an indication of its therapeutic importance in the treatment of reported diseases. Beside, a species with high fidelity level indicates its potentially in healing.

The uses of leaves of the three species of Ipomea in curing common tropical diseases (Table 2) and also for dietary purposes revealed that they may contain different phytochemical.

Table 3: Physicochemical characterization of leaves of selected Ipomea plants.

Parameters	*Ipomea aquatica*	*Ipomea batatus*	*Ipomea carnea*
Moisture content (%)	12.27 ± 0.029	15.41 ± 0.121	22.73 ± 0.011
Solid content (%)	87.72 ± 0.029	84.44 ±0.096	77.27 ± 0.011
Total ash content (g% dwt)	12.25 ± 0.003	9.26 ± 0.032	4.85 ± 0.027

*Means were calculated from triplicate observations
*Values with ± indicate standard error of mean

Table 3 shows moisture, solid and ash content of leaves of selected Ipomea plants. From analysis of the data it was noticed that *Ipomea carnea* contains the highest amount of moisture content (22.73 ± 0.011%) and *Ipomea aquatica* contains the least amount of moisture content (12.27 ± 0.029 %) and *Ipomea batatus* contains moderate amount of moisture content (15.41 ± 0.121 %). The solid content of *Ipomea aquatica, Ipomea batatus and Ipomea carnea* were 87.72 ± 0.029%, 84.44 ±0.096% and 77.27 ± 0.011%, respectively. Maximum amount of total ash content was found to contain in *Ipomea aquatica* (12.25 ± 0.003 g% dwt) following *Ipomea batatus* and *Ipomea carnea* it was 9.26 ± 0.032 g% dwt and 4.85 ± 0.027 g% dwt, respectively.

Since the ash obtained may be derived from the plant itself (physiological ash) as well as extraneous matter, especially sand and soil adhering to the surface of the drug (non-physiological ash), it represents the inorganic part or earthly

matter along with the plant. It also gives an idea about the quality and purity of the drug [45].

Table 4: Quantification of leaf pigments of selected Ipomea plants.

Leaf pigments	*Ipomea aquatica*	*Ipomea batatus*	*Ipomea carnea*
Chlorophyll a (mg g^{-1} dwt)	2.20 ± 0.191*#	0.99 ± 0.042	1.10 ± 0.005
Chlorophyll b (mg g^{-1}dwt)	2.82 ± 0.129	1.54 ± 0.087	1.25 ± 0.030
Total chlorophyll (mg g^{-1}dwt)	4.85 ± 0.286	2.54 ± 0.051	2.37 ± 0.037
Carotenoids (mg g^{-1}dwt)	0.55 ± 0.068	0.63 ± 0.019	0.64 ± 0.039
Vitamins C (mg/100 gm dwt)	146.66 ± 6.64	32.36 ± 0.48	121.66 ± 1.85

*Means were calculated from triplicate observations
#Values with ± indicate standard error of mean

Table 4 shows quantification of leaf pigments of selected Ipomea plants. From analysis of the data it was noticed that *Ipomea aquatica* contains the highest amount of chlorophyll a (2.20 ± 0.191mg/g dwt) and *Ipomea batatus* contains the least amount of chlorophyll a (0.99± 0.042mg/g dwt) and *Ipomea carnea* contains moderate amount of chlorophyll a (1.10 ± 0.005 mg/g dwt). The chlorophyll b contain *Ipomea aquatica, Ipomea batatus and Ipomea carnea* were 2.82 ± 0.129 mg g^{-1}dwt, 1.54 ± 0.087 mg g^{-1}dwt and 1.25 ± 0.030 mg g^{-1} dwt, respectively. Maximum amount of total chlorophyll was found to contain in *Ipomea aquatica* (4.85 ± 0.286 mg g^{-1}dwt) following *Ipomea batatus* and *Ipomea carnea* it was 2.54 ± 0.051 mg g^{-1}dwt, 2.37 ± 0.037 mg g^{-1}dwt, respectively. *Ipomea aquatica* leaves were found to contain the highest amount of chlorophyll. According to Vasu and coworkers [46] out of the three aquatic plants studied by them, *Ipomea aquatica* was reported to contain highest amount of chlorophyll. This could be an indication that *Ipomea aquatica* having the quality to capture more sunlight and make it available to plant system for its cultivation for photosynthesis rather than the two other Ipomea plants studied.

The quantity of Carotenoids was less in *Ipomea aquatica* (0.55 ± 0.068 mg/g dwt) and maximum with *Ipomea carnea* (0.64 ± 0.039 mg/g dwt) and in *Ipomea batatus* the quantity was moderate (0.63 ± 0.019 mg/g dwt).Traditionally, vitamins are not essential for short-term well-being, but there is increasing evidence that modest long-term intakes can have favourable impacts to prevent the incidence of cancers and many chronic diseases, including cardiovascular disease and type II diabetes, which are occurring in western population with increasing frequency [47]. Besides preventing vitamins A malnutrition [48], Carotenoids are also good source of antioxidant. In the present study, it was noticed that *Ipomea carnea* is a good source of Carotenoids. So, these plants help to enhance the immune response which is further associated with lower risk of developing degenerative diseases than the other two Ipomea plants [49, 50].

Maximum amount of vitamin C content was found to contain in *Ipomea aquatica* (146.66 ± 6.64 mg/100gm dwt) following *Ipomea batatus* and *Ipomea carnea* it was 32.36 ± 0.48 mg/100 gm dwt and 121.66 ± 1.85 mg/100 gm dwt, respectively. Ascorbic acid (Vitamin C- water soluble) an essential dietary requirement in man, is widely distributed in the plants and due to its universal presence in the actively metabolizing cells. Isher Wood and Mapsenin 1962 [51] have suggested the role of ascorbic acid in the plant growth and metabolism. Chinoy (1984) [52] also suggested the important role of ascorbic acid during the juvenile phase of growth of a plant. The presence of high amount of vitamin C in *Ipomea aquatica* (146.66 ± 6.64mg/100g dwt) showed that this plant material having antioxidant activity facilitates wound healing, production of collagen, formation of red blood cells, calcification of teeth and bone and boosts immune system [53].

Table 5: Quantification of leaf pigments of selected Ipomea plants.

Primary metabolites	*Ipomea aquatica*	*Ipomea batatus*	*Ipomea carnea*
Soluble sugar(g% dwt)	0.65 ±0.06 *#	2.98 ± 0.07	8.47 ± 0.02
Insoluble sugar(g% dwt)	10.47 ± 0.04	4.59 ±0.02	9.24 ± 0.07
Total carbohydrate (g% dwt)	11.12 ± 0.04	7.57 ± 0.01	17.71 ± 0.01
Soluble protein (g% dwt)	3.35 ± 0.006	1.62 ± 0.01	2.25 ± 0.02
Insoluble protein (g% dwt)	22 ± 0.02	25.63 ± 0.02	23.67 ± 0.01
Total protein (g% dwt)	25.35 ±0.02	27.25 ±0.02	25.92± 0.01
Total free amino acid (mg/100 gm dwt)	42±2.30	58±7.21	144±7.02

*Means were calculated from triplicate observations
#Values with ± indicate standard error of mean

Table 5 shows quantification of primary metabolites from leaves of selected Ipomea plants. From analysis of the data, it was found that *Ipomea carnea* contains the highest amount of soluble sugar (8.47 ± 0.02 g% dwt) and *Ipomea aquatica* contains the least amount of soluble sugar (0.65 ±0.06 g% dwt) and *Ipomea batatus* contains moderate amount of soluble sugar (2.98 ± 0.07 g% dwt). The insoluble sugar content of *Ipomea aquatica, Ipomea batatus and Ipomea carnea* were 10.47 ± 0.04 g% dwt, 4.59 ±0.02 g% dwt and 9.24 ± 0.07 g% dwt, respectively. Maximum amount of total carbohydrate was found to contain in *Ipomea carnea*(17.71 ± 0.01 g% dwt) followed by *Ipomea aquatica* and *Ipomea batatus*(11.12 ± 0.04 g% dwt and 7.57 ± 0.01 g% dwt), respectively.

The soluble protein content were less in *Ipomea batatus*(1.62 ± 0.01 g% dwt) and maximum in *Ipomea aquatica* (3.35 ± 0.006 g% dwt) and in *Ipomea carnea* the quantity was moderate (2.25 ± 0.02 g% dwt). The minimum insoluble protein content was recorded in *Ipomea aquatica* (22 ± 0.02 g% dwt) and maximum in *Ipomea batatus* (25.63 ± 0.02 g% dwt) while in *Ipomea carnea* it was 23.67 ± 0.01 g% dwt. The highest amount of total protein (27.25 ±0.02 g% dwt) was found in *Ipomea batatus* and least amount of total protein (25.35 ±0.02 g% dwt) in *Ipomea aquatica* plant. While in *Ipomea carnea* the quantity was moderate (25.92± 0.01 g% dwt).

The total free amino acid content were less in *Ipomea aquatica* was 42±2.30 mg/100 gm dwt and maximum with *Ipomea carnea* (144±7.02 mg/100 gm dwt) and in *Ipomea batatus* the quantity was moderate (58±7.21 mg/100 gm dwt).

Carbohydrates are the major energy source of the body and they also play an important role in the structure and function of the body organs and nerve cells [54, 55]. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life activities [56]. Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [58]. In the present study it was noticed that *Ipomea carnea* contains high amount of carbohydrate suggesting that it may be a good source of energy [57].

Proteins are essential for growth of the body, produce enzyme, hormone and also help repairing and formation of cell structure. Table 4 shows *Ipomea batatus* leaves contain

highest amount of protein. In the year 2010 Abubakar *et al*., [58] reported that sweet potato leaf soup contains highest protein content than the other part. The presence of high amount of protein in *Ipomea batatus* leaves suggest that consumption of these leaves may be suitable for human body that provides calorific diet. *Ipomea carnea* contain highest amount of total free amino acid its indicate that Ipomea carnea have the ability to synthesis protein and other metabolic substances than the other two plants.

Table 6: Quantification of secondary metabolites from leaves of selected Ipomea plants.

Secondary metabolites	*Ipomea aquatica*	*Ipomea batatus*	*Ipomea carnea*
Alkaloids (mg g^{-1}dwt)	18.3 ± 0.85	17.66 ± 0.96	19.26±0.66
Phenolics (mg g^{-1} dwt)	16.83 ± 0.87	20.25 ± 1.13	15.33 ± 0.17
Flavonoids (mg g^{-1}dwt)	12.71 ± 0.03	12.63 ± 0.03	11.41 ± 0.05

*Means were calculated from triplicate observations
*Values with ± indicate standard error of mean

Table 6 shows quantification of secondary metabolites from leaves of selected Ipomea plants. From analysis of the data, it was found that the alkaloids content of *Ipomea aquatica, Ipomea batatus and Ipomea carnea* were 18.3 ± 0.85 mg g^{-1}dwt, 17.66 ± 0.96 mg g^{-1}dwt and 19.26±0.66 mg g^{-1}dwt, respectively. According to the present study, *Ipomea carnea* contains highest amount of alkaloids (19.26±0.66 mg g^{-1}dwt). Alkaloids repel or deter the feeding of many animals because of bitter or pungent taste or if ingested they are toxic [59]. Rois *et al*., 2008 [60] also confirmed the goats were fed with fresh leaves of *Ipomea carnea* for 45-60 days then Hirsute coat, depression, difficulty to stand up, ataxia, hypermetria wide-based stance, in coordination of muscular movements, intense tremors, spastic, hypersensitivity to sound, head tilting and loss of equilibrium were observed in all treated animals. This could be an indication that frequent consumption of *Ipomea carnea* leaves is not suitable for animals' body. Due to its bitter taste, goat avoids the plant for eating purposes and that is why local people frequently used the plant for fencing.

Ipomea batatus contains the highest amount of phenolics (20.25 ± 1.13mg g^{-1}dwt), *Ipomea carnea* contains the least amount of phenolics (15.33 ± 0.17 mg g^{-1}dwt) and *Ipomea aquatica* contains moderate amount of phenolics (16.83 ± 0.87mg g^{-1}dwt).Phenolics are involved in defense against ultraviolet radiation, provide mechanical support and are therefore an integral part of the human dietary system [60]. Table 6 shows the highest amount of phenolics found in leaves of *Ipomea batatus*. In the year 2008, Padda and Picha[61] reported that Ipomea batatus also contain a unique blend of phenolic compounds, including hydroxyl cinnamic acids, which represent the primary phenolic antioxidant in most commercially available cultivars. Islam *et al*., (2002) further reported *Ipomea batatus* leaves as an excellent source of ant oxidative polyphenolics compared to other commercial vegetables [62]. This could be indication that *Ipomea batatus* having antioxidant activity because they exhibit strong superoxide radical scavenging activity prevent chronic disease [48, 63, 64, 65]. High level of phenolics in the intact plants is perhaps to provide the chemical resistance (Allelopathic effect) to invading microorganisms [66].

Maximum amount of flavonoids was found to contain by *Ipomea aquatica*(12.71 ± 0.03 mg g^{-1}dwt) following *Ipomea batatus* and *Ipomea carnea* (12.63 ± 0.03 mg g^{-1}dwt and 11.41 ± 0.05mg g^{-1}dwt), respectively. The flavonoids have ability to relieve fever, eczema, sinusitis and asthma while certain flavonoids also can protect low-density lipoproteins from being oxidized, thereby play in important role in atherosclerosis [67]. Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk [68]. There have been an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant and anti-cancer agents [70]. The large amount of flavonoids found in *Ipomea aquatica* infers that the plant has biological functions such as protection against allerigies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepotoxins, viruses, tumors [69].and inhibition of hydrolytic and oxidative enzyme [70].

In conclusion, the results indicate that the ethno medicinal significance of the selected plants for the study corresponds to the presence of diverse primary and secondary metabolites they contain. It is therefore pertinent that further studies be carried out on screening and separation of specific metabolites to undergo pharmacological processes and become a potent drug.

Furthermore, there is need to focus research on the plants with antimicrobial properties which is necessary because of the microbial resistance manifested by some of the pathogenic microorganisms against the common antibiotics [71].

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