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Phytochemical investigations on the therapeutic properties of *Ensete glaucum* (Roxb.) Cheesman

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The traditional *Khasi* tribal community of North-East India cite the use of pseudostem sap from *Ensete glaucum* (Roxb.) Cheesman for therapeutic purpose, especially for diarrhoea. This preliminary study has been conducted to evaluate the curative properties of *Ensete glaucum* pseudostem sap by screening for the presence of amino acids, cardiac glycosides, flavonoids, polyphenols, alkaloids, reducing sugars, starch, saponins, tannins, terpenoids and oils and fats. Standard tests confirmed the presence of flavonoids, reducing sugars, terpenoids, saponins, cardiac glycosides and alkaloids, which together contribute to the curative property of the sap as discussed. Polyphenol content was found to be 10.59 mg GAE mL^{-1} and total antioxidant capacity estimated is 54.538 mg AAE mL^{-1}, whereas, total flavonoids were measured at 2.52 mg QE mL^{-1} of fresh sap.

Keywords: Antidiarrhoea, Antioxidant, *Ensete glaucum*, Sap, Traditional knowledge

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Family Musaceae representing bananas comprises of genera *Musa*, *Ensete* and *Musella*[1]. *Musa* includes most of the Musaceae species, whereas *Musella*, a monotypic genus endemic to the northern province of China and southern Sichuan[2,3]. However, *Ensete* occurs in Madagascar, tropical Asia and Africa[4]. The genus, *Ensete* is considered as an old and relict, with few good species mostly tall and herbaceous. Among the two species reported in India, the distribution *of E. glaucum* is limited to the Northeast states, while *E. superbum* to Southern states[5].

In traditional medicine, different varieties of illness, including leprosy, fever, digestive disorders, haemorrhage, hysteria, haemorrhoids, epilepsy and insect bites[6], are treated using banana sap. Research on bananas has validated its medicinal properties in treating diarrhoea[7], wounds, cuts to stop bleeding[8] and as an anti-inflammatory and antimicrobial agent[9]. Rabbani *et al.*[10] reported that green banana supplemented diet helped cure symptoms of acute childhood diarrhoea. The tribal communities of North East India have been using traditional medicines from available natural resources even before the advent of modern medicine, with the knowledge has been passed down through oral tradition. The Nishi and Tagin tribes of Arunachal describes the use of banana pseudostem sap for treating diabetes and stomach ailments[11]. Singh *et al.*[12] reported the possession of both, hyper and hypo-glycaemic effects in pseudostem sap of banana. The pseudostem extract of *Musa paradisiaca* is determined to be antidiabetic because of its hypoglycaemic properties[13]. Ponnambalam and Sellappan[14] reported on anti-urolithiatic and diuretic activity in *Musa balbisiana*. Similarly, Yakubu *et al.*[16] reported the use of *M. paradisiaca* sap to treat hysteria, diarrhoea, dysentery and epilepsy[15] with specific anti-diarrhoeal activity in Wistar rats[16].

Additionally, the study of Pothavorn *et al.*[17] also confirmed the presence of phenolic and aromatic amino compounds in pseudostem sap of both wild species and cultivars that might account for its medicinal properties. Similar findings on the presence of various phytochemicals and their medicinal properties in banana pseudostem sap, explains the use of these plants in treating different ailments[18,19].

The *Ensete* plant, known as Kait Marwei in Khasi dialect, is believed to be of high medicinal value. The pseudostem sap of *Ensete glaucum* has been

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traditionally used specifically for the treatment of diarrhoea and dysentery in the Khasi community. The ayurvedic system of medicine describes the use of *E. superbum* pseudostem and seed to treat various human ailments like kidney stones, stomach ache, diabetes, leucorrhoea, measles and debility[20,21]. Though the potential use of banana plants in treating different ailments and diseases has been well documented, yet, the documentation on the use of Genus *Ensete* as a medicinal plant is limited. Therefore, this study aims to assess various phytochemicals present in the sap of *E. glaucum* pseudostem to help understand and validate its therapeutic nature.

Methodology

Collection of plant material

Ethnobotanical information regarding the use of *Ensete glaucum* was collected from 20 respondents residing in Kyrdem village, Umraling village and Bhoirymbong village under Umsning Block, Ri-Bhoi District, Meghalaya. The plant was identified at ICAR Research Complex – NEH Region, Umiam, Meghalaya. The respondents were between the age group 25 to 55 years. The extraction of *Ensete* sap was demonstrated at Umraling village (25.7025°N, 92.1204°E, 882 m amsl) as presented. The sap was extracted by piercing and slitting around a small portion of the bark using a clean sharp knife and collected into a clean jar rinsed with DDW. However, other respondents from Kyrdem village also reported its extraction using a small hollow piece of bamboo which is pierced into the pseudostem. The sap is a thick liquid with a bright orange colour, slightly sticky and stainable. The extracted sap pH was found to be 6.4 and used immediately for all tests or stored at 4^{o}C for further analysis.

Phytochemical screening

Investigations were carried out on fresh extracts of *Ensete* sap using standard protocols to identify the presence of phytochemicals as per the methods described by Sofowara[22], Trease and Evans[23], and Harborne[24]. Crude sap extract was centrifuged at 8000 rpm to avoid plant particles that might have come while collecting the sap and proceeded for the tests.

Fehling's test for reducing sugars

Fehling's reagents consist of two solutions, Fehling's solution A [$CuSO_4$ (aq)] and solution B (alkaline $KNaC_4H_4O_6$). In a 2 mL of the sap, 2 mL of both Fehling's A and Fehling's B solution was added individually and placed in a boiling water bath and observed for colour reaction and precipitation.

Salkowski's test for terpenoids

A 0.5 mL of the sap was made up to the final volume of 5 mL using double-distilled water (DDW) water, to which 2 mL of chloroform added, followed by slow addition of 3 mL conc. H_2SO_4 to form a layer. The presence of a reddish-brown colour at the interface confirms the test positive for terpenoids.

Test for saponins

It is the general characteristic of saponins in a plant to cause persistent foam when the aqueous solution is agitated. To 0.5 mL of the sap, DDW of 5 mL was added and vigorously shaken. The formation of a stable persistent froth confirmed the presence of saponins.

Test for flavonoids

To 0.5 mL of the sap, 5 mL of 10% ammonia solution was added followed by Conc. H_2SO_4 for the appearance of yellow coloration, which is indicative of the presence of flavonoids in the sample.

Ferric Chloride test for tannins

Phenols form a violet complex with Fe $(III)^{+}$. About 0.5 mL of the sap is boiled in 10 mL of DDW and filtered. Then a few drops of 0.1% $FeCl_3$ were added and observed for indicative colour changes.

Keller Kilani's test for cardiac glycosides

To 5 mL diluted sap, 2 mL of Glacial acetic acid and 1 drop of $FeCl_3$ (1%) solution was added. The appearance of a brown ring at the interface upon the addition of 1 mL of sulfuric acid indicated the presence of glycosides.

Ninhydrin test for amino acids

The chemical Ninhydrin (2,2-dihydroxyindane-1,3-dione) is often used in chemical assays to detect both primary and secondary amines. In 2 mL of plant sap, two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added. The solution was observed for indicative colour changes.

Wagner's test for alkaloids

The presence of Alkaloids in the sample solution reacts with Potassium iodide and iodine solution, which produce a reddish-brown precipitate. A few drops of Wagner's reagent (6 g potassium iodide and 2 g iodine in 100 mL of DDW) were added to 2 mL of the plant sap and observed for the formation of a reddish-brown precipitate for the presence of alkaloids.

Iodine test for starch

Elemental iodine dissolved in an aqueous solution of potassium iodide and produces the triiodide anion (I_3^-). This anion reacts with starch to give an intense "blue-black" colour. Two drops of iodine solution were added to 2 mL of the test solution and thereafter observed for colour changes.

Saponification test for the presence of fats

Saponification test confirms the presence of fats. Phenolphthalein is used as an indicator as partial neutralization of KOH occurs. To 1 mL of the plant sap, few drops of 0.5 N ethanolic potassium hydroxide solution was added followed by a drop of phenolphthalein. The mixture was heated on a water bath for 2 min and observed for persistent frothing or partial neutralization of alkali.

Total phenolic content

Phenol content was estimated through Folin-Ciocalteau (FC) reagent as described by Singleton *et al*.[25]. Initially, 0.5 mL of the plant sap was made up to the final volume of 3 mL with DDW, to which added 1 mL of both DMSO and 10% FC reagent and vortexed. Following 3 min later, an addition of 3 mL 20% Na_2CO_3 and incubated at room temperature for 2 h. Absorbance was measured at 760 nm. The Phenolic contents were calculated based on the standard curve for gallic acid (50-300 µg/mL) and expressed as Gallic acid equivalents (GAE), in milligrams per millilitre of sap.

Total antioxidant capacity

The estimation of total antioxidant capacity was made by phosphomolybdenum assay[26]. To 0.3 mL of the sap, 3 mL of phosphomolybdenum solution containing 0.6 M H_2SO_4, 28 mM KH_2PO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$, was added. The tubes were then incubated at 95^{o}C for 1 h. The absorbance was taken at 695 nm when the solution reached room temperature. Using the standard curve of ascorbic acid (20-100 µg/mL) prepared and total antioxidant capacity is expressed as gallic acid equivalents (GAEs), in milligrams per millilitre of sap.

Total flavonoids content

Total flavonoid content was estimated as per the method described by Kusirisin*et al*.[27]. First, 500 µL of sap was diluted to 2 mL using DDW. To this 150 µL of 5% $NaNO_2$ was added and incubated for 5 min at room temperature. After which, 150 µL of 10% $AlCl_3$ solution was added and incubated for 6 min at room temperature. 1 mL of 1 M NaOH was immediately added and made the total volume to 5 mL with DDW. Absorbance was then measured at 510 nm. A standard curve of quercetin was plotted (50-250 µg/mL) and the total flavonoids content was expressed as mg quercetin equivalent (mg QE) per millilitre of sap.

Statistical analysis

Biochemical experiments were carried out in triplicates and the results were expressed as mean ± SD. All data obtained in the present investigation were statistically validated by one-way analysis of variance (ANOVA) with the Data Analysis tool pack in Microsoft Excel 2016 (Microsoft, Seattle, USA) software package.

Results

Traditional use of Ensete pseudostem sap

All the respondents interviewed cited the use of pseudostem sap extracted from *Ensete glaucum*, in treating diarrhoea and dysentery. The sap is taken orally, about 2-3 tbsp for children and 5 tbsp for adults, during diarrhoea or severe dysentery, until the symptoms of the sickness subside.

Phytochemical screening of Ensete sap

The analysis showed that *Ensete* sap contains reducing sugars, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids (Table 1). Of all, tannin, amino acids, starch, oils and fats were absent. Similar results were also observed in *M. paradisiaca*[18].

Discussion

Sarin and Bafna[28] reported that plants containing chemical constituents such as alkaloids, tannins, flavonoids, diterpenes, sesquiterpenes, terpenes and terpenoids, may be responsible for antidiarrheal activities. The presence of tannin, however, was not detected in *Ensete* sap.

Diarrhoea results from imbalances between secretory and gastrointestinal absorptive mechanisms leading to excessive intestinal fluid and electrolytes loss. But diarrhoea provides a physiological process to flush out harmful luminal substances from the gastrointestinal (GI) tract[29], which becomes pathological when the loss of fluids and electrolytes exceeds the body's ability to replace the losses. Since *Ensete* sap contains a higher amount of potassium, therefore, it would be a potential source to restore the electrolyte balance of a cell.

Total flavonoids content in the sap was calculated to be 2.52 mg QE mL^{-1} (Table 2). Prostaglandins are inflammatory mediators known to induce intestinal

Table 1 — Phytochemical screening and evaluation of *Ensete* sap

Sl. No.	Secondary Metabolites	Observation	Presence/Absence
1	Reducing Sugars	On heating, the solution turned to muddy brown colour with the presence of reddish precipitate on top	+
2	Terpenoids	Milky hazy precipitate at the top and brown-reddish ring between the interface of the liquid	+
3	Saponins	Stable frothing has been observed	+
4	Flavonoids	Sample changes to yellow colouration	+
5	Tannin	No reaction	-
6	Cardiac Glycosides	Brown ring formed between the interface of the clear liquid and violet portion at the bottom	+
7	Amino acids	No reaction	-
8	Alkaloids	A muddy reddish-brown precipitate was formed	+
9	Starch	No significant reaction observed	-
10	Oils and fats	No soapy solution was observed	-

Table 2 — Biochemical properties of *Ensete glaucum* sap

Total Phenols	10.59 mg GAE mL^{-1}+0.24
Total Antioxidant capacity	54.538 mg AAE mL^{-1}+3.38
Total Flavonoids	2.52 mg QE mL^{-1}+0.30

Values are mean of triplicates + SD

secretions during diarrhoea. Both flavonoids and terpenoids have shown inhibitory effects against prostaglandins E_2-induced intestinal secretion in animal models[30-32]. Studies conducted on albino rats have found that the fruit *A. leptophyllum* flavonoidal portion is responsible for the anti-diarrhoeal activity[33] which includes Na^+-K^+ ATPase and NO activity in reabsorption of electrolytes[34]. The antidiarrheal activity in *Musa paradisiaca* sap is reported as a function of alkaloids, phenols, flavonoids and/or saponins in the sap which enhances fluid and electrolyte absorption through de novo synthesized and/or reduced NO levels[16]. The digestive system is one major source of NO which play a crucial role in processes such as digestion, motility, secretion, and absorption[35]. Mascolo *et al.*[36] found that by preventing NOS, diarrhoea and intestinal secretion reduced significantly. Di Carlo *et al.*[37] had also reported the ability of different flavonoids to variably reduce intestinal transit in mice and influenced by its structure. Flavonoids have also been reported to inhibit contractions induced by spasmogens[38].

The presence of saponins has been indicative in *Ensete* sap. Histamine overproduction by mast cells may lead to diarrhoea[39]. Histamine is also a critical mediator in irritable bowel syndrome and food allergy[41]. In the GI tract, histamine is involved in modulation of GI motility and alteration of mucosal ion secretion[42,43]. Since saponins prevent histamine release, therefore, act as an anti-diarrheal agent[40].

The presence of reducing sugar glucose in oral rehydration therapy (ORT) is an established method to help intestine in absorbing water and sodium - a process where glucose absorption remains largely unperturbed during acute diarrhoea[44,45]. This allows the absorption of enough water and sodium to compensate for fluid losses. The indicative presence of cardiac glycosides in the pseudostem sap also highlights the medicinal attributes of *Ensete* sap. Cardiac glycosides are a class of steroids used in treating congestive heart failure in pharmacology[46].

A high capacity for antioxidative functions is determined in the sap as presented in Table 2. Total phenol content and total antioxidant capacity of banana sap measured was 10.59 mg GAE mL^{-1} and 54.538 mg AAE mL^{-1}, respectively. Clinical studies and trials of a complete complex of polyphenols extracted from pomegranate and green tea have shown that the bioactive polyphenol dietary supplement (LiveXtract) with oral rehydration solution (ORS), has the potential to treat paediatric patients suffering from acute diarrhea[47,48]. This polyphenol supplement is hypothesized to boost innate immune functions[48]. The presence of polyphenols like flavonoids in the plants itself indicates for the role of antioxidative functions due to their innate ability to act as high redox potentials[49]. Piuzza and Bullitta[50] have also established clear correlations between phenolic content and antioxidant activity in plant extracts. Thus, the antidiarrheal activity of *Ensete glaucum* sap may be attributed to the presence of these secondary metabolites. However, the qualitative and quantitative analyses of these phytochemicals need to be ascertained for presenting a clearer picture of its validity as an antidiarrheal agent. Further studies in animal models can confirm the anti-diarrhoeal activity and the mechanism of action in details.

Conclusion

The study results will serve as a record for traditional practices and beliefs by the local ethnic populations, which otherwise might have been lost. The results indicated that sap from the pseudostem of *Ensete* contains a variety of phytochemical constituents. Therefore, it is concluded that the presence of reducing sugars, terpenoids, saponins, flavonoids, cardiac glycosides and alkaloids not only attribute to the anti-diarrhoeal property of the *Ensete* sap in ethnomedicinal use among the Khasi community but also, can be a potent antioxidant.

However, further investigations on metabolic regulation, *in vivo* antioxidant activity, and biological properties of phytochemicals are needed to recommend its use in food products and pharmaceuticals. This work is only a preliminary validation study undertaken from a myriad of traditional knowledge on other crops or plants. Documentation of traditional information, scientific screening and evaluation provide the basic knowledge for modern ethnopharmacological research which can eventually catalyze the development of new drugs.

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Conflict of Interest

The authors declare there is no conflict of interest regarding the publication of this research paper.

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