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RESEARCH ARTICLE

Antioxidant, α -Amylase, α -Glucosidase-Inhibitory Activity and Composition of Phenolic, Flavonoid and Minerals in few Ethnomedicinal Antidiabetic Plants of North-East India

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Abstract

Ten ethnomedicinal antidiabetic plants of North-East India were evaluated for their α -amylase and α -glucosidase inhibitory as well as antioxidant activities. Phenolic, flavonoid and mineral contents in these plants were also analyzed. α -amylase inhibitory activity (IC₅₀ 25.455-52.833 μ g/mL) was detected in only four while α -glucosidase inhibitory activity (0.444-450.233 μ g/mL) was detected in seven of these plants. *Lagerstroemia speciosa* (25.455 μ g/mL) and *Parkia timoriana* (25.655 μ g/mL) showed highest α -amylase inhibitory activity, while α -glucosidase inhibitory activity was highest in *Euryale ferox* (0.444 μ g/mL) and *P. timoriana* (0.563 μ g/mL). Six species namely, *Lysimachia candida*, *Antidesma acidum*, *Dillenia indica*, *E. ferox*, *L. speciosa*, and *P. timoriana* showed higher antioxidant activity (in DPPH, ABTS and reductive assay) and these plants also contained phenolic and flavonoid in higher quantity compared to the remaining four plants. Calcium, magnesium, sodium, potassium, phosphorus, iron, copper and zinc were present in all the plants but potassium and zinc were found to be the most abundant macro and micro element. The heavy metals were not detected in these plants. Thus the preliminary data on the ethno medicinal antidiabetic plants show the potential of *L. speciosa*, *P. timoriana*, *E.ferox*, *L. candida* for carrying out further bioactivity guided evaluation and pre-clinical studies in future.

Keywords: Ethnomedicinal; Antioxidant; Phenolics; α -Amylase, α -Glucosidase, Minerals

1 Introduction

Throughout human history, medicinal plants have been used in treatment of various ailments and are sometimes the only affordable source of healthcare for people in remote and economically poor regions. Traditional herbal medicine is an important component of primary health care systems in many developing countries and also an important part of their cultural heritage(1). In recent times, the use of herbal medicine as an alternative in the management of various chronic diseases has gained a lot of interest, mainly due to their very less or no adverse side effects compared to those of

synthetic drugs(2; 3). Diabetes mellitus is a metabolic disorder of multiple etiology characterized mainly by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism along with oxidative stress(4). One of the therapeutic approaches for treating diabetes is to decrease the post-prandial hyperglycaemia by retarding glucose absorption through inhibition of two carbohydrate hydrolyzing enzymes, i.e., α -amylase and α -glucosidase in the digestive tract(5). Although, various available pharmacological agents in the treatment of diabetes is widespread, the use of medicinal plants as complementary treatment on traditional health care system is also visible(6). Traditional

knowledge on medicinal plants get transmitted through generations and forms the basis of valuable information in the discovery of bioactive compounds with therapeutic effects from them(7; 8). Various studies have indicated that medicinal plants are able to inhibit digestive enzymes due to the presence of various secondary metabolites, such as polyphenols and terpenes(9; 10; 11). Antioxidant components of the medicinal plants also play a significant role in antidiabetic activity(12; 13; 14; 15). The Sub-Himalayan region of North East India is endowed with rich resources of medicinal plants due to both climatic and geographical variations and ethnic communities of this region use these widely available plant resources both as dietary and medicinal items(16; 17). In this study, we carried out preliminary evaluation of the medicinal plants based on the limited information in literature reported earlier. Specifically, we examined the inhibitory capacities of ten ethno medicinal antidiabetic plants against α -amylase and α -glucosidase along with antioxidant activities and content of phenolic, flavonoid and mineral in them.

2 Plant Summary

Allium hookeri Thwaites (Amaryllidaceae) *A. hookeri* belongs to Liliaceae family, locally known as “maroinapakpi” in Manipur, is a wild herb which grows well in a wide range of soils. The whole plant grounded with Zingiber officinale roscoe, mixed with salt and water and juice is used to cure ulcer and stomach ailments and also it is used by the local healers to reduce weight of obese people. The leaf decoction is used to control blood pressure(18; 19). As it contains significant amount of phytosterols it must have a positive effect on lowering of LDL cholesterol concentrations. Literature survey has also revealed the use of this plant for the treatment of cough, cold, wound, vomiting and also on healing(20; 21; 22; 23). The methanolic and ethanolic extract and compounds isolated from the extract of *A. hookeri* were found to possess anti-inflammatory, antidiabetic, antioxidant and antimicrobial activity(24). The roots of this plant has been used as a home remedy by the Mizo community of Mizoram in their folklore medicine as a cardioprotective (mainly against high blood pressure) agent(25).

Allium ramosum L. (Amaryllidaceae)

A. ramosum is an herb, locally known as Maroi Nakupi in Manipur. The leaves of the plant are boiled to extract soup which is used to garnish traditional chutney, and pakora, etc. Soup of the leaves is traditionally used to administer to patients with urinary disorder especially in scanty urination. Fresh leaf juice is regarded beneficial for nourishing scalp and hair growth.

Antidesma acidum Retz. (Phyllanthaceae)

A. acidum is a shrub in North-East India, Meitei community use boiled extract of leaf for treatment of diabetes(26). The pharmacological activities of *A. acidum* have been reported specifically for its anti-HIV and immunomodulating actions. *A. acidum* is a folk plant, which is used for management of low backache, muscle pain, neuralgia by folklore practitioners which is mainly associated with inflammation at different parts of body(27). Despite having strong medicinal aspects, the potential role of *A. acidum* in the therapeutic interventions of T2DM has not yet been

systematically explored(17).

Cheilocostus speciosus (J.Koenig) C.D.Specht (Costaceae) *C. speciosus*, a perennial herb having tuberous rhizome is locally known as Jomlakhuti in Assam. The rhizome of this plant is traditionally used for cure of diabetes and jaundice in different parts of Assam(28).

Dillenia indica L. (Dilleniaceae)

D. indica, commonly known as elephant apple is a widely used evergreen plant of Northeast India including Assam. It is locally known as Outenga and fruit of this plant is a popular constituent of Assamese (people from state of Assam, India) cuisine. Apart from its use as food items, it has several other ethnomedicinal uses for relief of stomach related disorders(29).

Euryale ferox Salisb. (Nymphaeaceae)

E. ferox (Makhana) is a rooted macro-hydrophyte and commonly found in the lakes, ponds and ditches. Traditionally, different parts of the plant like tender leaves, petioles, fruits and seeds are advocated for patients to consume directly to bring relief to the problems associated with diabetes(30).

Kaempferia galanga L. (Zingiberaceae)

K. galanga L. belonging to the family Zingiberaceae is an endangered medicinal plant with potent medicinal activities(31). The leaves, rhizome and root tubers of the plant possess a number of medicinal applications. This economically important plant is over exploited to the extent that there is always scarcity of propagating material (rhizomes) as rhizomes of the plant is consumable part. It contains a component which is used in over 59 ayurvedic medicines. It is also extensively used in preparation of ayurvedic drugs, perfumery, cosmetics and as spice ingredients. Extracts of *K. galanga* have anti-inflammatory, analgesic, anti-diarrheal, anti-bacterial, sedative, cytotoxic, insecticidal and anthelmintic properties(32). *K. galanga*,

locally known as Gathion in Assam is known to have several traditional uses. Rhizome of the plant is mainly used for pneumonia, bronchial complaints, stomach disorders, food poisoning and for wound healing in Assam and Meghalaya(33).

Lagerstroemia speciosa (L.) Pers. (Lythraceae)

L. speciosa is commonly known as crape myrtle belonging to the Lythraceae family(34). *L. speciosa* or Banaba is a medicinal tree traditionally used to lower blood sugar in the body. Its high content of corosolic acid makes it an effective anti-diabetic drug. The plant is also recommended for kidney, bladder problems and hypertension. Leaves of the species have been traditionally used over thousands of years in folkloric treatment by the native Indians and Japanese for illness and ailments particularly for lowering blood sugar levels and losing body weight. The flower extracts of the species have been reported to exhibit pharmacological properties such as antioxidant and anti-microbial activities, whereas fruit extracts reported anti-nociceptive, anti-diarrhea and cytotoxic activities. Research on leaf extracts of this plant showed anti-bacterial anti-viral, anti-inflammatory, anti-obesity, anti-fibrotic, anti-diabetic and xanthine oxidase, inhibition, diuretic, decongestant activities. Root of this plant is used for treating mouth ulcers(35; 36; 37; 38; 39). In addition, bark is used to relieve the abdominal pains. The species also has essential metals like sodium, potassium, iron, zinc and

magnesium which were clinically proven to exert beneficial effect on human health.

***Lysimachia candida* Lindl. (Primulaceae)**

L. candida is a folklore medicinal plant that grows in East Asia, Eastern Himalayas at altitudes of 100-2100 m, Western Ghats, Manipur, Assam, Burma and Java is commonly known as Kengoiin Manipur and Loosestrife in English. The genus *Lysimachia* L. comprises about 200 species, both wild and cultivated and is native to temperate regions of Eurasia. This genus has traditionally been assigned to the family Primulaceae. The chemical constituents isolated from *L. candida* comprised of different compounds namely, kaempferol, astragalol, kaempferol-3-O-D-glucopyranosyl (1→2)-D-glucopyranoside, quercetin, quercetin-3-O-D-glucopyranoside, methyl-(R)-2-hydroxy-3-phenylpropanoate, (R)-2-hydroxy-3-phenylpropanoic acid, spinasterol, stigmasterol, palmitic acid, primulagenin A (candidoside), protoprimulagenin A and alpha-spinasterol-glucopyranoside(40). These isolated compounds are reported for their different biological activities such as anticancer, antidiabetic, anti-inflammatory, and anti-obesity, etc. Triterpene saponins isolated from butanol fraction namely, Lysimanoside, Lysikokianoside and Anagallisin C have been reported to exhibit antifungal activity(41). During our ethnomedicinal survey, the local healers informed that it is recommended for the treatment of diabetes and related complications.

***Parkia timoriana* (DC.) Merr. (Leguminosae)**

P. timoriana, commonly known as Yongchak in Manipur is traditionally used as food item as well as for various other ethnomedicinal purpose. The fruits (pods) of this leguminous plant are considered to be nutritious and are known to be effective in the management and treatment of diabetes(42)

3 Material And Methods

3.1 Chemicals And Reagents

α -amylase, α -glucosidase, acarbose, p-nitrophenyl alpha-D-glucopyranoside, 1,1-diphenyl-2-picryl hydrazyl (DPPH), ascorbic acid, gallic acid, Folin ciocalteu reagent, quercetin and Tris-HCl were obtained from Sigma Aldrich. All other reagents, buffers, chemicals and solvents are of analytical grade and were procured from Himedia, India and Milli-Q quality water was used for all experiments.

3.2 Plant Material

The Plants were collected during the month of October 2018 to April 2019 from habitats of these plants located in different regions of North East India. These specimens were deposited at Botanical Survey of India (BSI), Eastern Regional Centre, Shillong and voucher specimens were obtained.

3.3 Extraction Of Plant

Plant materials were washed thoroughly with tap water followed by distilled water to remove any contamination. Washed plant materials were dried under shade at room temperature to remove the moisture. The plants were considered to be dried when weight of the representative plant samples (100 g) were found to be constant for three consecutive days. Air dried plant materials were grinded coarsely

by using mixer grinder and was macerated with methanol. After 48 h, the filtrate was collected by filtration through Whatman No. 1 filter paper and the marc was immersed in methanol. The process was repeated until the color of the filtrates faded. Filtrates obtained were concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at below 50°C. Concentrated extracts were dried completely using lyophilizer (Labconco, USA) at -80°C.

3.4 Total Phenol Content (TPC)

TPC was determined using the Folin-Ciocalteu method [46]. Quantitative estimation in the plant extracts was performed using a standard calibration curve of gallic acid. TPC was expressed in terms of gallic acid equivalents (GAE) (mg of GAE/g of plant).

3.5 Total Flavanoid Content (TFC)

TFC was determined by using the aluminum chloride calorimetric method(43). Quantitative estimation of TFC in the plant extracts was performed using a standard calibration curve of quercetin. TFC was expressed in terms of quercetin equivalents (QE) (mg of QE/g of plant).

3.6 DPPH Radical-Scavenging Activity

The free radical scavenging activity of extract of a plant was measured in terms of hydrogen donating ability using DPPH radical as described by the method of Berg et al. 1999(44). Ascorbic acid was used as positive control. The free radical scavenging activity was calculated by using the formula below: % radical scavenging activity = [(Abs control - Abs sample) / (Abs Control OD)] × 100 (1)

3.7 ABTS Assay

The ABTS free radical scavenging activity of a plant extract was determined by following the method described by Shanab et al., 2012(45) with some minor modifications. The ABTS free radical scavenging activity was calculated by using the formula 1 given above.

3.8 Reducing Power Assay

The reducing power of test sample was determined on the basis of the ability of their antioxidant principles to form colour complex with potassium ferricyanide, TCA and FeCl₃ due to transformation of Fe³⁺ to Fe²⁺. Reductive ability of the plant extracts measured by following the method described by Oyaizu, 1986(46). Ascorbic acid was used as standard in this assay.

3.9 α -Amylase Inhibition Assay

The α -amylase enzyme inhibition assay was done by following starch-iodine method described by Hansawasdi et al., (2000)(47) and Hamdan et al., (2004)(48). Acarbose was used as positive control and the uninhibited enzyme was taken as negative control (DMSO control). The inhibition (%) of α -amylase activity was calculated by using the formula 1 given above.

3.10 α -Glucosidase Inhibition Assay

The α -glucosidase enzyme inhibition assay was performed by following the method described by Kumar et al., 2012(49). Acarbose was used as positive control and the uninhibited enzyme was taken as negative control (DMSO).

The inhibition (%) of α -glucosidase activity was calculated by using the formula 1 given above.

3.11 Mineral Analysis

The plant samples were washed with tap water followed by Milli-Q water thoroughly in order to remove surface contamination. They were then air dried in a clean drying chamber and oven dried at 80°C. 1 g of the powdered dried sample of each plant was taken in porcelain crucibles and placed in a muffle furnace at 500°C overnight. 5 mg of ash of each sample were dissolved in 20 % HCl and warmed to dissolve the residue. The dissolved solutions (containing 5 mg of ash of each sample) were taken in 50 ml volumetric flask and the volume was made up to the mark with deionized water. Diluted solution was taken for analysis of elements like Ca, Mg, Na, K, P, Fe, Cu, Zn, Pb, As, Cd, and Hg by Perkin Elmer AAnalyst-700 atomic absorption spectrometer.

3.12 Statistical Analysis

All the experiments were performed in triplicate, except minerals and heavy metals, which were carried out in duplicate. The results were expressed as the mean of the values obtained for the replications. The correlation coefficients were calculated with Pearson's test using the Graph Prism v8 statistical software. The results were considered statistically significant if the P value ($= 0.05$) is greater than the significance level.

4 Results And Discussion

Chemical constituents such as phenolic compounds in plants act as a good source of natural antioxidant agents(50). The chemical structures, mechanisms of actions, and pharmacological properties of the chemical constituents vary depending upon the plant species and also the geographical origin of the sample(51; 52).The values of total phenol content and antioxidant property parameters assayed using DPPH, ABTS and Reducing Power methods for all the 10 plant species used in this study is presented in Table 1. Phenolic compounds possess an ideal structural chemistry for free radical scavenging activity and contribute to the overall antioxidant potential of plants mainly due to their redox properties. Folin-Ciocalteu method is a rapid and widely used assay for estimation of TPC. The amounts of total phenols and total flavonoids varied between plant species and it ranged from 9.333 to 103.111 GAE (mg/mg dw) and from 7.700 to 50.667 QE (mg/mg dw), respectively. The highest total phenol value was observed in *A. acidum*, followed by *E. ferox* and *L. speciosa* and lowest phenolic content was found in *C. speciosus*. In terms of the scavenging activity of the different species determined by DPPH, *E. ferox* (IC₅₀ = 7.997 μ g/mL) showed the highest scavenging activity followed by *A. acidum* (IC₅₀ = 29.313 μ g/mL), *P. timoriana* (IC₅₀ = 46.203 μ g/mL) and *L. speciosa* (IC₅₀ = 69.582 μ g/mL). Antioxidant activity of *E. ferox* is comparable with that of the standard vitamin C ($P > 0.05$). *A. hookeri* (IC₅₀ = 789.233 μ g/mL) was the lowest antioxidant activity exhibiting species. *E. ferox*, *P. timoriana*, *L. candida* and *A. acidum* exhibited high radical scavenging activities as quantified by ABTS, and the IC₅₀ values for the species were found to be 54.737, 79.200, 111.333 and 117.720 μ g/mL, respectively. The IC₅₀ values of these

plants was significantly higher compared to the remaining species used in this study ($P > 0.05$). The species with the lowest radical scavenging activity was *C. speciosus* (IC₅₀ = 356.333 μ g/mL). One of the medicinal plants used in this study, namely *E. ferox* was also examined earlier by Ojo et al. (2019)(53) using methanolic extract and the IC₅₀ value was much higher (68.3 μ g/ml and 308 μ g/ml) than that observed in this study.

Reductive ability is a measure of the electron donating ability of a plant extract. In this assay, the presence of reducers (antioxidants) in the extracts caused the reduction of the Fe³⁺/ferric cyanide complex to the ferrous form. The amount of Fe²⁺ complex was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increase in absorbance at 700 nm indicates an increase in reducing ability. The values obtained revealed that the RP of each species was concentration dependent (Figures 1). *D. indica*, *L. speciosa*, *E. ferox*, *L. candida*, and *P. timoriana* showed higher reducing power with approximate absorbance value of 0.194 ± 0.005 , 0.188 ± 0.003 , 0.170 ± 0.006 , 0.161 ± 0.002 and 0.153 ± 0.002 , respectively, at a maximum concentration of 100 μ g/mL in comparison to ascorbic acid (1.238 ± 0.091). Previous study showed that Fe³⁺ reducing ability (0.454 ± 0.005 mgAAE) of *A. acidum* mature fruit was more than that of ripened fruits (0.418 ± 0.000 mgAAE)(54).

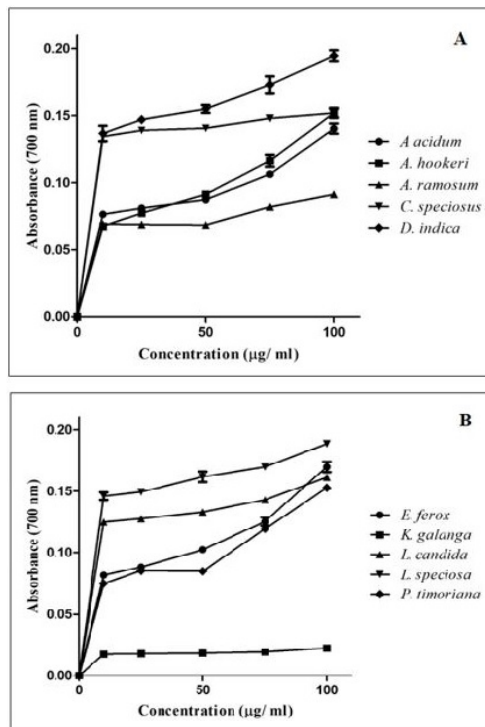


Figure 1: Reductive ability of the methanol extracts of the medicinal plant species measured in terms of formation of Fe³⁺/ Ferric cyanide complex at 720 nm

Secreted from saliva and pancreas, α -amylase catalyses the cleavage of α -1,4 glycosidic bonds to convert polysaccharides into smaller oligosaccharides such as mal-

Table 1: Antioxidant activity in terms of IC50 determined by DPPH and ABTS method and total phenolic (TPC) and flavonoid (TFC) content of the methanol extracts of the ten ethno-medicinal plants

Name of the Plant	Antioxidant activity		TPC (mg/g GAE)	TFC (mg/g QE)
	IC50 ($\mu\text{g/mL}$)			
	DPPH	ABTS		
<i>A. acidum</i>	29.313 \pm 0.729b	117.720 \pm 6.012d	103.111 \pm 2.776e	11.717 \pm 1.640c
<i>A. hookeri</i>	789.233 \pm 4.966g	170.800 \pm 1.312e	34.889 \pm 2.341b	24.247 \pm 0.979e
<i>A. ramosum</i>	203.827 \pm 1.9458d	>1000	30.900 \pm 1.353b	9.570 \pm 0.308bc
<i>C. speciosus</i>	205.177 \pm 6.025d	356.333 \pm 23.454f	9.333 \pm 0.667a	6.630 \pm 0.149a
<i>D. indica</i>	265.833 \pm 2.129e	126.370 \pm 2.650d	71.222 \pm 1.953d	50.667 \pm 1.137f
<i>E. ferox</i>	7.997 \pm 0.203a	54.737 \pm 0.0451b	101.778 \pm 2.694e	16.667 \pm 0.702d
<i>K. galanga</i>	384.100 \pm 4.004f	177.133 \pm 0.808e	31.333 \pm 4.163b	21.833 \pm 1.258e
<i>L. candida</i>	269.433 \pm 18.141e	111.333 \pm 2.309cd	51.300 \pm 1.300c	7.700 \pm 0.010ab
<i>L. speciosa</i>	69.582 \pm 0.278c	321.567 \pm 32.908f	73.178 \pm 2.664d	22.343 \pm 0.388e
<i>P. timoriana</i>	46.203 \pm 3.964b	79.200 \pm 0.705bc	71.697 \pm 3.864d	19.103 \pm 0.194d
Vitamin C	6.537 \pm 1.887a	1.807 \pm 0.035a	-	-

tose, maltotriose, and a number of α -1,4 and α -1,6-oligoglucans. These fragments are subsequently involved in further degradation by α -glucosidase located in the brush border of the small intestine. This enzyme hydrolyses the terminal non-reducing 1,4 linked α -glucose residues and release absorbable monosaccharides to enter the blood stream(55; 56; 57). Therefore, inhibitor of these enzymes delay digestion of carbohydrates and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting of postprandial hyperglycemia(58). The ability of a test substance to inhibit these two enzymes can be considered as a potential anti-diabetic property of the substance. The inhibitory activity of the methanolic extracts of all the species against these two enzymes in terms of IC50 is presented in Table 2. Four plants have shown α -amylase inhibitory activity and their IC50 values were in the range of 25.455 to 52.833 $\mu\text{g/mL}$, while the remaining plant species did not exhibit inhibitory activity even at the maximum concentration tested. α -Amylase inhibitory activity of *L. speciosa* (IC50 25.455 $\mu\text{g/mL}$) and *P. timoriana* (IC50 25.655 $\mu\text{g/mL}$) were significantly higher than those of the standard (acarbose) (IC50 52.870 $\mu\text{g/mL}$) and the remaining plant species tested (P_i 0.05). The inhibitory activity of *D. indica* (IC50 42.478 $\mu\text{g/mL}$) and *A. acidum* (IC50 52.833 $\mu\text{g/mL}$) were comparable with the standard ($P > 0.05$). Methanolic extract of 7 plants showed- glucosidase activity. Their IC50 values varied widely and were in the range of 0.444 to 450.233 $\mu\text{g/mL}$. α -glucosidase inhibitory activity of *E. ferox* (0.444 $\mu\text{g/mL}$) was found to be highest followed by that of *P. timoriana* (0.563 $\mu\text{g/mL}$), *A. acidum* (4.309 $\mu\text{g/mL}$), *D. indica* (8.155 $\mu\text{g/mL}$), *L. speciosa* (36.880 $\mu\text{g/mL}$), *L. candida* (117.237 $\mu\text{g/mL}$) and *A. hookeri* (550.233 $\mu\text{g/mL}$). Importantly, -glucosidase inhibitory activity of all the plant extracts except *A. hookeri* was significantly higher than that of the standard (acarbose) (625.759 $\mu\text{g/mL}$) ($P < 0.05$). Thus in this study, we have observed that except *L. speciosa*, the methanolic extracts of the medicinal plants exhibit strong -glucosidase inhibitory activity with weak or no α -amylase inhibitory activity even at highest concentration tested. The natural herbal products with weak -amylase inhibitory

but strong -glucosidase inhibitory potential are promising alternative to the present day marketed α -amylase enzyme inhibitors prescribed for slowing carbohydrate metabolism. These enzyme inhibitors cause abdominal distention, flatulence, meteorism and diarrhoea. These effects are due to the strong inhibition of pancreatic α -amylase resulting in abnormal bacterial fermentation of undigested carbohydrates in the colon(59; 60).

Content of minerals elements in the ten plants are presented in Table 3. The mineral contents varied widely among samples, but in general, K, Fe, Cu and Zn were found in significant amount in most of the plants. These minerals are known for their role as structural components of tissues and function in cellular, basal metabolism, and maintenance of homeostasis. All the studied plants also contained the other essential elements i.e. Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Phosphorus (P), Iron (Fe), Copper (Cu) and Zinc (Zn) in significant amount. Among the essential macro elements, K was the most abundant element ranging from 0.0731 to 9.985 % w/w in different plant extracts. Its content was highest in *A. hookeri* (9.985 % w/w), followed by *A. ramosum* (8.031 % w/w), *K. galangal* (8.031 % w/w), *L. candida* (3.814 % w/w), *P. timoriana* (2.3173 % w/w), *E. ferox* (1.245 % w/w) and *D. indica* (1.129 % w/w) respectively. None of the plants were found to contain any of the heavy metals such as As, Cd, and Hg except traces of lead in *A. hookeri* species. Heavy metal content in medicinal plants particularly in their Ayurvedic formulation is always a serious concern and the plant materials of this study are free from them and could be used in further research.

5 Conclusion

Among the ten ethnomedicinal antidiabetic plants of NE India, evaluated for their comparative inhibitory capacities against α -amylase and α -glucosidase along with relative content of phenolic, flavonoid, mineral antioxidant potential, methanolic extracts of *L. speciosa*, *E. ferox* and *P. timoriana*, showed highest α -amylase inhibitory activity, while those of *E. ferox* and *P. timoriana* highest α -glucosidase inhibitory activity. These three species also showed promis-

Table 2: α - amylase and α - glucosidase activity of the ethno-medicinal plant extracts of the ten ethno-medicinal plants in terms of IC50 values

Name of the Plant	α - amylase	α - glucosidase
	IC 50 ($\mu\text{g}/\text{mL}$)	IC 50 ($\mu\text{g}/\text{mL}$)
<i>A. acidum</i>	52.833 \pm 7.215b	4.309 \pm 0.119a
<i>A. hookeri</i>	>1000	550.233 \pm 1.168c
<i>A. ramosum</i>	>1000	>1000
<i>C. speciosus</i>	>1000	>1000
<i>D. indica</i>	42.478 \pm 15.187ab	8.155 \pm 0.738a
<i>E. ferox</i>	>1000	0.444 \pm 0.0285a
<i>K. galanga</i>	>1000	>1000
<i>L. candida</i>	>1000	117.237 \pm 1.301b
<i>L. speciosa</i>	25.455 \pm 0.847a	36.880 \pm 1.330a
<i>P. timoriana</i>	25.655 \pm 0.745a	0.563 \pm 0.161a
Acarbose	52.870 \pm 7.927b	625.759 \pm 75.661c

Table 3: Mineral (%) and Heavy metals (ppm) content of the ethno-medicinal plant extracts

Species	Ca	Mg	Na	K	P	Fe	Cu	Zn	Pb	As	Cd	Hg
<i>A. hookeri</i>	0.0445	0.054	0.0335	9.9849	0.13	32.03	50.84	142.56	68.82	ND	ND	ND
<i>A. ramosum</i>	0.0486	0.0737	0.0371	8.0307	0.0344	29.02	4.82	59.71	ND	ND	ND	ND
<i>A. acidum</i>	0.1098	0.1002	0.0242	0.4061	0.0424	41.8	27.86	33.44	ND	ND	ND	ND
<i>C. speciosus</i>	0.0325	0.0162	0.041	0.0731	0.021	19.55	22.45	28.62	ND	ND	ND	ND
<i>D. indica</i>	0.0434	0.079	0.0355	1.1299	0.0374	60	5.08	13.79	ND	ND	ND	ND
<i>E. ferox</i>	0.0511	0.102	0.041	1.2451	0.0146	55.03	8.63	18.11	ND	ND	ND	ND
<i>K. galanga</i>	0.036	0.0752	0.0371	8.0307	0.0344	30.04	6.72	41.53	ND	ND	ND	ND
<i>L. speciosa</i>	0.0731	0.2216	0.1018	3.8137	0.0309	44.7	37.22	43.05	ND	ND	ND	ND
<i>L. speciosa</i>	0.0322	0.0306	0.0022	0.646	0.0154	29.63	4.08	31.13	ND	ND	ND	ND
<i>P. timoriana</i>	0.0011	0.014	0.0161	2.3173	0.0265	33.18	6.52	41.11	ND	ND	ND	ND

ing antioxidant activity along with *A. acidum*, *D. indica* and *L. candida*. Potassium and Zinc were found to be most abundant mineral in the ten plants and none of the plant contained any heavy metals. Overall this study has screened few important antidiabetic ethnomedicinal plants of NE India to search for plant based lead molecules and for development of alternative/complimentary medicine against diabetes and related complications in future research.

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References

- [1] K. Sowjanya, K. Narendra, J. Swathi, and K. Satya, "Phytochemical extraction and antimicrobial efficiency of crude leaf extract of medicinal plant, *Cascabela thevetia*," *International Journal of Research in Pharmacy and Biomedical Science*, vol. 4, no. 2, pp. 465–470, 2013.
- [2] WHO, "Traditional Medicine Strategy (2002-2005)," WHO publications, Tech. Rep., 2002.
- [3] T. B. Klepser and M. E. Klepser, "Unsafe and potentially safe herbal therapies," *American Journal of Health-System Pharmacy*, vol. 56, no. 2, pp. 125–138, 1999, publisher: Oxford University Press.
- [4] K. Nowotny, T. Jung, A. Höhn, D. Weber, and T. Grune, "Advanced glycation end products and oxidative stress in type 2 diabetes mellitus," *Biomolecules*, vol. 5, no. 1, pp. 194–222, 2015, publisher: Multidisciplinary Digital Publishing Institute.
- [5] H. Ali, P. Houghton, and A. Soumyanath, "Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*," *Journal of ethnopharmacology*, vol. 107, no. 3, pp. 449–455, 2006, publisher: Elsevier.
- [6] C. Leduc, J. Coonishish, P. Haddad, and A. Cuerrier, "Plants used by the Cree Nation of Eeyou Istchee (Quebec, Canada) for the treatment of diabetes: a novel approach in quantitative ethnobotany," *Journal of Ethnopharmacology*, vol. 105, no. 1-2, pp. 55–63, 2006, publisher: Elsevier.
- [7] V. Gulati, I. H. Harding, and E. A. Palombo, "Enzyme inhibitory and antioxidant activities of traditional medicinal plants: potential application in the management of hyperglycemia," *Bmc complementary and alternative medicine*, vol. 12, no. 1, pp. 1–9, 2012, publisher: Springer.
- [8] R. A. Halberstein, "Medicinal plants: historical and cross-cultural usage patterns," *Annals of epidemiology*, vol. 15, no. 9, pp. 686–699, 2005, publisher: Elsevier.
- [9] J.-S. Kim, C.-S. Kwon, and K. H. Son, "Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid," *Bioscience, biotechnology, and biochemistry*, vol. 64, no. 11, pp. 2458–2461, 2000, publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.
- [10] G. J. McDougall, F. Shpiro, P. Dobson, P. Smith, A. Blake, and D. Stewart, "Different polyphenolic components of soft fruits inhibit -amylase and -glucosidase," *Journal of agricultural and food chemistry*, vol. 53, no. 7, pp. 2760–2766, 2005, publisher: ACS Publications.

- [11] Y. Tan, S. K. Chang, and Y. Zhang, "Comparison of -amylase, -glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera," *Food chemistry*, vol. 214, pp. 259–268, 2017, publisher: Elsevier.
- [12] S. Bajaj and A. Khan, "Antioxidants and diabetes," *Indian journal of endocrinology and metabolism*, vol. 16, no. Suppl 2, p. S267, 2012, publisher: Wolters Kluwer–Medknow Publications.
- [13] P. M. Mazumder, P. Rathinavelusamy, and D. Sasmal, "Role of antioxidants in phytomedicine with special reference to antidiabetic herbs," *Asian Pacific Journal of Tropical Disease*, vol. 2, pp. S969–S979, 2012, publisher: Elsevier.
- [14] H. Nasri, H. Shirzad, A. Baradaran, and M. Rafeian-Kopaei, "Antioxidant plants and diabetes mellitus," *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, vol. 20, no. 5, p. 491, 2015, publisher: Wolters Kluwer–Medknow Publications.
- [15] J. O. Unuofin and S. L. Lebelo, "Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review," *Oxidative medicine and cellular longevity*, vol. 2020, 2020, publisher: Hindawi.
- [16] L. Deb, S. Laishram, N. Khumukcham, D. Ningthoukhongjam, S. S. Nameirakpam, A. Dey, D. S. Moirangthem, N. C. Talukdar, and T. R. Ningthoukhongjam, "Past, present and perspectives of Manipur traditional medicine: a major health care system available for rural population in the North-East India," *Journal of ethnopharmacology*, vol. 169, pp. 387–400, 2015, publisher: Elsevier.
- [17] Y. Sheikh, B. C. Maibam, D. Biswas, S. Laisharm, L. Deb, N. C. Talukdar, and J. C. Borah, "Anti-diabetic potential of selected ethno-medicinal plants of north east India," *Journal of ethnopharmacology*, vol. 171, pp. 37–41, 2015, publisher: Elsevier.
- [18] P. Sanglakpam, R. R. Mathur, and A. K. Pandey, "Ethnobotany of chothe tribe of Bishnupur district (Manipur)," 2012, publisher: NISCAIR-CSIR, India.
- [19] L. Devi, "Allium hookerii (Maroi Napakpi)," *Folklore Medicines of Ethnobiological Importance in Manipur*, vol. 1, no. 1, 1990.
- [20] C. P. Kala, "Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India," *Journal of ethnobiology and Ethnomedicine*, vol. 1, no. 1, pp. 1–8, 2005, publisher: Springer.
- [21] V. Gupta, "Plants used in folklore medicine by Bangnis of East Kameng, Arunachal Pradesh," 2006, publisher: CSIR.
- [22] L. Bhuyan, "Some ethnomedicinal plants of Arunachal Pradesh," *Bull Arunachal Forest Res*, vol. 23, pp. 45–50, 2007.
- [23] G. Sharma, R. Gohil, and V. Kaul, "Cytological status of Allium hookeri thwaites (2n= 22)," *Genetic Resources and Crop Evolution*, vol. 58, no. 7, pp. 1041–1050, 2011.
- [24] N.-S. Kim, B.-K. Choi, S.-H. Lee, H.-H. Jang, J.-B. Kim, H.-R. Kim, D. K. Kim, Y.-S. Kim, J.-H. Yang, and H.-J. Kim, "Effects of Allium hookeri on glucose metabolism in type II diabetic mice," *Korean Journal of Pharmacognosy*, vol. 46, no. 1, pp. 78–83, 2015, publisher: The Korean Society of Pharmacognosy.
- [25] H. Lalramnghinglova, *Ethno-medicinal plants of Mizoram*. Bishen Singh Mahendra Pal Singh, 2003.
- [26] W. I. Devi, G. S. Devi, and C. B. Singh, "Traditional herbal medicine used for the treatment of diabetes in Manipur, India," *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 2, no. 4, pp. 709–715, 2011.
- [27] O. Boutaud, D. M. Aronoff, J. H. Richardson, L. J. Marnett, and J. A. Oates, "Determinants of the cellular specificity of acetylaminophen as an inhibitor of prostaglandin H2 synthases," *Proceedings of the National Academy of sciences*, vol. 99, no. 10, pp. 7130–7135, 2002, publisher: National Acad Sciences.
- [28] B. Bhuyan and C. Chetia, "An ethnomedicinal study focusing the genus Costus in Assam with emphasis on traditional medicine," *J Med Plants Stud*, vol. 6, no. 2, pp. 178–188, 2018.
- [29] P. Kamboj, N. C. Talukdar, and S. K. Banerjee, "Therapeutic benefit of Dillenia indica in diabetes and its associated complications," *Journal of diabetes research*, vol. 2019, 2019, publisher: Hindawi.
- [30] A. V. SINGH and P. Singh, "7. Nutritional Ethnotherapeutics and Socio Economic Relevance of Euryale Ferox Salisb," *LIFE SCIENCES LEAFLETS*, vol. 22, pp. 1104–to, 2011.
- [31] M. Heinrich, "Plant resources of south-east asia no. 12 (1). medicinal and poisonous plants 1-ls de padua, n. bunyapraphatsara and rhmj lemmens. backhuys, leiden, 1999, pp. 711, numerous botanical and some chemical line drawings, bibliography, indexes (compounds, pharmaceutical terms, scientific plant names, vernacular plant names), hardcover, isbn 90 5782 042 0 (350 dutch guilders-ca us 180)," *Phytochemistry*, vol. 5, no. 53, pp.619 – –620, 2000.
- [32] H. J. Shetu, K. T. Trisha, S. A. Sikta, R. Anwar, S. S. B. Rashed, and P. R. Dash, "Pharmacological importance of kaempferia galanga (zingiberaceae): A mini review," *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, vol. 3, no. 3, pp. 32–39, 2018.
- [33] S. Basak, G. C. Sarma, and L. Rangan, "Ethnomedicinal uses of Zingiberaceous plants of Northeast India," *Journal of ethnopharmacology*, vol. 132, no. 1, pp. 286–296, 2010, publisher: Elsevier.
- [34] A. Al-Snafi, "Medicinal value of Lagerstroemia speciosa: An updated review," *International Journal of Current Pharmaceutical Research*, vol. 11, no. 5, pp. 18–26, 2019.
- [35] A. Bhaumik, B. Ramu, F. Rahman, S. Basheer, and J. Mastanaiah, "The bioactive compounds of fruit of Lagerstroemia speciosa L. act as potential antimicrobial agent," *Int J Pharm Res Health Sci*, vol. 2, pp. 476–80, 2014.
- [36] M. M. Nutan, T. Goel, T. Das, S. Malik, S. Suri, A. K. S. Rawat, S. K. Srivastava, R. Tuli, S. Malhotra, and S. K. Gupta, "Ellagic acid & gallic acid from Lagerstroemia speciosa L. inhibit HIV-1 infection through inhibition of HIV-1 protease & reverse transcriptase activity," *The Indian journal of medical research*, vol. 137, no. 3, p. 540, 2013, publisher: Wolters Kluwer–Medknow Publications.
- [37] A. Morshed, M. H. Hossain, S. Shakil, K. Nahar, S. Rahman, D. Ferdousi, T. Hossain, I. Ahmad, M. H. Chowdhury, and M. Rahmatullah, "Evaluation of antinociceptive activity of two Bangladeshi medicinal plants, Kalanchoe pinnata (Lam.) Pers. and Lagerstroemia speciosa (L.) Pers," *Adv Nat Appl Sci*, vol. 4, no. 2, pp. 193–7, 2010.
- [38] E. W. C. Chan, L. N. Tan, and S. K. Wong, "Phytochemistry and pharmacology of Lagerstroemia speciosa: A natural remedy for diabetes," *International Journal of Herbal Medicine*, vol. 2, no. 2, pp. 100–105, 2014, publisher: AkiNik Publications.
- [39] G. Pavithra, S. Siddiqua, A. S. Naik, P. K. TR, and K. Vinayaka, "Antioxidant and antimicrobial activity of flowers of Wendlandia thyrsoidea, Olea dioica, Lagerstroemia speciosa and Bombax malabaricum," *Journal of Applied Pharmaceutical Science*, vol. 3, no. 6, p. 114, 2013, publisher: Open Science Publishers LLP.
- [40] Z. X. R. P. S. Lin and X. S. C. D. L. Sheng, "Saponins from Lysimachia candida," *ACTA BOTANICA SINICA*, p. 05, 1999.
- [41] X. Xia, X. Wei, and L. Lin, "Triterpenoid saponins from Lysimachia candida Lindl." *Pharmacognosy Journal*, vol. 5, no. 3, pp. 119–122, 2013, publisher: Elsevier.
- [42] Y. Sheikh, B. C. Maibam, N. C. Talukdar, D. C. Deka, and J. C. Borah, "In vitro and in vivo anti-diabetic and hepatoprotective effects of edible pods of Parkia roxburghii and quantification of the active constituent by HPLC-PDA," *Journal of ethnopharmacology*, vol. 191, pp. 21–28, 2016, publisher: Elsevier.
- [43] A. Rebaya, S. I. Belghith, B. Baghdikian, V. M. Leddet, F. Mabrouki, E. Olivier, J. Cherif, and M. T. Ayadi, "Total phenolic, total flavonoid, tannin content, and antioxidant capacity of Halimium halimifolium (Cistaceae)," *Journal of applied pharmaceutical science*, vol. 5, no. 1, pp. 52–57, 2014.

- [44] R. van den Berg, G. R. Haenen, H. van den Berg, and A. Bast, "Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures," *Food Chemistry*, vol. 66, no. 4, pp. 511–517, 1999, publisher: Elsevier.
- [45] S. M. Shanab, S. S. Mostafa, E. A. Shalaby, and G. I. Mahmoud, "Aqueous extracts of microalgae exhibit antioxidant and anticancer activities," *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 8, pp. 608–615, 2012, publisher: Elsevier.
- [46] M. Oyaizu, "Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine," *The Japanese journal of nutrition and dietetics*, vol. 44, no. 6, pp. 307–315, 1986, publisher: The Japanese Society of Nutrition and Dietetics.
- [47] C. Hansawasdi, J. Kawabata, and T. Kasai, "-Amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea," *Bioscience, biotechnology, and biochemistry*, vol. 64, no. 5, pp. 1041–1043, 2000, publisher: Taylor & Francis.
- [48] I. Hamdan, F. Affi, and M. Taha, "In vitro alpha amylase inhibitory effect of some clinically-used drugs," *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, vol. 59, no. 10, pp. 799–801, 2004, publisher: Avoxa-Mediengruppe Deutscher Apotheker GmbH.
- [49] D. Kumar, H. Kumar, J. Vedasiromoni, and B. C. Pal, "Bio-assay guided isolation of -glucosidase inhibitory constituents from *Hibiscus mutabilis* Leaves," *Phytochemical Analysis*, vol. 23, no. 5, pp. 421–425, 2012, publisher: Wiley Online Library.
- [50] M. Carochi and I. C. Ferreira, "A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives," *Food and chemical toxicology*, vol. 51, pp. 15–25, 2013, publisher: Elsevier.
- [51] F. Pistollato and M. Battino, "Role of plant-based diets in the prevention and regression of metabolic syndrome and neurodegenerative diseases," *Trends in food science & technology*, vol. 40, no. 1, pp. 62–81, 2014, publisher: Elsevier.
- [52] Y. S. Oh, "Bioactive compounds and their neuroprotective effects in diabetic complications," *Nutrients*, vol. 8, no. 8, p. 472, 2016, publisher: Multidisciplinary Digital Publishing Institute.
- [53] M. C. Ojo, F. O. Osunsanmi, G. E. Zaharare, R. A. Mosa, N. D. Cele, M. O. Oboh, and A. R. Opoku, "In-vitro Anti-diabetic and Antioxidant Efficacy of Methanolic Extract of *Encephalartos ferox* leaves," *Pharmacognosy Journal*, vol. 11, no. 3, 2019.
- [54] P. Patil and V. Jadhav, "Nutritional Assessment of Fruits of *Antidesma Acidum* Retz."
- [55] K. Alagesan, P. K. Raghupathi, and S. Sankarnarayanan, "Amylase inhibitors: potential source of anti-diabetic drug discovery from medicinal plants," *Int J Pharm Life Sci*, vol. 3, no. 2, pp. 1407–1412, 2012.
- [56] S. Chiba, "Molecular mechanism in -glucosidase and glucoamylase," *Bioscience, biotechnology, and biochemistry*, vol. 61, no. 8, pp. 1233–1239, 1997, publisher: Taylor & Francis.
- [57] P. M. Sales, P. M. Souza, L. A. Simeoni, P. O. Magalhães, and D. Silveira, "-Amylase inhibitors: a review of raw material and isolated compounds from plant source," *Journal of Pharmacy & Pharmaceutical Sciences*, vol. 15, no. 1, pp. 141–183, 2012.
- [58] S. Kumar, S. Narwal, V. Kumar, and O. Prakash, "-glucosidase inhibitors from plants: A natural approach to treat diabetes," *Pharmacognosy reviews*, vol. 5, no. 9, p. 19, 2011, publisher: Wolters Kluwer–Medknow Publications.
- [59] H. B. B. AG, "Pharmacology of -glucosidase inhibition," *European journal of clinical investigation*, vol. 24, no. S3, pp. 3–10, 1994, publisher: Wiley Online Library.
- [60] J.-L. Chiasson, R. G. Josse, R. Gomis, M. Hanefeld, A. Karasik, M. Laakso, and STOP-NIDDM Trial Research Group, "Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial," *The Lancet*, vol. 359, no. 9323, pp. 2072–2077, 2002, publisher: Elsevier.