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ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF IMPORTANT TRADITIONAL MEDICINAL PLANTS FROM NORTHEASTERN INDIA

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ABSTRACT: The antidiabetic and antioxidant efficacy of traditional medicinal plants from North-Eastern India remains largely unexplored. This study seeks to address this gap by examining the properties of 11 such plants. The plant samples were subjected to extraction using four different solvents. Their antidiabetic potential was evaluated through *in-vitro* α-glucosidase and α-amylase inhibition bioassays, while the antioxidant activity was assessed using the DPPH Scavenging assay. The results were compared to the standard antidiabetic drug, acarbose. Out of the 11 plants, five species demonstrated significant antidiabetic properties, inhibiting more than 50% of α-glucosidase and α-amylase enzyme activities. The petroleum ether tuber extract of *Stephania glandulifera* exhibited the highest α-glucosidase inhibition (99%), followed by the methanol leaf extract of *Begonia roxburghii* (94%) and the water leaf extract of *Antidesma acidum* (85%). In the α-amylase inhibition assay, *Equisetum debile* methanol extracts showed a 64% inhibition, followed by the leaf extract of *A. acidum* with a 62% inhibition in the methanol leaf extracts. Comparatively, methanol and water leaf extracts of *Debregeasia longifolia* displayed 64% and 67% inhibitions, respectively. The water extract of *B. roxburghii* demonstrated significant antioxidant activity (57%), while the other samples showed less than 50% effectiveness. Notably, the α-glucosidase inhibition consistently exceeded the α-amylase inhibition across the samples. This study confirms the antidiabetic properties of five out of the 11 ethnomedicinal plants, as evidenced by the *in-vitro* α-glucosidase and α-amylase inhibitory assays. These findings support the traditional use of these plants in the management of diabetes and emphasize the need for further *in-vivo* studies, which could contribute to the development of novel herbal formulations or therapeutic drugs for the treatment of diabetes.

INTRODUCTION: Diabetes mellitus, also known as hyperglycemia, is a long-term metabolic condition that disrupts the metabolism of carbohydrates, proteins, and fats. This disruption can lead to various organ-specific diseases affecting the eyes, kidneys, and blood vessels, while also diminishing the quality of life for countless individuals globally [1, 2].

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It can also cause secondary health issues like high lipid levels, heart disease, kidney failure, strokes, nerve damage, eye conditions, and blindness [3]. Diabetes arises either from reduced insulin production (Type-1 diabetes) or from the body's resistance to insulin (Type-2 diabetes) [4].

A prevalent treatment strategy for Type-2 diabetes involves inhibiting enzymes that break down carbohydrates, such as α-glycosidase and α-amylase, which helps delay glucose absorption, thereby reducing blood sugar spikes after meals and controlling hyperglycemia [5, 6]. Diabetes ranks among the top five critical health issues in developed nations, with approximately 60 million

cases in India alone [7]. Type-2 diabetes is the most common form, leading to persistent high blood sugar levels due to the body's insensitivity to insulin, especially when faced with excessive glucose [8, 9]. The rise in diabetes cases is linked to dietary habits that may lead to obesity-a major risk factor for Type-2 diabetes-and varies with social and cultural lifestyle differences [10]. When diet and exercise are insufficient for controlling blood sugar levels, medication with oral hypoglycemic agents becomes necessary. Among these medications, inhibitors of α-glucosidase and α-amylase are employed to slow down the rise in blood sugar after meals by decelerating carbohydrate metabolism and glucose release. Alpha-amylase, a key enzyme in pancreatic juice and saliva, breaks down complex starch molecules into simpler sugars like dextrin, maltotriose, maltose, and glucose [11, 12]. Conversely, α-glucosidase, located in the small intestine's lining, completes the digestion of starch and disaccharides prevalent in our diet [13].

Inhibitors of these enzymes reduce carbohydrate absorption and extend the digestion time in the small intestine, effectively lowering post-meal blood sugar levels [12, 14]. These enzymes are thus targeted for Type-2 diabetes treatments [15]. Recent phytochemical research has explored the antidiabetic potential of various plants, with findings showing that compounds like alkaloids, flavonoids, terpenoids, saponins, polysaccharides, and glycosides from medicinal plants exhibit antidiabetic properties [16, 17]. Ethnic communities' use of traditional herbal remedies plays a crucial role in diabetes care, particularly in developing and low-income nations [18].

It's estimated that 80% of the global population depends on naturally derived medicines, and it's advised to support this reliance, especially where modern diabetes treatments are scarce [19]. In recent years, there's been a surge in interest in plant-based antioxidants and blood sugar-lowering agents for managing diseases linked to oxidative damage. Antioxidants combat oxidative harm through four primary actions: diminishing reactive oxygen species (ROS), halting chain reactions, neutralizing free radicals, and binding metals that facilitate the formation of free radicals [20]. The body's inherent antioxidant system contributes to functions like anti-aging, and protection against mutations and cancer [21]. During metabolic processes, ROS are produced, but their overproduction leads to oxidative stress, disrupting the balance between oxidants and antioxidants and damaging essential biomolecules such as lipids, proteins, and DNA [22, 23]. ROS particularly attack unsaturated fatty acids in cell membranes, causing lipid peroxidation, reduced membrane fluidity, enzyme and receptor dysfunction, and ultimately cell damage [24, 25]. Antioxidant substances work by postponing the oxidation of other molecules and preventing the start or spread of oxidizing chain reactions caused by free radicals, thus shielding the body from oxidative harm that can lead to chronic illnesses like diabetes, cancer, and heart disease [26, 27]. The North-Eastern region of India is home to diverse tribal groups, each with unique languages, rituals, attire, medicinal knowledge, and living styles [28].

Many medicinal plants found in this area, both wild and cultivated, are believed to have significant industrial and medical value. Traditional healthcare practices using local medicinal plants continue to be prevalent among these communities for treating various health issues, including diabetes. Reports indicate that over 200 medicinal plants are utilized in the region for diabetes management [29-31]. In this study, we address the challenge of undocumented and unverified traditional medicinal practices passed down through generations, which may have led to changes in the original use of specific plants. The risk of misapplication is heightened by the morphological similarities among plants and their identical names in various ethnic languages. Our research aims to systematically validate the antidiabetic and antioxidant properties of selected ethnomedicinal plants from the North-Eastern region using *in-vitro* α-glucosidase and α-amylase inhibition assays, along with the DPPH Assay, to ensure their efficacy and safety in traditional healthcare applications.

MATERIALS AND METHODS:

Collection and Identification of Plant Samples: Eleven significant ethnomedicinal plants, utilized for treating and managing diabetes by various ethnic communities in Assam, Arunachal Pradesh, Manipur, Meghalaya, and Sikkim in Northeastern India, were selected and collected for the present study, as indicated in **Table 1**. The plant specimens were identified with the help of

taxonomists and floras [32-34]. The plant names were authenticated based on the plant list (https://wfoplantlist.org/). Herbarium specimens were prepared for 11 plant species as per standard protocols [35]. The herbarium specimens (Voucher no.KK01, 04-05, 07-14) were deposited in the Herbarium of the Department of Forestry, NERIST, Nirjuli, Arunachal Pradesh.

TABLE 1: LIST OF ETHNOMEDICINALLY IMPORTANT PLANT SPECIES FROM NORTH EASTERN INDIA

Sl. no.	Botanical Name	Family	Local Name (States)*	Parts Used
1	*Antidesma acidum* Retz.	Phyllanthaceae	Heikum/ Chingyensin (MN)	Leaves
2	*Begonia roxburghii* A. DC.	Begoniaceae	Siltetoi (AR/AS)	Leaves
3	*Cuscuta reflexa* Roxb.	Cuscutaceae	Akash lota (AS)	Whole plant
4	*Cyperus esculentus* L.	Cyperaceae	Kaothum (MN)	Tuber, Rhizome
5	*Debregeasia longifolia* (Burm.f.) Wedd.	Urticaceae	U-khajing (MN)	Root, Leaves
6	*Equisetum debile* Roxb. ex Vaucher	Equisetaceae	Lai utong (MN)	Whole Plant
7	*Ruellia simplex* C.Wright.	Acanthaceae	Langdhrei (MN)	Whole plant
8	*Hydrolea zeylanica* (L.) Vahl	Hydroleaceae	Langali (AS)	Leaves, Twigs
9	*Litsea cubeba* (Lour.) Pers.	Lauraceae	Siltimmur (ML, SK)	Fruits
10	*Paederia scandens* (Lour.) Merr.	Rubiaceae	Padurilata (AS)	Leaves
11	*Stephania glandulifera* Miers	Menispermaceae	Tubukilota (AS)	Tuber

(*AS=Assam; AR=Arunachal Pradesh; MN=Manipur; ML= Meghalaya; SK=Sikkim).

Preparation of Plant Sample for Analysis: Approximately 200 grams of diverse fresh plant specimens, including leaves, tubers, flowers, stems, and fruits, were meticulously cleaned, rinsed, and dehydrated in a hot air oven at temperatures ranging from 45-50°C for 12-24 hours to eliminate excess moisture. Following drying, the botanical materials were finely ground, carefully labeled, and stored at 4°C for subsequent analysis.

Sequential solvent extraction was performed on the samples, commencing with petroleum ether (P), followed by ethyl acetate (E), methanol (M), and concluding with water (W). Subsequently, the samples underwent agitation on a rotary shaker for 6-12 hours at 100 rpm and ambient temperature. The resulting supernatants were then filtered using Whatman filter papers No.1 and 4.

The filtrates were concentrated and desiccated under a vacuum at a reduced pressure of 5°C utilizing a Vacuum Rotary Evaporator with Chiller (RV10, IKA) set to 80 to 100 rpm and a water bath temperature of 30°C. Samples that were difficult to dry using the rotary evaporator were alternatively dried in a hot air oven placed on Petri dishes or glass slides at temperatures ranging from 35-45°C.

A solubility assessment was conducted by dissolving 1mg of the extract in 1 ml of the respective solvents (P, E, M, and W). Extracts demonstrating solubility in any of the four solvents were subsequently selected for the screening of antidiabetic and antioxidant attributes via standard *in-vitro* assays.

***In-vitro* Antidiabetic Assay:**

α-Glucosidase Inhibitory Assay: Screening of anti-diabetic properties of selected plants was done following the 96-well microplate-based α-glucosidase inhibitory assay described by Kumar *et al.*[36]. For the preparation of test samples, 1mg of the dried extract was dissolved in 20µl of solvent (in which the extract was soluble) and made up the volume to 1000µl with sterile water (MilliQ) respectively in a 1.5/ 2ml centrifuge tube.

The α-glucosidase assay was performed in a reaction volume of 75µl using a 96-well microplate. A sample solution of 25µl was mixed with 25µl of the α-glucosidase enzyme (0.5U) and pre-incubated at 37°C ± 1°C for 10 min. After pre-incubation, 25µl of the substrate (0.5mM, p-nitrophenyl α-D glucopyronoside) was added to the reaction mixture and incubated at 37°C ± 1°C for 30 min.

The reaction was terminated by adding 100µl of 0.2M sodium carbonate solution. The amount of p-nitrophenol released from PNPG (yellow color) was quantified on a 96-well microplate at 405nm in a UV visible spectrophotometer/ microplate reader (Mustikan GO, Thermo-Scientific, Finland). Appropriate sample blanks and controls were included for each sample treatment. All reactions were performed in three replicates. The percentage

of α-glucosidase inhibition activity was calculated by using the formula.

α-glucosidase inhibition (%) = [(Control OD-Sample OD)/ Control OD] ×100

[Control OD = OD of the control reaction without inhibitor-Blank OD; Sample OD = Sample OD-Sample blank OD].

α-Amylase Inhibitory Assay: The α-amylase inhibitory assay was carried out following the standard protocol with slight modifications [37, 38] glass test tube (10 x 60 mm) containing 200 µl of 0.1 % starch was preincubated at 37°C for 5 minutes.

Then, 5 µl of the α-amylase enzyme (0.5U), and 25 µl of the sample (1mg/ml) were added and a final volume of 500 µl was made up with assay buffer (Tris-HCl buffer, pH 6.9). The reaction mixture was then incubated at 37°C for 15 min.

After incubation, 500 µl of 50% acetic acid was added to stop the reaction. Then 1000 µl of iodine solution (254 mg Iodine crystals + 4 g KI in 1000 ml sterile water) was added to each test tube. The tubes were vortexed for 30 seconds and the absorbance of the upper supernatant was measured at 565 nm using a UV-visible spectrophotometer (Multiskan GO UV-Vis spectrophotometer, Thermo-Scientific, Finland).

A control reaction without an enzyme was included. Acarbose was also included as a standard inhibitor drug for comparison. Appropriate blanks were included for each sample treatment. A minimum of three replicate reactions was run for each sample experiment. The percentage of α-amylase enzyme activity inhibition was calculated by using the formula.

α-amylase inhibition (%) = (Test OD/ Control OD) x 100

[Control OD= Positive Control OD-Blank OD; Test OD=Blank OD -Sample OD].

***In-vitro* Antioxidant Assay:**

DPPH Scavenging Assay: The antioxidant activity of the selected plant samples was evaluated using a modified DPPH assay [39, 40]. A volume of 1ml DPPH (0.33 mM) was taken in a test tube (10x60mm), then 20 µl of the test sample was added. Then, the test tubes were gently shaken and incubated in the dark for 30 minutes. A volume of 200µl from each reaction mixture solution containing test samples and standard antioxidants was transferred in 96-well microplates and the absorbance was recorded at 517 nm in a UV-visible spectrophotometer (Multiskan GO, Thermo-Scientific, Finland). Ascorbic acid (100 ppm) and gallic acid (50 ppm) were also included as standard antioxidants in the different tubes for comparison with the test samples.

A reaction containing 1 ml of DPPH and 20µl of distilled water or methanol was also included as a control. All test samples and blanks were analyzed in quadruplicates. Appropriate sample blanks were included in four replicates for test samples and standard antioxidants. The percentage of DPPH scavenging activity (antioxidant activity) was calculated as follows:

DPPH scavenging activity (%) = [(Control OD-Sample OD)/Control OD] ×100

[Control OD= OD of Blank; Sample OD= OD of the samples-Blank Sample OD].

Statistical Analysis: All data presented were analyzed in triplicate or quadruplet and mean values were presented in the table and text with respective standard deviations (SD).

RESULTS: The yields (dry weights) of samples of 11 medicinal plant species extracted in four different solvents are presented in **Table 2**. A minimum of 53.10 mg was recovered in *R. simplex* and a maximum of 461.10 mg was obtained from *A. acidium* among the petroleum ether extracts. In the case of ethyl acetate, the highest yield was recorded from *H. zeylanica* (271.60 mg) while the lowest (98.13 mg) was obtained from *S. glandulifera*.

The yield of the methanol extract was maximum in *H. zeylanica* (321.80 mg) and a minimum of 3.90 mg was obtained from *A. acidium*. Among the water extracts, the highest yield was recorded from *C. reflexa* (388.30 mg) and the lowest from *A. acidium* (2.40 mg). The total weight of all four extracts of each plant species varied from a minimum of 561.73 mg in *R. simplex* to a maximum of 1147.90 mg in *C. reflexa* respectively.

TABLE 2: LIST OF PLANTS AND YIELDS OF THEIR EXTRACTS IN DIFFERENT SOLVENTS

Sl. no.	Medicinal plant species (Plant parts used)	Yield of extracts (mg)				Total weight (mg)
		Petroleum ether (P)	Ethyl acetate (E)	Methanol (M)	Water (W)	
1	*A.acidum* (leaf)	461.10	245.70	3.90	2.40	713.10
2	*B.roxburghii* (leaf)	321.20	241.10	179.80	294.90	1037.00
3	*C.esculentus* (tuber)	71.30	152.60	161.10	176.20	561.20
4	*C.reflexa* (whole plant)	239.50	229.70	290.40	388.30	1147.90
5	*D.longifolia*(leaf)	119.10	251.30	130.30	346.40	847.10
6	*R.simplex* (Whole plant)	53.10	128.00	101.20	279.43	561.73
7	*E.debile* (whole plant)	128.40	181.60	141.30	259.50	710.80
8	*H.zeylanica* (leaf)	170.20	271.60	321.80	342.40	1106.00
9	*L.cubeba* (fruits)	98.89	114.62	121.00	382.50	717.01
10	*P.scandens* (leaf)	280.60	181.50	151.10	252.30	865.50
11	*S.glandulifera*(tuber)	89.21	98.13	110.10	278.60	576.04

Antidiabetic Properties of Plant Extracts: α-glucosidase inhibition properties of the 11 plant samples in different solvents are shown in **Fig. 1.** The petroleum ether extract of the tuber from *S. glandulifera* was found to show the highest inhibition of enzyme activity (99%) as compared to the standard α-glucosidase inhibitor drug, acarbose (72%). Other plant samples did not show the enzyme inhibition property. The ethyl acetate extracts of the plant samples showed a maximum of 39% in *P. scandens* followed by 22% in *C. reflexa*. A minimum of 10% inhibition was recorded in *S. glandulifera* against acarbose (74%). Among the methanol extracts, *B. roxburghii* displayed a maximum inhibition of 94% followed by 23% in *S. glandulifera* and 22% in *C. reflexa* as compared to acarbose. Methanol extracts from eight plant samples have shown no inhibition property against the α-glucosidase activity. In the case of water extracts, the highest α-glucosidase inhibition activity was recorded in the leaf extract of *A. acidum* (85%) which was followed by *C. reflexa* (24%) as compared to acarbose (76%). Six of the plant samples (*C. esculentus, D. longifolia, R. simplex, E. debile, H. zeylanica,* and *L. cubeba*) have not shown α-glucosidase inhibitory properties.

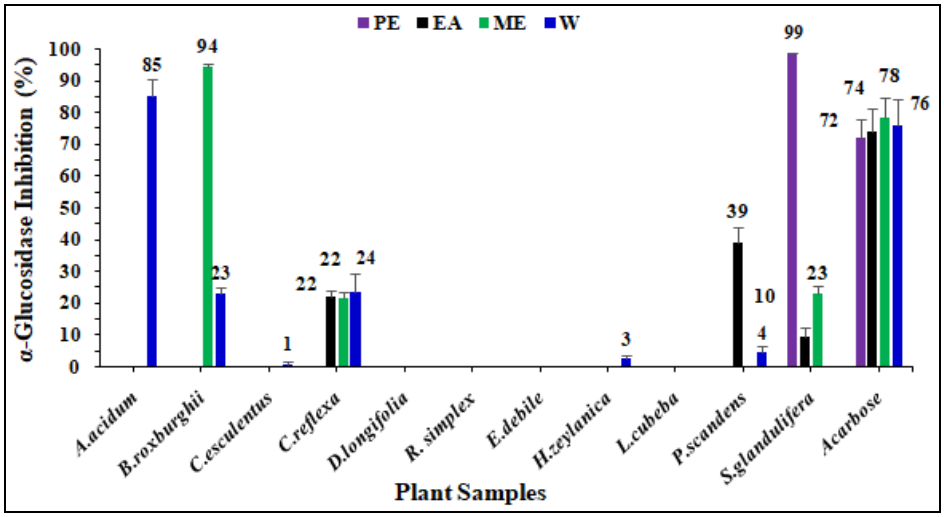

FIG. 1: α -GLUCOSIDASE INHIBITORY PROPERTIES OF ELEVEN MEDICINAL PLANTS IN VARIOUS SOLVENT EXTRACTS

The α-amylase inhibitory properties of 11 medicinal plants in four different solvents are shown in **Fig. 2**. The petroleum ether extracts of the 11 medicinal plants did not exhibit α-amylase activity inhibition properties. Ethyl acetate extracts of only 4 plant samples (*P. scandens*, *H. zeylanica*, *B. roxburghii*, and *C. reflexa*) have shown very low enzyme activity inhibition properties as compared to acarbose (88%). Eight of the 11 methanol extracts have shown enzyme inhibition properties

with the highest (64%) recorded in *D. Longifolia* and *E. debile* followed by 62% (*A. acidum*). Other plant samples have shown low enzyme inhibition properties. *D. longifolia* (67%) and *E. debile* (64%) were recorded with the highest enzyme inhibition properties among water extracts. The remaining plant samples displayed enzyme inhibition properties between 27% and 49% as compared to acarbose (87%). No enzyme activity inhibition properties were recorded from the water extracts of four plant species (*R. simplex, S. glandulifera, L. cubeba,* and *P. scandens*).

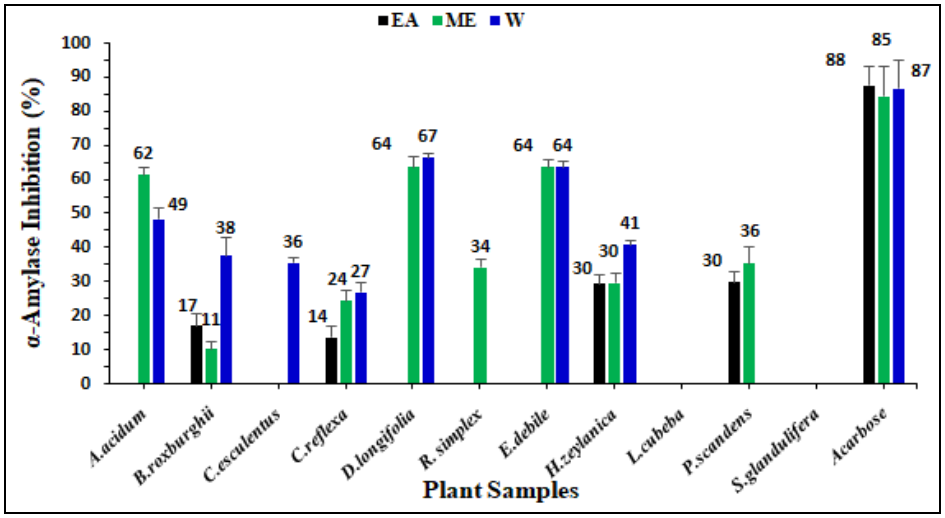

FIG. 2: α - AMYLASE INHIBITORY PROPERTIES OF ELEVEN MEDICINAL PLANTS IN VARIOUS SOLVENT EXTRACTS

Antioxidant Activity of Plant Extracts: The antioxidant activities of plant extracts in four solvents as shown in **Fig. 3**. The petroleum ether and ethyl acetate extracts of all plants showed between 5% and 28% antioxidant properties. The methanol extracts displayed antioxidant properties between 7% in *H. zeylanica* to 41% in *C. reflexa.* The water extract of *B. roxburghii* had shown the highest antioxidant activity (57%) followed by *A. acidum* (42%) and 41% in *P. scandens* and *S. glandulifera*. The antioxidant activities of all plant samples in different solvents were comparatively lower than the standard antioxidants, ascorbic acid, and gallic acid.

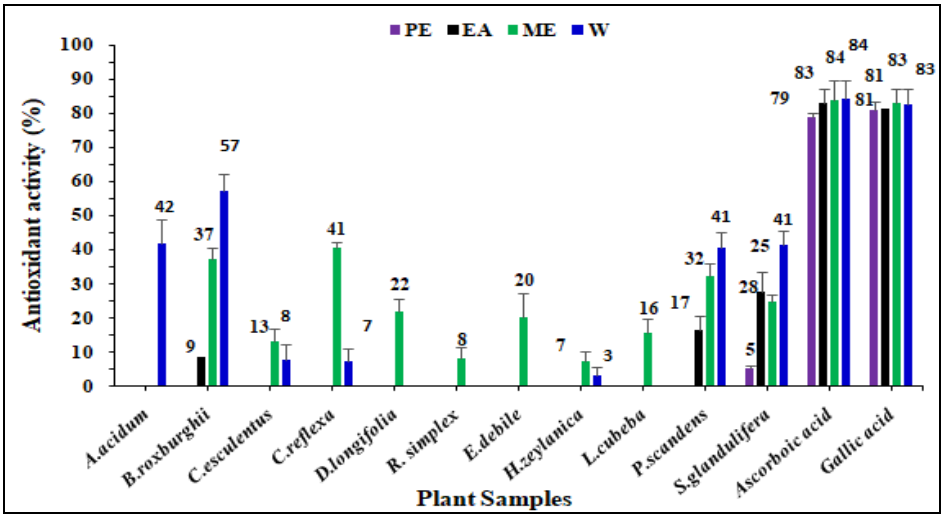

FIG. 3: ANTIOXIDANT ACTIVITY OF ELEVEN MEDICINAL PLANTS IN VARIOUS SOLVENT EXTRACTS

DISCUSSION: In North-Eastern India, various ethnic groups traditionally use medicinal plants to treat and manage numerous health conditions. These plants provide an accessible healthcare option for the region's economically disadvantaged ethnic populations [41, 42]. This study focused on 11 plants known within these communities for their diabetes treatment potential, examining their

antidiabetic and antioxidant capabilities through *in-vitro* enzyme inhibition and DPPH scavenging assays. Out of these, five species- *A. acidum*, *B. roxburghii*, *D. longifolia*, *E. debile*, and *S. glandulifera*- showed notable antidiabetic effects, inhibiting over 50% of α-glucosidase and α-amylase enzyme activities. Remarkably, *S. glandulifera* petroleum ether tuber extract and *B. roxburghii* methanol leaf extract inhibited over 90% of α-glucosidase activity, with the former's efficacy against α-glucosidase being documented for the first time in this research. Additionally, it's reported that Arunachal Pradesh's ethnic communities consume the fresh tuber extract of *S. glandulifera* for a duration of 2 to 3 weeks as a diabetes remedy [42]. Additional species within the same genus, including *S. glabra*, *S. japonica*, *S. hernandifolia*, and *S. tetrandra*, have demonstrated the ability to lower blood sugar levels in diabetic mice and rats induced with alloxan and streptozotocin [43-45]. In this research, both methanol and aqueous extracts from the leaves of *A. acidum* (Syn. *A. bunius*) exhibited strong antidiabetic effects by blocking the enzymes α-glucosidase and α-amylase.

Notably, the methanol extract of *A. acidum* leaves from North-East Thailand has shown similar antidiabetic effects in diabetic rats [46]. Further studies have confirmed the antidiabetic benefits of methanol extracts from the leaves, stems, and bark of this plant, as per references [47, 48]. However, the methanol extract of the fruit showed minimal antidiabetic activity [49]. In Manipur, the boiled leaf water extract of *A. acidum* is widely recognized as an effective antidiabetic remedy [31, 50]. Meanwhile, *B. roxburghii*, a relatively obscure medicinal plant found from Nepal to Arunachal Pradesh and some regions of Bangladesh in the Eastern Himalayas, has been identified to have antidiabetic properties [51-53]. Recent research has also uncovered its analgesic, anti-arthritic, thrombolytic, and cytotoxic properties [52]. Tribal groups in Arunachal Pradesh have traditionally consumed the fresh leaf juice extract of a certain plant twice daily for 2 to 3 weeks as a diabetes treatment [42, 51]. Until now, there has been no scientific confirmation of how this plant's antidiabetic effects work. However, this study found that the methanol leaf extract of *B. roxburghii* strongly inhibits α-glucosidase activity, even more so than the standard drug acarbose, marking the first scientific backing for the antidiabetic properties of *B. roxburghii* through α-glucosidase enzyme inhibition. Similarly, the leaves and roots of *D. longifolia* are used by some Manipur communities for diabetes management[31], but no scientific evidence has supported its mechanism of action until now. This research shows that the methanol and water leaf extracts of *D. longifolia* moderately inhibit α-amylase activity by 62 to 67% *in-vitro*. Out of the 11 medicinal plants studied, *L. cubeba* was the only one without detectable antidiabetic effects. Yet, studies have indicated that the methanol and ethanol fruit extracts of *L. cubeba* do possess antidiabetic qualities due to their ability to inhibit α-glucosidase and α-amylase activities [54]. The antioxidant potential of these plants was assessed through DPPH scavenging assays, revealing that *B. roxburghii* has significant antioxidant properties. The highest antioxidant activities were observed in *B. rex-cultorum* (Baby rainbow) and *B. malabarica*, with a positive correlation between anthocyanin levels and antioxidant capacity among the cultivars [55].

CONCLUSION: This investigation into the antidiabetic efficacy of traditional medicinal plants from North-Eastern India has substantiated the claims of various ethnic and tribal communities regarding the therapeutic potential of five out of eleven plants studied. The significant inhibition of α-glucosidase and α-amylase activities by these plants supports their role in diabetes management. Notably, this study has unveiled the antidiabetic properties of *B. roxburghii*, *D. longifolia*, and *S. glandulifera* for the first time, with *B. roxburghii* also demonstrating antioxidant capabilities. The absence of antidiabetic activity in *Litsea cubeba* highlights the specificity of plant-based treatments. The findings pave the way for future research to isolate active compounds from these ethnomedicinal plants, which could lead to the creation of new herbal remedies or pharmaceuticals for diabetes care. The promise shown by these plants underscores the rich medicinal heritage of North-Eastern India and its potential contribution to contemporary healthcare solutions.

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