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ETHNOMEDICINAL VALUES AND ANTIDIABETIC POTENTIAL OF *CLERODENDRUM* SPP. OCCURRING IN NORTHEASTERN REGION

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ABSTRACT: The present study was undertaken to systematically analyze, document the traditional knowledge of *Clerodendrum* species use for the treatment of various human ailments from NER. The information was collected by literature survey as well as by consulting questionnaire with the villagers and local communities of NER. A total of twelve *Clerodendrum* species were collected, and among them, *C. colebrookianum, C. indicum, C. viscosum* were found to have multi-medicinal values that were widely used for the treatment of various diseases. Further antidiabetic properties of *Clerodendrum* species were evaluated by α-amylase and α-glucosidase assay. The result showed 4 species, *C. indicum, C. japonicum, C. serratum, C. viscosum* exhibited significant (>50%) α-amylase inhibition properties while 3 other species, *C. colebrookianum, C. inerme, C. viscosum* displayed significant α-glucosidase inhibition properties. Lowest IC_{50} values were observed in *C. serratum* of α-amylase (45µg/ml) and *C. viscosum* of α-glucosidase (47 µg/ml). A detailed scientific investigation of the biochemical compounds and metabolites with potential biological activities in *Clerodendrum* species may lead to the discovery of potential drug candidates against life-threatening diseases and will help in scientific understanding and proper utilization in traditional and modern health care systems of the country in particular and the world in general.

INTRODUCTION: *Clerodendrum* is a very large and medicinally important genus reported to have more than 400 species distributed in tropical and subtropical regions of the world [1]. There are about 23 species being reported in India where 16 species and 1 variety are reported from the state of Arunachal Pradesh alone [2].

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These plants are found abundantly growing in northeastern region (NER) and widely used continuously by the local peoples for treatment of many diseases such as anti-microbial, anti-helmintic, anti-inflammatory, anti-malarial, anti-diabetic, hepatoprotective, indigestion, high blood pressure, high fever, asthma, *etc*. Phytochemical study of *Clerodendrum* species reported to have major compounds of alkaloids, phenolics, flavonoids, steroids *etc*. and various biological activities like antibacterial, antimicrobial, insecticidal, antihypertensive, antioxidant *etc*. have been reported [1].

Type II diabetes (non-insulin-dependent diabetes mellitus) is a chronic endocrine disorder characterized by hyperglycemia in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin [3]. In the recent past, *in-vitro* screening of herbal-based inhibitors for alpha (α-) amylase and α-glucosidase enzymes have been important approaches to researchers for the discovery of antidiabetic drugs. α-amylase is the enzyme that hydrolyses the polysaccharide (starch) to oligosaccharides (maltose), and α-glucosidase catalyzes the final step to release the absorbable glucose. Hence, the inhibition of these enzymes leads to a decrease in blood glucose level, which plays an important role in the management of diabetic complications[4]. Since these enzymes play key roles in digestion and intestinal absorption of sugar in the diet, their inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [5]. *In-vitro* screening of α-amylase and α-glucosidase enzyme inhibitors have been reported from many plants including some species of *Clerodendrum* such as *C. bungei*, *C. multiflorum* and *C. volubile* [6, 7, 8] and other plants namely, *Artocarpus altilis, Artocarpus heterophyllus, Berberis aristata, Cassia auriculata, Cinnamomum zeylanicum, Piper betel, Terminalia arjuna etc*. [9, 10]

Therefore, the present investigation was carried out to document on the ethnobotany of North East *Clerodendrum* species and evaluation of an antidiabetic property by *in-vitro* α-amylase and α -glucosidase assay.

MATERIALS AND METHODS:

Study Area, Species Identification and Herbarium Preparation: Field surveys (2011-2015) were conducted in six states of NER (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Mizoram) and *Clerodendrum* species were collected, identified by examining the morphological and reproductive features with consulting various available floras [11, 12], Botanical Survey of India, Itanagar and available identifying keys [13, 2]. The plant list (http://theplantlist.org) was used for the valid names. Each of the herbarium was given a specific voucher number and was deposited in the Herbarium of Department of Forestry, NERIST, Nirjuli, Arunachal Pradesh.

Field Survey and Ethnobotany of *Clerodendrum* Species from NER: All peer-reviewed journals, book chapters, particularly on ethno-medicinal uses of Clerodendrum species from NER,, were selected for this review and collected by searching major scientific electronic databases including Google Scholar, PubMed, Science Direct, *etc*.

In terms of journal contribution to this review, the Journal of Traditional Knowledge (13nos.) and Journal of Economic and Taxonomic Botany (11nos.) provided the majority of articles carrying information on the ethnobotany of *Clerodendrum* species from NER. Further validation was done by making field trips to village areas of tribal communities, and information on medicinal uses of *Clerodendrum* species was collected through modified semi-structured questionnaire [14] (Appendix 1). A total of 100 informants were selected at random during house-to-house surveys. The knowledge of medicinal plants >30 years of age was taken into consideration.

Preparation of Plant Material for Bioassay: A total of the twelve *Clerodendrum* species were collected during the field survey. However, only seven (7) species viz. *C. colebrookianum* (CC), *C. inerme* (CIN), *C. indicum* (CI), *C. japonicum* (CJ), *C. philippinum* (CP), *C. serratum* (CS) and *C. viscosum* (CV) were selected for antidiabetic assay based on their medicinal values and availability of plant samples in wild habitats**.** Young, tender, and disease-free leaves of selected seven *Clerodendrum* species were rinsed with tap water followed by drying in a hot-air oven (60 °C) for 3-12 h, grounded into powder by using a kitchen grinder.

Preparation of Crude Methanol Leaf Extract: Dry powdered leaves (1kg) of seven *Clerodendrum* species were suspended in methanol (ME) and kept overnight in a rotary shaker (Scigenics Biotech, Chennai) at 100 rpm. The slurry was filtered on sterile Whatman filter paper (110mm), and the green-colored filtrate was vacuum dried (below 25 °C) in a rotary evaporator (RV-10, IKA, Germany). The yield of the extracts was recorded, and the samples were finally stored at -20º separately. The crude methanol extracts were screened for their inhibitory activities against α-amylase and α-glucosidase.

C**hemicals and Reagents:** α-amylase ex-porcine (SRL148188), α-glucosidase (Maltase) (SRL74854), p-nitrophenyl α-D-glucopyranoside (SRL144969) were purchased from SRL India. Acarbose (A8980) was purchased from Sigma. Starch, NaOH pellets, Na_2HPO_4, NaH_2PO_4, NaCl, calcium chloride, dimethyl sulphoxide, Tris base, and other chemicals and solvents were of highest purity grade and purchased from Merck, Mumbai, India. Milli-Q water was used for all the enzymatic assays.

Preparation of Enzyme, Substrate, and Buffer Solutions for α-amylase Assay:
Incubation Buffer: 0.05M Tris Hcl buffer (pH 6.9) containing 0.01M $CaCl_2$.

Enzyme Preparation (20U/ml): A stock solution of 1mg/ml (1mg=20U) α-amylase prepared in Tris-HCl buffer.

Substrate Starch Solution: Starch azure 0.1% in 0.05M Tris-Hcl buffer (pH 6.9) containing 0.01M calcium chloride and boiled for 5-10 min to properly dissolve starch in solution.

Positive Control Acarbose: A stock solution (1mg/ml) prepared in distilled water (Millipore Type I).

Test Sample: A stock solution (1mg/ml) prepared in DMSO and further diluted with water upto 1ml. DMSO concentration was below 2%.

Terminating Solution: 50% acetic acid.

Color Reagent: Iodine solution was prepared by dissolving 0.254g I_2 and 4g KI in 1L of distilled water.

Preparation of Enzyme, Substrate, and Buffer Solutions for α-glucosidase Assay:
Incubation Buffer: 0.1M phosphate buffer, pH 6.8

Enzyme Preparation (64U/ml): A working stock of 1mg/ml (1mg=64U) α-glucosidase prepared in phosphate buffer.

Substrate Preparation: 0.5mM of p-nitrophenyl α-D glucopyranoside (PNPG) prepared in phosphate buffer.

Positive Control Acarbose: 1mg/ml dissolved in distilled water (Millipore Type 1).

Test Sample: A stock solution (1mg/ml) prepared in DMSO and further diluted with water upto 1ml. DMSO concentration was below 2%.

Terminating Solution: 0.2M sodium carbonate solution.

α-amylase Inhibition Assay of Crude Leaf Extract: *In-vitro* α-amylase enzyme inhibition assay was done by following partial modification of the starch-iodine method [15, 16].

A volume of 200µl starch solution (0.1% starch in 0.05M Tris HCl buffer containing 0.01M $CaCl_2$) was taken in a test tube (10 × 100mm) and pre-incubation at 37 °C for 5 min. After pre-incubation, a sample extract solution of 25µl (1mg/ml), 5µl of the α-amylase enzyme (1U) and 270µl of buffer were added to the test tube to make the final reaction mixture of 500µl. The reaction tube was further incubated at 37 °C for 10minutes and stopped by adding 500µl of 50% acetic acid. The reaction mixture was then centrifuged at 3000 rpm for 5 min at 4°C. The upper centrifuged part of the reaction mixture was transferred into a clean and dry test tube. The reaction mixture was allowed to develop color by adding 1ml of iodine solution followed by vortexing for 30 sec. The absorbance was measured at 565nm in a spectrophotometer (Multiskan GO, Thermo-Scientific, Finland). Acarbose was used as standard inhibitor drug for α-amylase. Appropriate sample blanks and controls were included for each sample treatment. All reactions were performed in triplicate. The inhibition (%) of α-amylase activity was calculated by using the formula.

α -amylase inhibition (%) = Sample OD × 100/ Control OD

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

Dose-Response Analysis of α-amylase Inhibition Assay: The significant *Clerodendrum* species (showed >50% inhibition) were further selected for dose-dependent analysis by taking different con-centrations *i.e.*, 10-100µl≈20-200µg/ml, respectively.

α-glucosidase Inhibitory Assay of Crude Leaf Extract: The α-glucosidase enzyme inhibitory assay was performed in a chromogenic method in

which enzyme activity was estimated by measuring yellow color developed due to release of p-nitrophenyl after cleavage of glycosidic linkage of substrate p-nitrophenyl α-D glucopyranoside (PNPG)[4]. 25µl of sample solution was premixed with 25µl of the enzyme (0.5U) and incubated at 37 °C ± 1 °C for 10 min. After incubation, 25µl of the substrate (0.5mM, *p*-nitrophenyl α-D glucopyranoside) 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 was quantified on a 96 well microplate at 405nm in a microplate reader (Multiskan GO, Thermo-Scientific, Finland). Appropriate sample blanks and controls were included for each sample treatment. Acarbose was used as a standard inhibitor drug for α-glucosidase. All reactions were performed in triplicate. The inhibition (%) of α-glucosidase activity was calculated by using the formula.

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

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

Dose-Response Analysis of α-glucosidase Inhibition Assay: The significant *Clerodendrum* species (showed >50% inhibition) were further selected for dose-dependent analysis by taking different concentrations *i.e.* 5µl to 30µl≈67µg/ml to 400µg/ml respectively.

IC_{50} Calculation: The half-maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. IC_{50} values were calculated by using an online tool (www.ic50.tk) from the dose-response curve for both α-amylase and α-glucosidase assay.

RESULTS:

Diversity and Ethnobotany of *Clerodendrum* Species in NER: Present investigation on the taxonomic diversity of *Clerodendrum* species revealed the rich diversity with the occurrence of 12 numbers of species (*C. bracteatum*, *C. colebrookianum*, *C. inerme*, *C. indicum*, *C. japonicum*, *C. philippinum*, *C. serratum*, *C. speciosum*, *C. splendens*, *C. thomsonii*, *C. viscosum* and *C. wallichii*) from NER **Table 1**, **Fig. 1**.

Literature survey covered a total of 60 numbers of reports particularly on ethnomedicine of *Clerodendrum* species resulted in ten species representing 37 diseases used by 26 tribal communities in entire NER while field survey analysis recorded 9 *Clerodendrum* species representing 16 diseases used by 13 tribal communities of NER. In contrast, the single species, *C.thomsoniae* was found to be used as ornamental **Table 2**.

TABLE 1: DETAILS OF TWELVE CLERODENDRUM SPECIES, THEIR VERNACULAR NAMES, AND PLACE OF COLLECTION FROM NORTHEASTERN REGION

S. no.	*Clerodendrum* species	Vernacular name (Tribe/NE state)	Place of the collection (NE States)
1	*C.bracteatum* Wall. ex Walp.	Dom-bhetai (Assamese, Bodo Kachari/AS); Atsuksuba (Naga/NL)	Lower Subansiri, Papum Pare (AR)
2	*C. colebrookianum* Walp.	Tapen, Poto, Dringi (Adi, Nishi, Nocte, Singpho, Khampti, Tangsa /AR); Nefaphu (Assamese, Bodo Kachari/AS); Jaren, Sia-long, Dien-ja-rem-kyntheri, Jhr-khtung, Yay-iong / Khasi, Jaintia, Garo/ML); Kutab-manbi, Kuthab (Manipuri/MN); Phuinum (Mizo/MZ); Orematong, Umrem (Naga/NL)	Lower Subansiri, Papum Pare (AR); Lakhimpur, Kamrup (AS); Aizwal (MZ); East Imphal, Senapati (MN); Dimapur (NL); Ri Bhoi (ML)
3	*C. indicum* (L.) Kuntze	Akal bih (Assamese/AS); Charoidong (Manipuri/MN);Bamus gach (Garo/ML)	Lakhimpur (AS)
4	*C. inerme* (L.) Gaertn.	-	Papumpare (AR); Lakhimpur (AS)
5	*C. japonicum* (Thunb.) Sweet	Horaiphul (Assamese/AS)	Papumpare (AR); Lakhimpur (AS)
6	*C. philippinum* Schauer	-	Changlang, Lohit, Papumpare (AR); Lakhimpur (AS); East Imphal (MN)
7	*C. serratum* (L.) Moon	Nangal bhanga, Teuri-longphlang (Assamese, Kachari/AS); Bharung (Apatani/AR); Sam-seng, Hursymet, Rilong-phlang (Garo,	Lower Subansiri, Papumpare (AR); Lakhimpur (AS); Ri Bhoi (ML)

		Khasi/ML)	
8	*C. thomsoniae* Balf.f.	-	Papumpare (AR)
9	*C. viscosum* Vent.	Purimoli (Nishi/AR); Bhet tita, Dhopa tita, Reiwang, Makhna, Lwkhna, Mwkhwna (Assamese, Kuki, Bodo/AS); Chuikuima (Reang/TR); Dieng-Jarem-Synrang, Sam-makhi (Khasi, Garo/ML); Tokolam Naga/NL)	Changlang, Lohit, Papumpare, (AR); Lakhimpur (AS); Dimapur (NL)
10	*C.wallichii* Merr.	-	East Khasi Hill (ML)
11.	*C. speciosum* Dombrain	-	Lakhimpur (AS); Papumpare (AR)
12.	*C. splendens* G.Don	-	Lakhimpur (AS); Papumpare (AR)

AR-Arunachal Pradesh; AS-Assam; MN-Manipur; MZ-Mizoram; ML-Meghalaya; NL-Nagaland

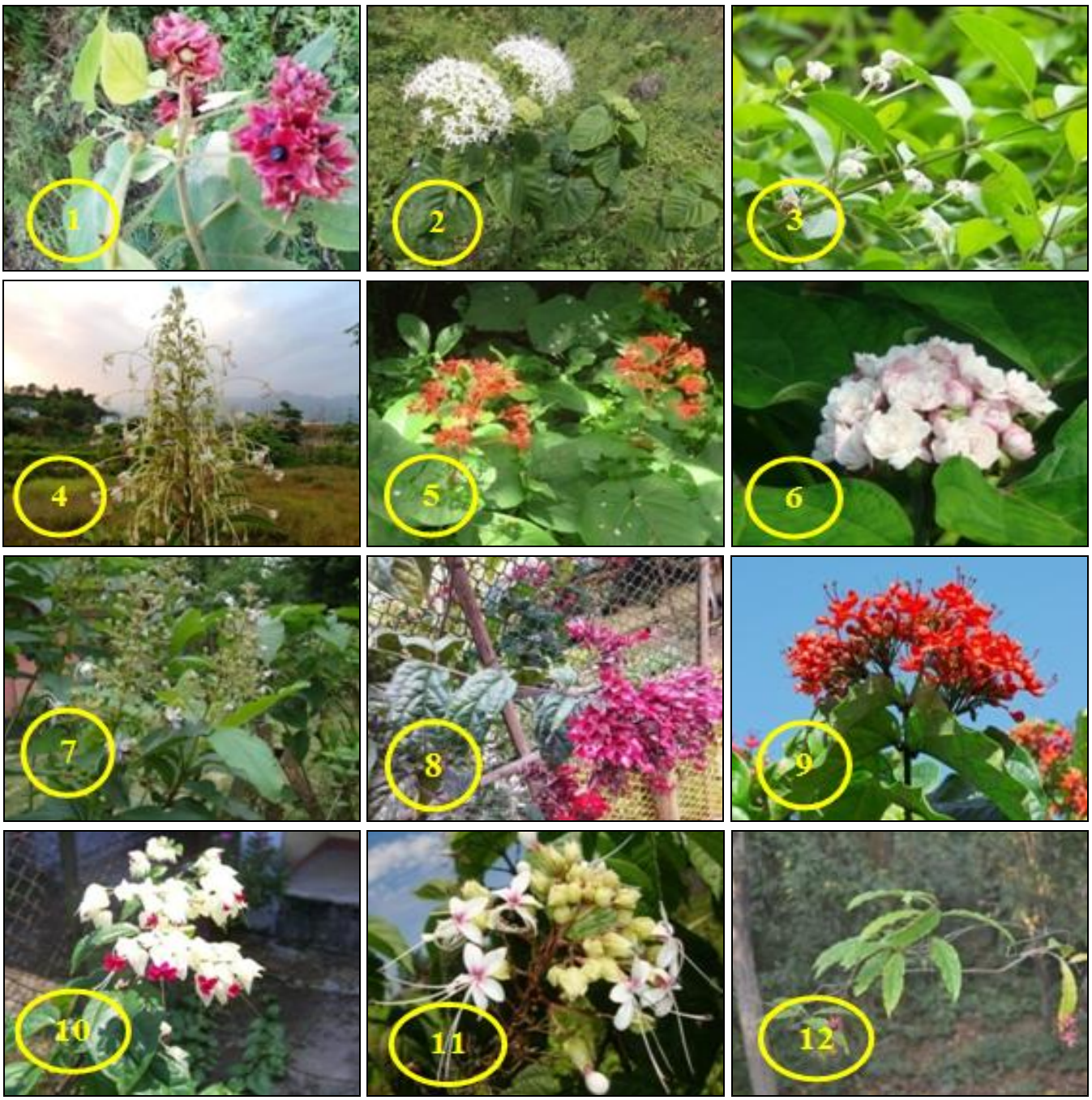

FIG. 1. *CLERODENDRUM* SPECIES IN NATURAL HABITAT (1) *C. BRACTEATUM* (2) *C. COLEBROOKIANUM* (3) *C. INERME* (4) *C. INDICUM* (5) *C. JAPONICUM* (6) *C. PHILIPPINUM* (7) *C. SERRATUM* (8) *C. SPECIOSUM* (9) *C. SPLENDENS* (10) *C. THOMSONII* (11) *C. VISCOSUM* (12) *C. WALLICHII*

TABLE 2: *CLERODENDRUM* SPECIES USED IN DIFFERENT AILMENTS AND DISEASES BY VARIOUS TRIBES OF NORTHEASTERN REGION (LITERATURE SURVEY REPORT)

Species	Diseases/ ailments	NE State	Tribe/ Communities	Parts used	Mode of uses
C. bracteatum	Brain tonic	AS	Bodo	R	Juice [26]
	Diarrhea and dysentery	ML	Mizo	L,R	Decoction [27]
	Fever	AS	Bodo	R	Juice [26]
C. colebrookianum	Fever	NL	Naga		mixed with dried and pounded meat of tortoise and decoction [28]
	Headache	AR	Hill Miri	L	brushing around the forehead [29]
	Blood pressure	AR	Tangsa	L, TW	boiled in water and mixed with a few ground pieces of *Allium sativum* L. and salt, are prescribed orally as soup or decoction for a consecutive period of three months, twice a day, on alternate days at 200g per dose [30]
		AR	Bangni	L	decoction or juice given orally [31]
		AR	Nishi	L	Decoction [32]
		AR	Apatani	L	boiled or infusion is taken orally [33]
		AR	Hill Miri	L	Juice [29]
		AR	Memba	L	Decoction [34]
		AR	Adi	L	decoction taken 3-4 teaspoonful twice daily [2]
		AR	Adi	L	[35]
		AR	Monpa	L	decoction with sugar [36]
		AS	Hmar	L	leaf juice [37]
		AS	Jaintia, Lusai	L	taken raw [38, 39]
		AS	Dimasa Kachari	L	leaf extract [40]
		AS	Moran	L	Decoction [41]
		AS	Zeme	L	Soup [42]
		AS	Mishing	L	Boil [43]
		AS	Assamese	TW	Boil [44]
		AS	Barman	L	Boil [45]
		AS	Barman	L	Boil [46]
		NL	Naga	L, TW	eaten raw or in soup; boiled in water and the extract is taken daily for few days [28, 47]
		ML	Mizo	L	Decoction [48, 38]
		ML	Khasi	L	Decoction [49]
		ML	Khasi, Jaintia	L	Extract [50]
		NL	Naga	L	Decoction of dry leaf [51]
		NL	Naga, Kuki	L	Decoction [52]
	Malarial fever	NL	Naga	R, B, L	decoction, eaten raw or in soup [28, 53]
	Reduce weight	AR	Hill Miri	L	- [29]
	Rheumatism	ML	Khasi, Garo	L	- [53]
		AS, ML, NL	Khasi, Jaintia, Naga	L	made into paste and massaged for a long time in rheumatic pain and gout [54]
	Stomachache	AR	Adi, Hill Miri	L	- [28, 29]
	Abdominal pain	AS	Tai-Ahom	L	three teaspoonful leaf extract is mixed with small amount of common salt and is taken thrice daily [55]
	Anthelmintic	AS	Assamese	L	- [53]
	Antidote	NL	Naga	L	infusion of leaves mixed with bark paste of the “menpan plant” is drunk [28]
	Blood purifier	AS	Lushai	L	- [39]
	Colics in infants	ML	Mizo	L	juice of 5 ml twice daily [48]
	Cough	AR	Adi, Nishi	R	- [2]
	Diabetes	AS	Jaintia	L	taken raw [38]
		AS	Barman community	L	Decoction [45]
		AS	Zeme	L	Decoction [42]
		ML	Mizo	L	Decoction [48]
	Diarrhoea and dysentery	AR	Adi, Nishi	L	Juice [53]

		AS	Dimasa Kachari	R	Extract [40]
	Gastric disorders	AR	Bangni	L, S	decoction or juice [31]
	Fever	AR	Adi	L	boiled and extract [53]
	Heart trouble	NL	Naga	L, TW	eaten raw or in soup [28]
C. hastatum	Skin infection	AS	Assamese	L	paste of 3-4 leaves applied on infected skin area for 8-10 hours [56]
C. indicum	Vermifuge	AR	Adi	L	- [2]
	Cough	AR	Adi, Nishi	L	- [53]
		AS	Mikir	L	smoke of dry leaf [13]
	Fever	AR	Adi	L	- [2]
	Rheumatism	AS	-	L	- [57]
	Asthma	AR	Adi, Nishi	R	- [2]
		AS	Assamese	R	two teaspoonfuls juice twice daily regularly for a month [58]
	Diabetes	MN	Meitei	L	boiled extract along with *Justicia adhatoda* leaves [59]
	Jaundice	AR	-	R	soaked in water for overnight and extract taken orally for 7-15 days [60]
C. paniculatum	Typhoid	TR	Reang	R	cut into pieces and pounded together with the roots of *Tamarindus indica* and *Ananas comosus* and made into paste. The decoction of the mixture is taken internally twice a day [61]
	Fever	TR	Reang	L	juice taken orally [62]
C. philippinum	Body pain	SK	-	R	Juice [63]
	Headache	SK	-	R	Juice [63]
C. serratum	Cephalalgia and ophthalmania	AR	Adi, Miri	L	- [2]
	Dropsy	AR	Adi, Miri	S	- [2]
	Fever	AR	Adi, Miri	L	- [2]
		AS	Jaintia	WP, L	ground with water [38]
	Headache	AR	Hill Miri	L	brushing around the forehead [29]
	Rheumatism	AR	Nishi, Adi	R	- [2]
	Malaria	AS	-	R	Extract [64]
	Irregular menstruation	NL	Naga	L	Decoction [28]
	Jaundice	AR		L	Juice [60]
C. villosum	Liver disorder	NL	Naga	WP	decoction of the plant [28]
	To kill lice	NL	Naga	WP	juice applied on the scalp [28]
C. viscosum	Blood purifier	AR	Singpho	L	Boil [33]
		AR	Nishi	F, L	- [32]
	Skin diseases and tumor	AS	Assamese	R	- [65]
		MZ	Mizo	L,R	juice applied externally [48]
	Snake bite	AS	Assamese	SH	- [66]
	Toothache	AS	Bodo	SH	Chewed [67]
	Vomiting	AS	Sarania	L	crushed to make juice and one teaspoonful taken internally [68]
	Asthma	AS	Jaintia	L	raw [38]
	Abdominal pain	AS	-	L	- [65]
	Body inflation	AS	Bodo	L	Juice twice daily for 3-4 days [69]
	Cut and wounds	AS	Hmar	L	Paste [37]
	Malarial fever	AS	Nath	L	Infusion [70]
		AS	Assamese	SH	[66]
		AS	Assamese	L	Decoction [64]
		NL, ML	Naga, Khasi	WP	decoction of whole plant along with black pepper [54]
	Deworming	AS	Barman	L	- [45]
		AS	Bengali	L	mixed with rice flour [46]
	Diabetes	AS	Jaintia	L	Raw [38]
		AS	Bengali	L	Juice [46]
		MN	Meitei	L	Boil [59]

	Diarrhoea and dysentery	AS	Khasi	L	Juice [71]
		AS	Bodo		[72]
		AS	Jaintia, Lushai	R	crushed and decoction taken orally thrice a day [67]
		AS	Jaintia, Lushai	L	Boil [46]
		MZ	Mizo	L	Juice [64]
	Scabies	MZ	Mizo	R	Decoction [48]
	Dog bite, snakebite	TR	Reang	L	paste [62]
C. wallichii	Skin infection	ML	Khasi, Jaintia	L	pounded with slaked lime and applied on skin infections [73]
	Abdominal tumor	MZ	Mizo	R	paste of roots mixing with leaves of *Ardisia paniculata*, *Claoxylon khasianum*, *Phlogacanthus thyrsiflorus* applied externally every day for 7 days [74]

B- Bark; F- Flower; L- Leaf; R- Root; S- Seed; S-Stem; T-twig; SH-Shoot; WP- Whole plant; TW-Twig. AS-Assam; AR-Arunachal Pradesh; MN-Manipur; MZ-Mizoram; ML-Meghalaya; NL-Nagaland; TR-Tripura; SK-Sikkim.

Among the species, *C. colebrookianum* has been found to be used in a total of 17 different diseases where a maximum of 21 northeast tribes used for curing of blood pressure, 7 tribes used for diabetes, 4 tribes used for rheumatism, 3 tribes used for diarrhea and dysentery, fever respectively reported from a literature survey. While in field survey, the species has been used in 13 different diseases where a maximum number of informants (90) belonging to 12 different tribal communities used for controlling of blood pressure, 4 tribes used in gastric trouble, fever, dysentery and abdominal pain, 3 tribes in stomach trouble, diabetes, heart trouble, headache and cough, 2 tribes in malaria, 1 tribe in jaundice and sinusitis respectively **Table 3**. Young and tender leaves were used in the majority of diseases and ailments in both literature and field survey **Fig. 2**. Mode of administration was found to be leaf boil in both literature and field survey **Fig. 3**.

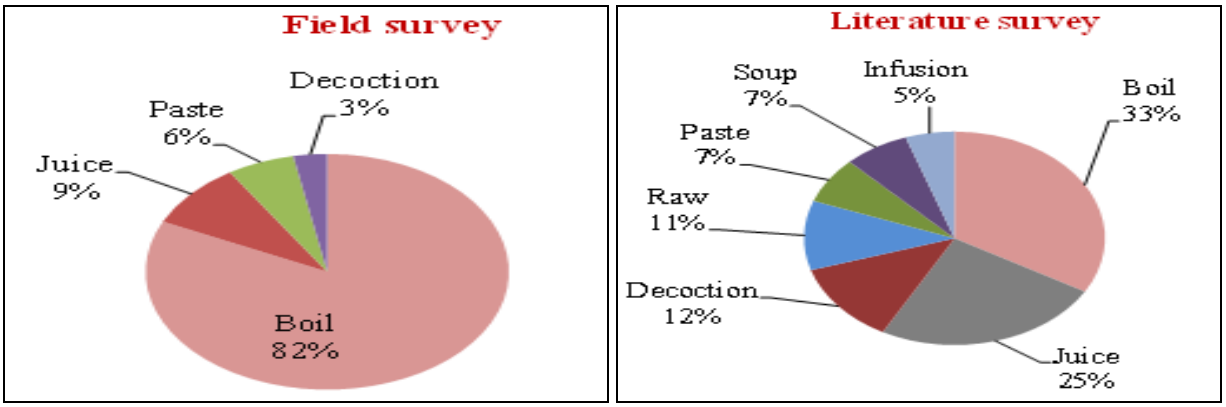

FIG. 2: DIFFERENT PLANT PARTS OF *CLERODENDRUM* SPECIES USED IN TRADITIONAL MEDICINE (LITERATURE SURVEY AND FIELD SURVEY)

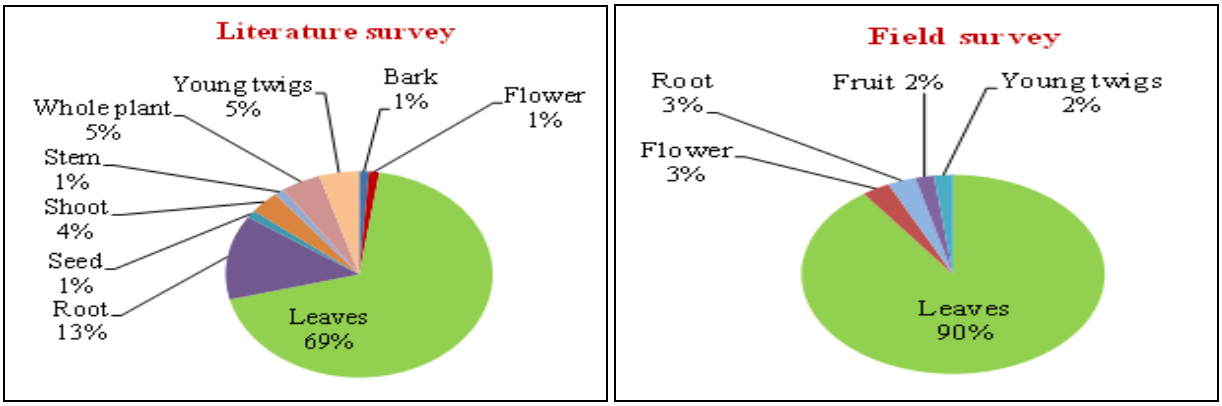

FIG. 3: DIFFERENT WAYS OF HERBAL PREPARATION OF *CLERODENDRUM* SPECIES USED IN TRADITIONAL MEDICINE (LITERATURE SURVEY AND FIELD SURVEY)

TABLE 3: ETHNO MEDICINAL USES OF *CLERODENDRUM* SPECIES BY VARIOUS TRIBAL COMMUNITIES OF NORTHEASTERN REGION (FIELD SURVEY REPORT)

Species	Parts used	Diseases/ ailments	Mode of preparations	NE State	Tribes/Communities
C. bracteatum	L	fever	juice	AR	Nishi
C. colebrookianum	L	blood pressure	boil with bamboo shoot, garlic; dry leaves chewed	AR, AS, MN, NL, MZ, ML	Nishi, Adi, Apatani, Assamese, Khasi, Mishing. Mizo, Bodo, Khamti, Naga, Sonowal Kachari, Tagin
	L, F	gastric trouble	boil with bamboo shoot	AR, ML	Nishi, Adi, Apatani, Khasi
	L	diabetes	boil	AR	Nishi, Adi, Apatani
	L, F	stomach trouble	boil	AR, ML	Nishi, Adi, Apatani,
	L, FR	fever	boil	AR, NL, MZ	Nishi, Adi, Apatani, Mizo
	L	malaria	boil	AR	Nishi, Adi
	L	jaundice	boil	ML	Khasi
	L	heart trouble	boil	AR	Nishi, Adi, Apatani,
	L	diarrhoea and dysentery	boil	AR	Nishi, Adi, Apatani, Tagin
	L	cough	boil	AR	Nishi, Adi, Apatani,
	L	abdominal pain	boil	AR, MZ	Nishi, Adi, Apatani, Mizo
	L	headache	boil	AR	Nishi, Adi, Apatani,
	L	sinusitis	juice	ML	Khasi
C. inerme	L	cuts and wounds	paste	AR	Nishi, Adi, Apatani
C. indicum	L	jaundice	juice	AR	Nishi, Adi
	R	asthma	juice	AR, AS	Adi, Nishi, Assamese
	L	fever	boil	AS	Assamese, Bodo
C. japonicum	L	typhoid	juice	AS	Bodo
C.philippinum	L	cuts and wounds	paste	AR	Nishi, Adi, Apatani
	L	cough	juice	AS	Bodo, Khasi
C. serratum	L	jaundice	decoction	AS	Mishing
	L	cuts and wounds	paste	AS	Deori
	R	cuts and wounds	paste	AR, AS	Nishi, Khasi
	L	fever	juice	AR	Nishi
C. thomsoniae	-	ornamental	-	-	-
C. wallichii	L	abdominal pain	juice	ML	Khasi

F- Flower; FR-Fruit; L- Leaf; R- Root, AS-Assam; AR-Arunachal Pradesh; MN-Manipur; MZ-Mizoram; ML-Meghalaya; NL-Nagaland

Screening of α-amylase Inhibition Properties: The crude ME of four species (*C. serratum*, *C. japonicum*, *C. viscosum* and *C. indicum*) have shown significant inhibition of enzyme activity (>50%) while the other 3 species (*C. philippinum*, *C. colebrookianum*, and *C. inerme*) have shown insignificant inhibition activity (<50%) as compared to the standard inhibitor of α-amylase, acarbose (ACB) (80%) **Fig. 4**.

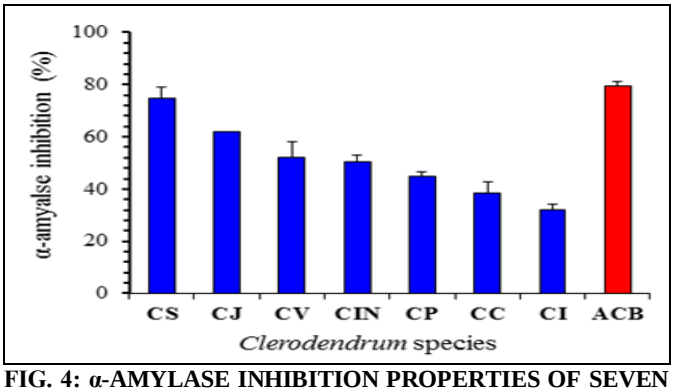

FIG. 4: α-AMYLASE INHIBITION PROPERTIES OF SEVEN *CLERODENDRUM* SPECIES AND STANDARD DRUG ACARBOSE. T-BARS ON THE HISTOGRAM REPRESENT STANDARD DEVIATION (SD)

Dose-Response Analysis of α-amylase Inhibition Assay: The inhibition was found to be increased with increasing concentrations in all species with the lowest inhibition by *C. serratum* (28% in 10µl) to highest inhibition by *C. indicum* and *C. serratum* (97% in 100µl) respectively. The inhibition activity for standard drug acarbose (ACB) was between 7 to 85% in 10 to 100µl concentrations **Fig. 5**.

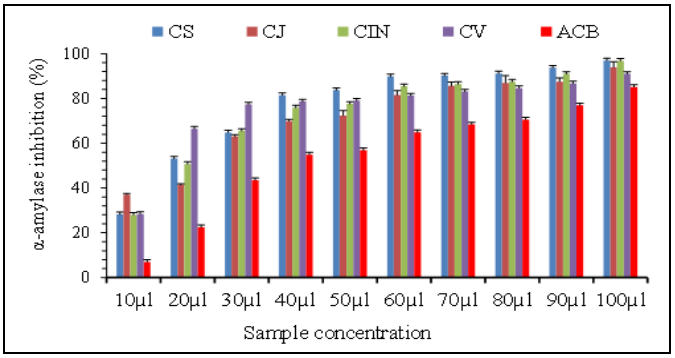

FIG. 5: COMPARISON OF α-AMYLASE INHIBITION PROPERTIES OF FOUR *CLERODENDRUM* SPECIES AND STANDARD DRUG ACARBOSE (ACB) AT DIFFERENT CONCENTRATIONS (10-100µL≅20-200µG/ML).T-BARS ON THE HISTOGRAM REPRESENT STANDARD DEVIATION (SD)

Screening of α-glucosidase Inhibition Properties: The crude ME of 3 species were found to possess significant inhibition (>50%) of α-glucosidase activity. *C. viscosum* was found to show the highest level of inhibition (73%) followed by *C. inerme* (68%) and *C. colebrookianum* (59%) in comparison with standard drug, acarbose (86%). Four species (*C. japonicum, C. serratum, C. philippinum and C. indicum)* showed no significant inhibition of the enzyme activity **Fig. 6**.

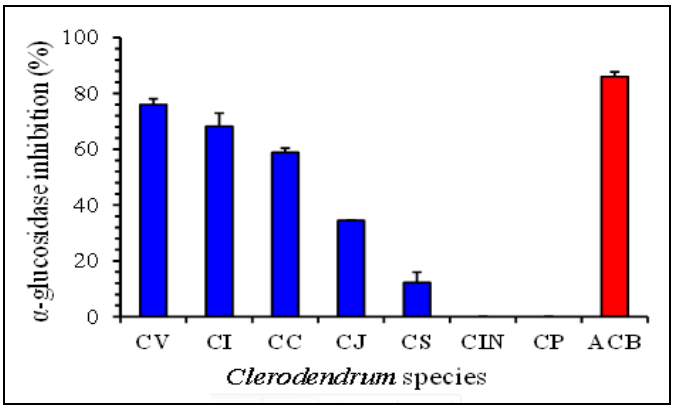

FIG. 6: α-GLUCOSIDASE INHIBITION PROPERTIES OF SEVEN *CLERODENDRUM* SPECIES AND STANDARD DRUG ACARBOSE. T-BARS ON THE HISTOGRAM REPRESENT STANDARD DEVIATION (SD)

Dose-Response Analysis of α-glucosidase Inhibition Assay: The α-glucosidase inhibition percentage was found to be increased with increasing concentrations of the leaf extract. *C. viscosum* showed the highest inhibition in all concentrations (23% in 5µl to 83% in 30µl). Similarly, *C. colebrookianum* and *C. inerme* also displayed enzyme inhibition properties between the lowest of 18% (5µl) to a maximum of 80% (30µl), respectively **Fig. 7**.

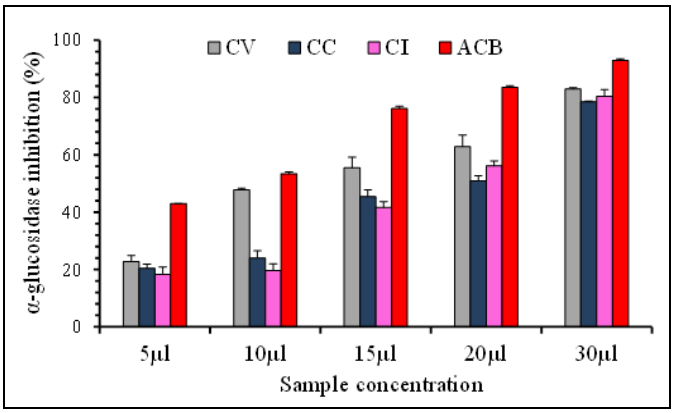

FIG. 7: COMPARISON OF α-GLUCOSIDASE INHIBITION PROPERTIES OF 3 *CLERODENDRUM* SPECIES AND STANDARD DRUG ACARBOSE AT DIFFERENT CONCENTRATIONS (5-30µL ≈ 67-400µg/mL). T-BARS ON THE HISTOGRAM REPRESENT STANDARD DEVIATION (SD)

50% Inhibition Concentration (IC_{50}) of Leaf Extracts: The IC_{50} values showed ranged from a minimum of 45µg/ml of *C. serratum* to a maximum of 71µg/µl of *C. japonicum* as compared to positive control acarbose (79µg/ml) for α-amylase. On the other hand, IC_{50} values for α-glucosidase ranged from 47µg/ml of *C. viscosum* to a maximum of 184µg/ml of *C. inerme* as comparison to acarbose (244µg/ml). Among the species, crude ME of *C. serratum* and *C. viscosum* showed the lowest IC_{50} values of α-amylase (45µg/ml) and α-glucosidase (47µg/ml) **Table 4**.

TABLE 4: IC_{50} VALUES OF LEAF EXTRACTS WITH POTENTIAL A-AMYLASE AND A-GLUCOSIDASE INHIBITION PROPERTIES FROM *CLERODENDRUM* SPECIES. VALUES WITHIN PARENTHESIS SHOW SD OF MEAN

***Clerodendrum* species**	**IC_{50} (µg/ml)**	
	α-amylase	**α-glucosidase**
C. serratum	45 (±-4.8)	-
C. indicum	47(± 8.1)	-
C. japonicum	71 (± 8.0)	
C. viscosum	59 (± 1.2)	47 (±1.3)
C. colebrookianum	-	175 (±0.86)
C. inerme	-	184 (±1.2)
Acarbose	79 (±1.3)	244 (±0.78)

DISCUSSION: From the present observation, it has been found that many *Clerodendrum* species growing in NER are continuously used by tribal communities for the treatment of many diseases in the form of special preparations or as vegetables. *C. colebrookianum, C. inerme, C. indicum, C. serratum* were found to have multi medicinal properties. To validate the traditional claims associated with *Clerodendrum* species, many scientific investigations were carried out by using *in-vitro* and *in vivo* assays. In the present study, 4 species of *Clerodendrum* (*C. serratum, C. japonicum, C. viscosum, C. indicum*) exhibited significant α-amylase inhibition properties while 3 other species (*C. viscosum, C. colebrookianum, C. inerme*) displayed significant α-glucosidase inhibition properties.

Previous studies have reported crude ME of *C. viscosum* possessed hypoglycemic property against streptozotocin and alloxan-induced diabetes in wister rats [17, 18]. *C. serratum* and *C. inerme* leaf extract were reported to have significant blood glucose-lowering potential in STZ-induced diabetic rats [19, 20, 21]. These findings are in agreement with significant *in-vitro* α-amylase, and α-glucosidase

inhibition properties of the methanol extracts of the three species (*C. serratum, C. viscosum* and *C. inerme*) reported in the present study.

The present study of *C. colebrookianum* methanol leaf extract was found to show moderately high percentage inhibition of α-glucosidase activity. A study on experimentally induced insulin resistance rats also revealed significant ameliorating role of aqueous leaf extract of *C. colebrookianum* [22]. *C. colebrookianum, C. indicum, C. inerme, C. japonicum, C. philippinum, C. viscosum* and *C. serratum* are reported to contain various bioactive compounds such as acacetin, acteoside, apigenin-6-C-β-l-fucopyranoside, apigenin-7-0-glucoside, isoacteoside, isoquercetin, hispidulin, hispidulin 7-O-glucuronide, kaempferol, luteolin, oleanic acid, quercetin, rutin, *etc*. [23, 24, 25, 1].

All these compounds isolated from other medicinal plants reported having antidiabetic properties; however isolation, particularly from *Clerodendrum* species together with antidiabetic properties, has not been established.

CONCLUSION: Therefore, it is concluded that four *Clerodendrum* species were found to possess highly significant antidiabetic properties as revealed by *in-vitro* α-amylase, and three other species have shown significant α-glucosidase inhibition properties. Despite large information available on phytochemical compounds and their correlation with antidiabetic properties from different species of the genus *Clerodendrum*, there is scarce information on the phytochemical characterization of the above species with potential antidiabetic properties reported in this study. A further investigation into phytochemical profiling and evaluation of their role in hypoglycemic activities of selected species, specially *C. viscosum* and *C. serratum* under *in-vitro* and *in-vivo* conditions may help in the identification of lead molecule for therapeutic use in treatment and management of diabetes.

The hypoglycemic effect of *Clerodendrum* species may be due to the presence of more than one antidiabetic bioactive compound or their synergistic properties. Further bioassay-guided fractionation and *in-silico* screening bioactive compounds from *Clerodendrum* species may serve as target inhibitors in the treatment and management of diabetes mellitus in the near future.

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CONFLICTS OF INTEREST: Nil

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