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Antidiabetic activity of *Abroma augustum* (L.) L.f. and *Cajanus cajan* (L.) Millsp. extracts benchmarked through phytochemicals, enzyme inhibition and glucose uptake assays

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The rising global burden of diabetes mellitus and the limitations of conventional therapies necessitate the exploration of safer, effective plant-based alternatives. Although *Abroma augustum* (L.) L.f. and *Cajanus cajan* (L.) Millsp. are traditionally used in Nagaland for diabetes management, scientific validation regarding their specific enzyme inhibition mechanisms and safety profiles remains limited. This study systematically evaluated their antidiabetic potential through phytochemical profiling, antioxidant activity, enzyme kinetics, and glucose uptake assays. Quantitative analysis revealed that 80% ethanol extracts were richer in bioactive constituents, particularly phenols and flavonoids, compared to aqueous extracts. *Cajanus cajan* consistently demonstrated superior antioxidant activity and potent, competitive inhibition of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) compared to *A. augustum*. Furthermore, *C. cajan* exhibited significantly higher glucose uptake (147.75%) in HepG2 cells. Cytotoxicity assessment in VERO cells indicated that while *C. cajan* (IC_{50} 139.98 µg/mL) was relatively safe, *A. augustum* (IC_{50} 115.79 µg/mL) possessed higher cytotoxicity. These findings scientifically validate the antidiabetic potential of *A. augustum* and *C. cajan*, supporting their traditional use and warranting further investigation for antidiabetic drug development.

Keywords: Alpha-glucosidase, Cytotoxicity, Ethnobotany, Enzyme kinetics, HepG2 cells, Oxidative stress

Introduction

Diabetes mellitus (DM) is a major global health challenge characterized by chronic hyperglycemia, arising from impaired insulin secretion or action, which leads to significant morbidity and mortality[1]. India carries a high burden, with over 77 million affected individuals facing severe complications like retinopathy and nephropathy, driven by rising obesity and sedentary lifestyles[2]. Despite advancements in conventional pharmacology, widespread limitations such as adverse effects, high costs, and accessibility issues underscore the urgent need for alternative therapeutic strategies[3].

Phytomedicines, naturally abundant in bioactive compounds like flavonoids and polyphenols, present a promising alternative due to their potential to modulate blood glucose, improve insulin sensitivity, and mitigate oxidative stress and inflammation key factors in DM pathogenesis[4]. A critical target for managing postprandial hyperglycemia (PPHG) is the inhibition of carbohydrate-hydrolyzing enzymes, pancreatic α-amylase and α-glucosidase[5]. By slowing down the digestion and absorption of carbohydrates, enzyme inhibition effectively curbs postprandial glucose spikes. Traditional medicinal systems, such as Ayurveda, have long utilized plant-based therapies for diabetes, offering cost-effective and sustainable adjuncts to conventional care[6].

The focus of this study, *Abroma augustum* (L.) L.f. and *Cajanus cajan* (L.) Millsp., are well-known in ethnomedicine. *A. augustum* has diverse traditional uses, primarily for gynecological issues and conditions like diabetes and rheumatism. Similarly, *C. cajan* (pigeonpea) is traditionally used to treat ailments including diabetes, coughs, and skin infections using various plant parts. Scientific interest in both species is growing rapidly due to their high content of polyphenolic and flavonoid compounds, which are known for their potent antioxidant, anti-inflammatory, and antidiabetic properties[7,8]. Specifically, *A. augustum*'s antidiabetic action is linked to α-glucosidase inhibition and the attenuation of Advanced Glycation End-products (AGEs)[9]. Its

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antioxidant potential correlates directly with its high flavonoid and polyphenol content, though its concentration-dependent cytotoxicity requires careful dosage consideration[9]. Likewise, *C. cajan*, particularly dark-seeded varieties, is rich in phenolic compounds like rutin and quercetin, contributing to its strong antioxidant profile and proven blood glucose-reducing efficacy, supporting its potential as a functional food for diabetes management[10-12].

While the ethnobotanical use of *A. augustum* and *C. cajan* by indigenous communities in Nagaland, India, for diabetes management is documented[13,14], specific scientific validation within this local context remains limited. Variations in phytochemical profiles due to environmental and geographical factors necessitate localized investigation. Crucially, no prior study has specifically investigated the mode of inhibition (competitive, non-competitive, or mixed) of α-amylase and α-glucosidase by extracts of these plants from this specific region.

Therefore, this research aimed to: (i) assess solvent efficacy for polyphenol-rich extract preparation from local *A. augustum* and *C. cajan*; (ii) systematically quantify their phytochemical compositions; (iii) evaluate their antioxidant and antidiabetic potential through enzyme inhibition kinetics; and (iv) assay extract cytotoxicity and their ability to enhance glucose metabolism using *in vitro* glucose uptake assays. This comprehensive evaluation seeks to provide data-driven insights into their potential as natural agents for diabetes management.

Materials and Methods

Plant materials, sample collection and preparation

Drawing upon existing ethnobotanical surveys documenting the antidiabetic potential of medicinal plants traditionally used in Nagaland[13], this study focused on two prominent species: *Abroma augustum* and *Cajanus cajan* (Fig. 1). The identity of the plant species was rigorously confirmed through both morpho-taxonomic analysis and DNA sequence-based molecular markers[15]. Comprehensive ethnobotanical information regarding these plants, including their traditional applications, local nomenclature, geographical coordinates of collection sites, and other pertinent details, is summarized in Table 1.

To ensure optimal concentrations of secondary metabolites, specific plant parts were harvested during their respective flowering seasons, a practice aligned with traditional knowledge. Following collection, plant materials were meticulously washed with tap water to eliminate surface contaminants and subsequently dried in a hot air oven at a controlled temperature of 50°C until a constant weight was attained. The dried biomass was then pulverized into a fine powder using a mechanical grinder and stored in hermetically sealed containers to maintain sample integrity until further processing.

Preparation of plant extracts

For phytochemical extraction, precisely weighed 50mg aliquots of each powdered plant sample were individually mixed with 10mL of either 80% (v/v) ethanol or ultrapure water at laboratory temperature of 25±2°C. To enhance the solubility and extraction of phytochemical compounds, the resulting mixtures were incubated in a temperature-controlled water bath maintained at 40°C for duration of 1 h. Following

Fig. 1 — Selected two ethnomedicinal plants a) *Abroma augustum* (L.) L.f. b) *Cajanus cajan* (L.) Millsp.

Table 1 — Information of studied plants, including their traditional uses, local names, GPS coordinates

Sl. No.	Scientific Name (Voucher Number)	Family	Local name in Nagaland	Habit	Plant part used	Method of use	GenBank Accession Nos.	GPS coordinates Latitude (N)	Longitude (E)	Elevation (mamsl)
1	*Abroma augustum* (L.) L.f. (NU/BOT/IS-CRD/0006)	Malvaceae	Ulatkambal	Shrub	Fresh leaves	Fresh leaves decoction	OQ913536	26°13'47.31″	94°28'19.91″	998.8
2	*Cajanus cajan* (L.) Millsp. (NU/BOT/IS-CRD/0007)	Fabaceae	Chiopi (Zeliang tribe)	Herb	Leaves	Boiled leaf is drink as tea	OQ915149	26°13'1.73″	94°28'30.90″	952.5

incubation, the samples were subjected to centrifugation at 10,000 rpm for 10 min to separate the solid residue from the liquid extract. The supernatants were then carefully collected and filtered through Whatman No. 1 filter paper to remove any remaining particulate matter. To concentrate the extracted phytochemicals, the filtrates were subjected to rotary evaporation under reduced pressure at 40°C, allowing for the controlled removal of the solvent while retaining the liquid extract. The percentage extraction yield was subsequently calculated using the following formula:

$$\text{Percent yield} = \frac{\textit{Final extract weight}}{\textit{Initial dry sample weight}} \text{x } 100$$

The percentage extraction yields determined in triplicate (n=3) to ensure accuracy, varied depending on the plant material and the solvent used. Ethanol extraction of *Cajanus cajan* resulted in a yield of 13.5%, while aqueous extraction yielded 9.2%. For *Abroma augustum*, the ethanol extract provided a yield of 11.8%, and the aqueous extract yielded 7.6%. The prepared extracts, containing residual solvent, were stored at 4°C in sterile containers to maintain their integrity until further analyses.

Phytochemical analysis

Determination total phenol content (TPC)

The TPC of the extracts was determined using a modified Folin-Ciocalteu method, as described by Genwali *et al.*[16]. Briefly, 200µL of each plant extract (prepared with both ethanol and water) was mixed with 2.8mL of distilled water (dH_2O), 2mL of a 7% (w/v) sodium carbonate (Na_2CO_3) solution, and 0.5 mL of 10% (v/v) Folin-Ciocalteu reagent. The resulting mixtures were then incubated in the dark at room temperature for 90 min. Following incubation, the absorbance of each reaction mixture was measured at 765 nm using a spectrophotometer. The TPC was quantified and expressed as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g DW) of the sample. All analyses were performed in triplicate for each solvent extract to ensure the reliability and reproducibility of the results.

Determination of total flavonoid content (TFC)

The TFC of the extracts was determined following the protocol described by Tan[17]. Briefly, 200µL of each plant extract was mixed with 0.15mL of 0.5 M sodium nitrite ($NaNO_2$) and 0.15mL of 0.3 M aluminum chloride ($AlCl_3$). The final volume of the reaction mixture was adjusted to 4.0mL by the addition of 30% ethanol (v/v), and the mixture was allowed to stand for 5 min. Subsequently, 1mL of 1 M sodium hydroxide (NaOH) was added to the reaction mixture, and the absorbance was measured at 415 nm using a spectrophotometer. The resulting absorbance values were used to determine the TFC concentration, expressed as milligrams of Quercetin equivalents per gram (mg QE/g DW), of dry weight of the sample, using a Quercetin standard curve.

Determination of total tannin content (TTC)

The TTC of the extracts was estimated using the Folin-Dennis method, as described by Longchar and Deb[18]. Briefly, reaction mixtures were prepared by combining 200µL of each plant extract with 3.8mL of distilled water (dH_2O), 0.5mL of Folin-Dennis reagent, and 0.5mL of a 20% (w/v) sodium carbonate (Na_2CO_3) solution. The mixtures were thoroughly vortexed, and the absorbance was measured at 775nm using a spectrophotometer. The TTC was calculated based on a calibration curve generated using tannic acid as a reference standard and expressed as milligrams of tannic acid equivalents per gram of dry weight of the sample (mg TAE/g DW).

Determination of total triterpenoid content (TTRC)

To quantify the TTRC, a standard curve was prepared using Ursolic acid following Longchar and Deb.[18] For this purpose, 40µL of each plant extract was mixed with 800µL sulfuric acid and 400µL 5% Vanillin-Glacial Acetic Acid reagents. The reaction mixtures were then incubated at 60°C for 15 min, allowing for chemical reactions followed by mixed 5ml of glacial acetic acid into the mixture, ensuring complete homogenisation. The absorbance was read at 545nm, and a standard curve was generated using Ursolic acid, with known concentrations linked to absorbance values at 545nm. By employing this curve, the TTRC in the samples were determined and expressed as milligrams of Ursolic acid equivalent per gram (mg UAE/gm) of the sample.

Determination of total alkaloid content (TAC)

To quantify TAC, a modified procedure based on Patel *et al.*[20] was employed. Briefly, powdered plant samples were extracted using 80% ethanol and distilled water (dH_2O) via Soxhlet apparatus. The resulting extracts were concentrated and evaporated to yield dry residues. These residues were then dissolved in 2N HCl. For quantification, 1mL of the test solution was transferred to a separating funnel,

followed by the addition of 5mL of 0.2 M phosphate buffer (pH 4.7) and 10^{-4} M Bromocresol Green solution. The alkaloid-dye complex was formed by adding variable volumes of chloroform (1-4mL), followed by vigorous shaking. The chloroform layers containing the complex were collected in a 10mL volumetric flask, and the final volume was adjusted with chloroform. The absorbance of the complex was measured at 470 nm using a spectrophotometer. The TAC was quantified as milligrams of Atropine equivalents per gram of dry weight of the sample (mg AE/g DW) using an Atropine standard curve. All analyses were performed in triplicate.

For calculation of TPC, TFC, TTC, TTRC, TAC following formula was used:

$$\text{TPC, TFC, TTC, TTRC, TAC} = \frac{(C \times V \times D)}{W},$$

where: C = Concentration of the compound in the sample (mg/mL), V = Volume of the extract used (mL), D = Dilution factor (if applicable), W = weight of the sample (g).

Studies of biological activity of extracts

Antioxidant activity assay

The antioxidant activity of the extracts was determined based on their ability to scavenge the DPPH and ABTS radicals and to reduce ferric ions in the FRAP assay. Results for DPPH and ABTS are expressed in Trolox equivalent (mg/g), while FRAP values are expressed in mM Fe^{2}/g of dry sample.

2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay

The radical scavenging activity of the sample extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following a modified method of Longchar and Deb[18]. Briefly, varying concentrations of the plant extracts were mixed with 3mL of a 0.1mM DPPH solution prepared in 80% methanol. The final volume of each reaction mixture was adjusted to 4mL with 80% methanol and incubated in the dark at room temperature for 30 min. Following incubation, the absorbance of the resulting solutions was measured at 517nm using a spectrophotometer. The percentage inhibition of DPPH radicals was calculated using the formula:

$$\text{Free radical inhibition (\%)} = \frac{Controlabsorbance - Sampleabsorbance}{Controlabsorbance} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was employed to evaluate the antioxidant capacity of the extracts, based on their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions in the presence of 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ)[18]. The FRAP reagent was freshly prepared by mixing 10mM TPTZ, 20mM ferric chloride, and 300mM sodium acetate buffer (pH 3.6) in a 10:1:1 ratio and warmed to 37°C. For the assay, sample extracts were mixed with 3mL of the FRAP reagent, and the final volume was adjusted to 4mL with distilled water. Following 30-min incubation in the dark at 37°C, the absorbance was measured at 593nm. A ferrous sulfate standard curve was used to calculate the FRAP values, expressed as mM Fe^{2+} equivalents per gram of dry sample.

2,2-Azino-(3-ethyl) Benzothiazoline-6-sulfonic Acid Diammonium Salt (ABTS) radical cation scavenging assay

The antioxidant capacity of the sample extracts was evaluated using a modified 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay[18]. A stable ABTS stock solution (7mM ABTS in water and 2.45mM potassium persulfate, 16 h dark incubation) was diluted with 80% ethanol to achieve an absorbance of 0.70 AU at 734nm. For the assay, sample extracts were mixed with 3mL of the ABTS working solution, with methanol as a control. Following a 30-min dark incubation, the absorbance was measured at 734nm to determine the antioxidant capacity.

Enzyme inhibition assays

α-Amylase inhibition assay

The α-amylase inhibitory activity of the plant extracts was evaluated using a modified protocol based on Hansawasdi *et al.*[20]. Starch azure (2mg) was suspended in 0.2mL of 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ to prepare the substrate solution. This solution was boiled for 5 min, followed by pre-incubation at 37°C for another 5 min. The 80% ethanol extracts of the plant samples were dissolved in DMSO to obtain concentrations ranging from 100 to 500 µg/mL. Subsequently, 0.2 mL of each plant extract concentration was added to the substrate solution. Porcine pancreatic α-amylase (0.1mL, 2 units/mL in Tris-HCl buffer) was then introduced to initiate the enzymatic reaction, which proceeded at 37°C for 10 min. The reaction was terminated by adding 0.5 mL of 50% acetic acid. The reaction mixture was then centrifuged at 3000 rpm for 5 min at

4°C, and the absorbance of the supernatant was measured at 595 nm using a spectrophotometer. The same procedure was followed for the aqueous plant extracts. Acarbose, a known α-amylase inhibitor, served as a positive control. All experiments were performed in triplicate, and the percentage inhibition was calculated using the formula:

$$\text{Percent Inhibition} = \frac{absorbance\ 595\ (Control) - absorbance\ 595\ (extract)}{absorbance\ 595\ (control)} \times 100$$

The α-amylase inhibitory activities were expressed as a percentage of inhibition (IC_{50}) determined through regression analysis based on a graph illustrating scavenging activity in relation to concentration.

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity of the plant extracts was determined using a method adapted from Indrianingsih *et al.*[21]. The enzyme was prepared by solubilizing α-glucosidase in a 100 mM phosphate buffer (pH 6.8), which served as the enzyme solution. *p*-Nitrophenyl-α-D-glucopyranoside (pNPG) was used as the substrate. The 80% ethanol and aqueous extracts of the plant samples were dissolved in DMSO to create a range of concentrations (100-500 µg/mL). For each assay, 10µL of the test extract solution was pre-incubated with 250µL of pNPG (3mM) and 490µL of phosphate buffer (100mM, pH 7) at 37°C for 5 min. Subsequently, 250µL of α-glucosidase enzyme solution (2units/mL) was added to initiate the reaction, which proceeded for 15 min at 37°C. The reaction was terminated by the addition of 1mL of 0.2 M sodium carbonate (Na_2CO_3). The absorbance of the resulting mixture was measured at 400nm using a spectrophotometer. Acarbose was used as a positive control. All assays were performed in triplicate. The percentage inhibition was calculated using the formula:

$$\text{Percent Inhibition} = \frac{absorbance\ 595 (Control) - absorbance\ 595\ (extract)}{absorbance\ 595\ (control)} \times 100$$

The IC_{50} values, representing the concentration of the extract required to inhibit 50% of the enzyme activity, were determined by non-linear regression analysis of the percentage inhibition data plotted against the logarithm of the inhibitor concentration.

Determining the mode of inhibition

For determining the mode of inhibition of α-Amylase and α-Glucosidase, α-Amylase and α-Glucosidase assay was carried out by varying the substrate concentration and using IC_{50} of sample extract as concentration and as the source of inhibitor. A Lineweaver-Burk plot of against was constructed to identify the mode of inhibition[21].

Cytotoxicity assay

Cell culture

The cytotoxicity of the plant extracts was evaluated using the VERO cell line, a non-tumorigenic cell line derived from the kidney of the African green monkey. VERO cells are widely utilized in cytotoxicity assays due to their well-characterized responses and relevance in predicting potential effects on human cells[22]. Their use allows for the assessment of the extracts' potential toxic effects on normal, healthy cells, a critical step in determining the safety profile of potential therapeutic agents.

MTT colorimetric assay

The cytotoxic effects of the plant extracts were quantitatively assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. This assay evaluates cellular viability by measuring the activity of mitochondrial dehydrogenase enzymes, which reduce the yellow MTT tetrazolium salt to purple formazan crystals. The quantity of formazan produced is directly proportional to the number of metabolically active, viable cells, thus serving as an indicator of cytotoxicity. Following a 24-h cell culture period, VERO cells were exposed to a range of concentrations of each plant extract (5, 10, 25, 50, 100, 250, and 500µg/well). After an 18-20 h incubation period, cellular viability was determined by adding 100µL of MTT reagent to each well, followed by 4-h incubation in a CO_2 incubator. Subsequently, the supernatant was removed, and 100µL of dimethyl sulfoxide (DMSO) was added to solubilize the formed formazan crystals. The absorbance of each well was measured at 570nm using a microplate reader, with higher absorbance values correlating with greater cell viability. All assays were performed in triplicate to ensure data reliability. The percentage of cell viability relative to the untreated control was calculated for each extract concentration. The half-maximal inhibitory concentration (IC_{50}) values, representing the concentration of extract required to inhibit 50% of cell viability, were determined using non-linear regression analysis in Microsoft Excel 2019 software.

Glucose uptake assay using HepG2 cells

Cell culture

The human hepatoma cell line (HepG2) was procured from the National Centre for Cell Sciences (NCCS), Pune. The HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37°C with 5% CO_2. The cells at 80–90% confluence were split and then used for further experiments.

Cell viability assay

The inhibitory effect of the different extracts on HepG2 cell growth was quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described by Akinrinde *et al.*[23], with extract concentrations ranging from 5-500µg/mL. HepG2 cells (5×10^4 cells/mL) were seeded in 96-well plates and allowed to adhere and grow for 24 h at 37°C. Subsequently, the cells were treated with varying concentrations of the test extracts for an additional 24 h. Following treatment, 10µL of MTT solution (5mg/mL in PBS) was added to each well, and the plates were incubated at 37°C for 3 h to allow formazan crystal formation. After incubation, the culture medium was carefully removed, and 100µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the intracellular formazan crystals. The absorbance of the resulting solutions was measured at 540 nm using a microplate reader, and the percentage of cell viability was calculated relative to the untreated control.

Glucose uptake assay

HepG2 cells were seeded in culture plates at a density of 5×10^4 cells/mL and allowed to adhere and grow for 24 h at 37°C in a 5% CO_2 incubator. Prior to the glucose uptake assay, cells were pre-incubated for 48 h at 37°C with varying concentrations of the plant extracts (31.25–500µg/mL). Subsequently, the culture medium was removed and replaced with 25µL of glucose uptake medium (DMEM supplemented with 8mM glucose, 0.1% bovine serum albumin (BSA), and phosphate-buffered saline (PBS), followed by incubation at 37°C for 3 h. After incubation, 10µL of the medium was collected from each well and transferred to a new 96-well plate. The glucose concentration in the collected medium was then quantified using a glucose assay kit (Sigma GAGO20) according to the manufacturer's instructions, with absorbance readings taken at 540nm. The amount of glucose utilized by the cells was determined by subtracting the glucose concentration in wells containing cells from that in cell-free control wells[24].

Statistical analysis

All the experiments were executed in triplicate and repeated thrice, and deviations were calculated as the standard error of the mean (SEM). In addition, One-Way Analysis of Variance Analysis (ANOVA) and the Least Significant Test were done at 95% confidence level ($p\leq0.05$) using Microsoft Excel 2019 Software.

Results

Phytochemical studies of extracts

Quantitative phytochemical analysis revealed variations in the content of total phenolics (TPC), total flavonoids (TFC), total tannins (TTC), total triterpenoids (TTRC), and total alkaloids (TAC) in the 80% ethanol and aqueous extracts of *Abroma augustum* and *Cajanus cajan* (Table 2). The ethanol extract of *C. cajan* exhibited the highest TPC (150.52mg GAE/g DW), while the ethanol extract of *A. augustum* showed the lowest (27.97 mg GAE/g DW). A similar trend was observed for TFC, with the ethanol extract of *C. cajan* yielding the highest content (166.64mg QE/g DW) and *A. augustum* the

Table 2 — Content of TPC, TFC, TTC, TTRC and TAC in the extract of *Abroma augustum* and *Cajanus cajan*

Sl. No.	Sample	TPC (mg GAE/g dry weight)		TFC (mg QE/g dry weight)		TTC (mgTA/g dry weight)		TTRC (mg Ursolic acid/g dry weight)		TAC (mg AE/g dry weight)	
		80% ethanol extract	Pure water extract	80% ethanol extract	Pure water extract	80% ethanol extract	Pure water extract	80% ethanol extract	Pure water extract	80% ethanol extract	Pure water extract
1	*Abroma augustum* (L.) L.f.	27.97±0.7	39.22±1.3	26.78±0.7	26.80±0.6	19.418±1.07	31.800±1.4	32.465±1.88	14.131±0.74	22.37±0.88	15.02±0.34
2	*Cajanus cajan* (L.) Millsp.	150.52±0.2	60.54±1.0	166.64±1.1	78.57±0.9	67.012±0.8	46.436±0.7	46.995±0.94	24.476±0.78	16.71± 0.84	8.48± 0.88

Data are expressed as Mean±Standard error; n=3.

lowest (26.78mg QE/g DW). Notably, the aqueous extract of *A. augustum* contained a slightly higher TFC (26.80mg QE/g DW) compared to its ethanol counterpart.

The highest TTC was found in the aqueous extract of *A. augustum* (31.80mg TAE/g DW), whereas the lowest was in its ethanol extract (19.42mg TAE/g DW). Conversely, *C. cajan* displayed higher TTC in its ethanol extract (67.01mg TAE/g DW) compared to the aqueous extract (46.44mg TAE/g DW). For TTRC, ethanol extraction proved more efficient for both species, with *C. cajan* exhibiting the highest content (46.99mg UAE/g DW) and the aqueous extract of *A. augustum* the lowest (14.13mg UAE/g DW). Similarly, the ethanol extract of *A. augustum* showed a significantly higher TAC (22.37mg AE/g DW) than its aqueous extract (15.02mg AE/g DW) (Fig. 2). *C. cajan* also demonstrated higher TAC in the ethanol extract (16.71mg AE/g DW) compared to the aqueous extract (8.48mg AE/g DW).

Studies of biological activity of extracts

Antioxidant activity

The antioxidant potential of the extracts was evaluated using DPPH, FRAP, and ABTS assays (Table 3). *Cajanus cajan* extracts consistently demonstrated superior antioxidant activity compared to *Abroma augustum* extracts across all assays. In the DPPH assay, *C. cajan* exhibited lower IC_{50} values for both ethanol (162.17mg/mL) and aqueous (158.03mg/mL) extracts, indicating stronger radical scavenging capacity than *A. augustum* (ethanol: 578.75mg/mL; aqueous: 389.83mg/mL). Similarly, the FRAP assay revealed higher reducing power for *C. cajan* (ethanol: 73.62mM Fe^{2+}/g; aqueous: 60.72 mM Fe^{2+}/g) compared to *A. augustum* (ethanol: 42.35 mM Fe^{2+}/g; aqueous: 35.18 mM Fe^{2+}/g). Consistent with these findings, *C. cajan* extracts displayed lower IC_{50} values in the ABTS assay (ethanol: 2.87mg/mL; aqueous: 6.35mg/mL) compared to *A. augustum* extracts (ethanol: 7.54mg/mL; aqueous: 9.78mg/mL), further confirming its higher antioxidant potential.

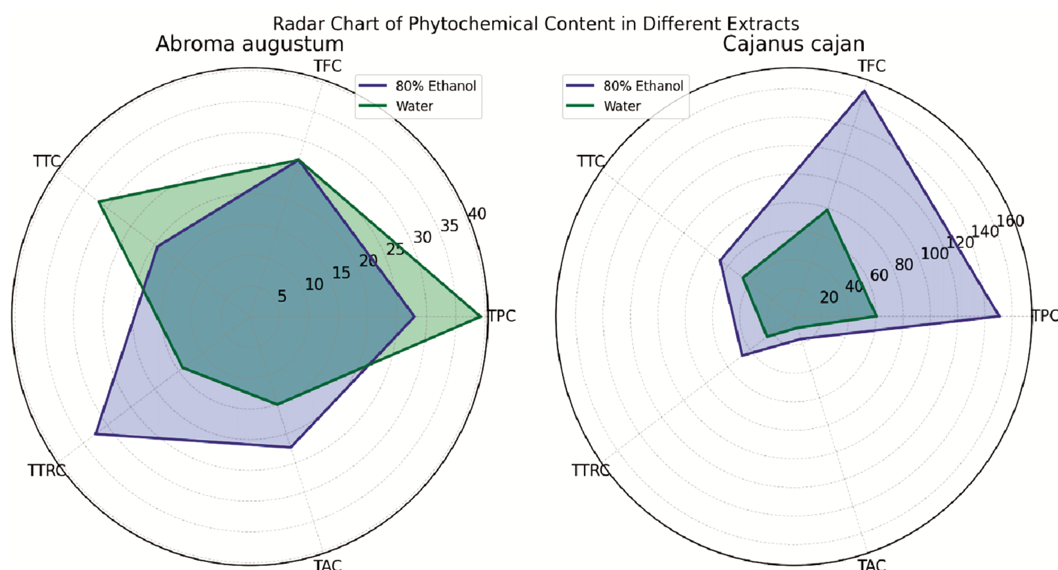

Fig. 2 — Radar chart comparing the total phytochemical content (TPC, TFC, TTC, TTRC, and TAC) in 80% ethanol and pure water extracts of *Abroma augustum* and *Cajanus cajan.*

Table 3 — Comparative analysis of antioxidant activities of *Abroma augustum* and *Cajanus cajan* through different analytical techniques and different extraction processes

Sl. No.	Anti-diabetic potential plants	80% Ethanol extract			Pure water extract		
		DPPH RSA IC_{50} value (mg/mL)*	FRAP (mM Fe^{2+}/gm)	ABTS IC_{50} values (mg/mL)*	DPPH RSA IC_{50} value (mg/mL)*	FRAP (mM Fe^{2+}/gm)	ABTS IC_{50} values(mg/mL)*
1	*Abroma augustum* (L.) L.f.	578.75±1.09^{a}	11.132±1.69^{b}	7.537±0.038^{a}	389.83±1.87^{a}	23.124±0.80^{b}	9.78±0.069^{a}
2	*Cajanus cajan* (L.) Millsp.	162.17±0.95^{b}	73.617±0.51^{a}	2.87±0.058^{b}	158.03±1.49^{b}	60.724±2.00^{a}	6.35±0.059^{b}

Note: RSA: Radical scavenging activity; Data represents the mean of three replicates ± Standard error from the mean. * *P* value has been calculated using one-way ANOVA.

Table 4 — α-amylase and α-glucosidase inhibitory activity of *Abroma augustum* and *Cajanus cajan*

Sl. No.	Sample plant	α-Amylase inhibitory activity (IC_{50} value, µg/ml)		α-Glucosidase inhibition (IC_{50} value, µg/ml)	
		80% ethanol extract (±SE)*	Pure water extract (±SE)*	80% ethanol extract (±SE)*	Pure water extract (±SE)*
1	*Abroma augustum* (L.) L.f.	357.57± 10.09[a]	394.99±08.76[a]	393.12±09.63[a]	422.35±13.62[a]
2	*Cajanus cajan* (L.) Millsp.	333.61±12.63[b]	375.50±10.84[b]	381.32±13.51[b]	403.65±11.85[b]
3	Acarbose	54.40816327±4.21		45.4330544±2.66	

Note: Data represents mean of three replicates ± Standard error from mean. * *P* value has been calculated using one-way ANOVA.

Inhibition of α-Amylase and α-Glucosidase enzymes

The inhibitory effects of *Abroma augustum* and *Cajanus cajan* extracts on α-amylase and α-glucosidase enzymes were assessed using their 80% ethanol and aqueous extracts (Table 4, Fig. 3 and 4). Both plant extracts exhibited notable inhibitory activity against both enzymes, with *C. cajan* generally showing stronger inhibition. For α-amylase, the IC_{50} values for the ethanol extracts were 357.57µg/mL for *A. augustum* and 333.61µg/mL for *C. cajan*. The aqueous extracts showed slightly higher IC_{50} values (394.99µg/mL for *A. augustum* and 375.50µg/mL for *C. cajan*). Similarly, for α-glucosidase inhibition, *C. cajan* exhibited lower IC_{50} values for both ethanol (381.32µg/mL) and aqueous (403.65 µg/mL) extracts compared to *A. augustum* (ethanol: 393.12 µg/mL; aqueous: 422.35µg/mL). However, the inhibitory potencies of both plant extracts were lower than that of the standard inhibitor, acarbose (α-amylase IC_{50}: 54.41µg/mL; α-glucosidase IC_{50}: 45.43µg/mL).

Mode of enzyme inhibition

Lineweaver-Burk plots revealed that both the ethanol and aqueous extracts of *Abroma augustum* and *Cajanus cajan* exhibited competitive inhibition of both α-amylase (Fig. 5) and α-glucosidase (Fig. 6) enzymes. The plots showed an increase in the apparent Michaelis-Menten constant (Km) with increasing extract concentrations, while the maximum reaction velocity (V_{max}) remained unchanged, as indicated by the intersection of the lines on the y-axis. This suggests that the bioactive compounds in the plant extracts compete with the substrates for binding to the active sites of both enzymes.

Cytotoxicity assessment

The cytotoxic potential of *Abroma augustum* and *Cajanus cajan* extracts was evaluated using the VERO cell line, and the IC_{50} values and cell viability at 500µg/mL are presented in Table 5. *Abroma augustum* exhibited a stronger cytotoxic effect, with a lower IC_{50} value (115.79µg/mL) and a lower cell viability (26.67%) at 500µg/mL compared to the

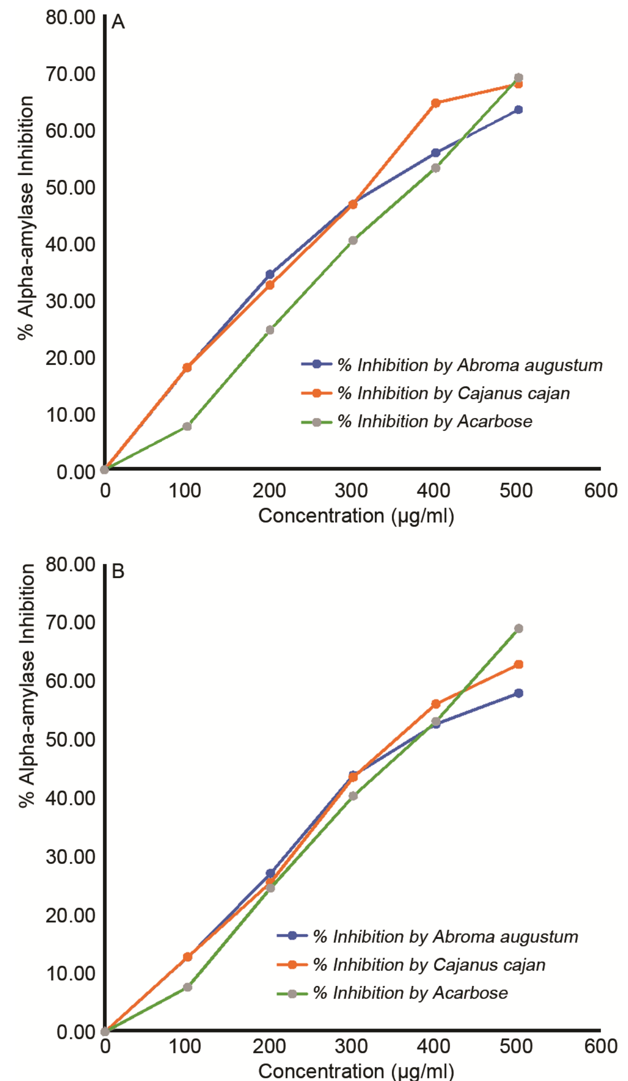

Fig. 3 — Percent inhibition of α-amylase by a. 80% EtOH extract and b. pure water extract.

control. In contrast, *Cajanus cajan* demonstrated a comparatively milder cytotoxic effect, with a higher IC_{50} value (139.98µg/mL) and a higher cell viability (47.84%) at the same concentration.

Glucose uptake activity in HepG2 cells

The effect of *Abroma augustum* and *Cajanus cajan* extracts on glucose uptake in HepG2 cells was

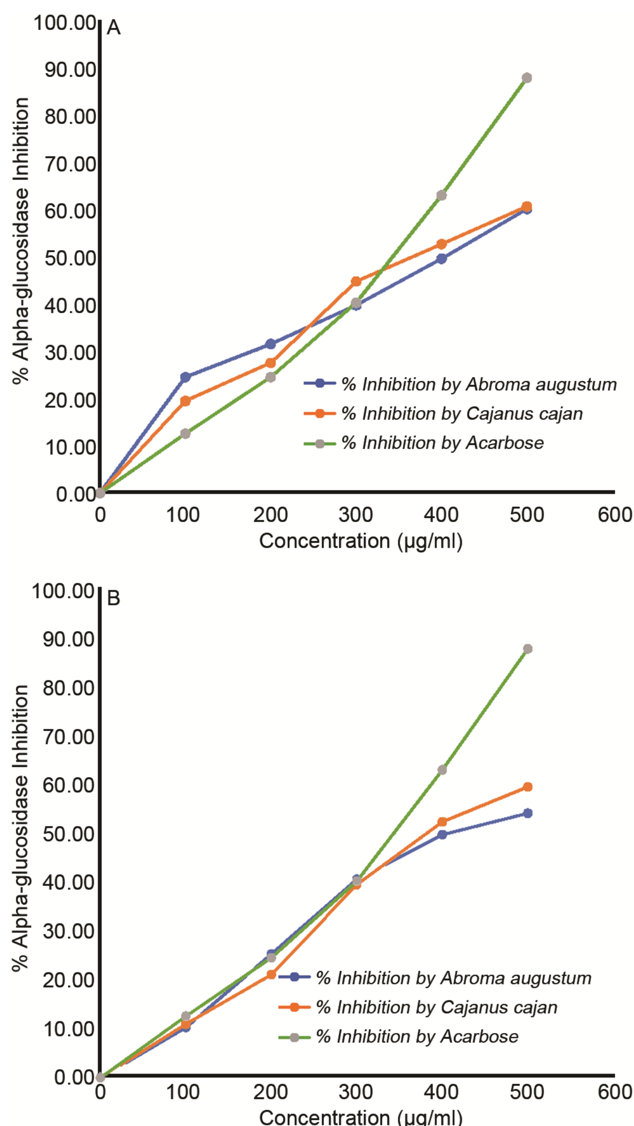

Fig. 4 — Percent inhibition of α-glucosidase by a. 80% EtOH extract and b. pure water extract.

assessed at concentrations ranging from 31.25 to 500µg/mL (Fig. 7a, 7b; Table 6). *Abroma augustum* extract showed a concentration-dependent increase in glucose uptake, with a significant enhancement observed at higher concentrations, reaching 139.06% uptake at 500µg/mL. *Cajanus cajan* consistently exhibited higher glucose uptake across all tested concentrations compared to *A. augustum*. At 500µg/mL, *C. cajan* induced the highest glucose uptake at 147.75%, which was significantly greater than that observed with *A. augustum*. However, both plant extracts were less effective in promoting glucose uptake compared to the positive control, Metformin (100µg/mL), which showed a significantly higher glucose uptake of 250.83%.

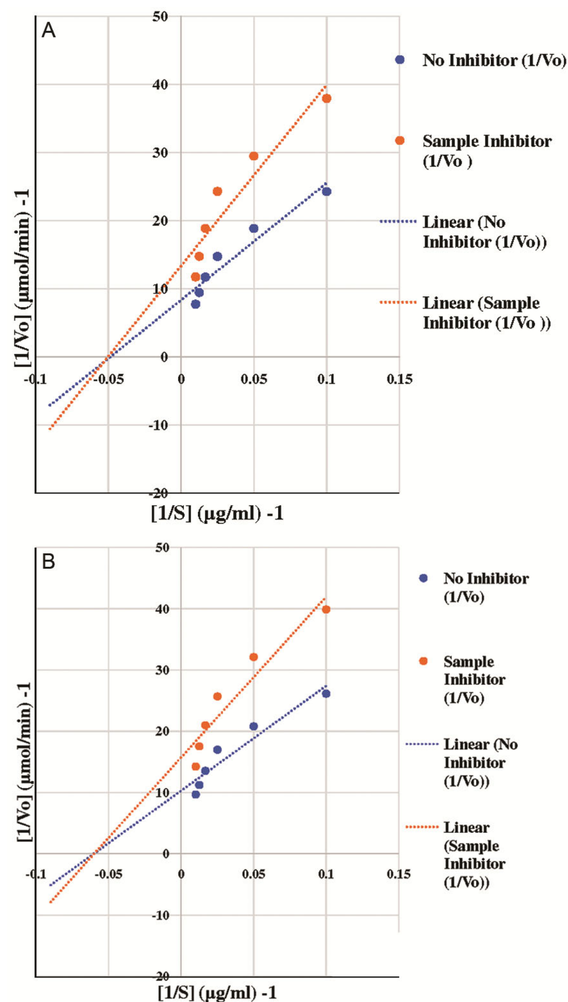

Fig. 5 — Mode of inhibition of a. α- amylase enzyme (competitive) and b. α- -glucosidase enzyme (competitive) by *Abroma augustum* extracts.

Discussions

Phytochemical profiling and solvent extraction efficiency

The exploration of safer and more effective antidiabetic agents from natural sources requires a meticulous evaluation of extraction methodologies and phytochemical profiling. The present study provides a comparative insight into the extraction efficiency of *Abroma augustum* and *Cajanus cajan*, revealing that 80% ethanol is significantly superior to aqueous solvents for isolating bioactive secondary metabolites. This finding is critical, as it aligns with the principle that moderately polar solvents like ethanol are more effective in solubilizing polyphenols and flavonoids, which are often trapped within the complex lignocellulosic matrices of plant tissues.

Our quantitative analysis demonstrated that the ethanol extract of *C. cajan* contained the highest

concentration of TPC (150.52 mg GAE/g DW) and TFC (166.64 mg QE/g DW), which directly correlated with its superior biological activity in subsequent assays. This observation is supported by recent phytochemical profiling of *C. cajan* leaves from other geographical regions (e.g., Panama), where ethanolic extraction similarly yielded high flavonoid concentrations, including bioactive compounds like lupenone and lupeol[11], which are known to exert antidiabetic effects[25]. Conversely, while *A. augustum* showed lower TPC and TFC values compared to *C. cajan*, the presence of these compounds confirms its ethnopharmacological relevance[8]. The detection of these metabolites in the ethanol extracts validates the traditional preparation methods often involving alcoholic or fermented tinctures in certain indigenous practices, suggesting that the community knowledge intuitively targets the most bioactive fractions[26,27]. potentially richer source of compounds contributing to its observed bioactivities.

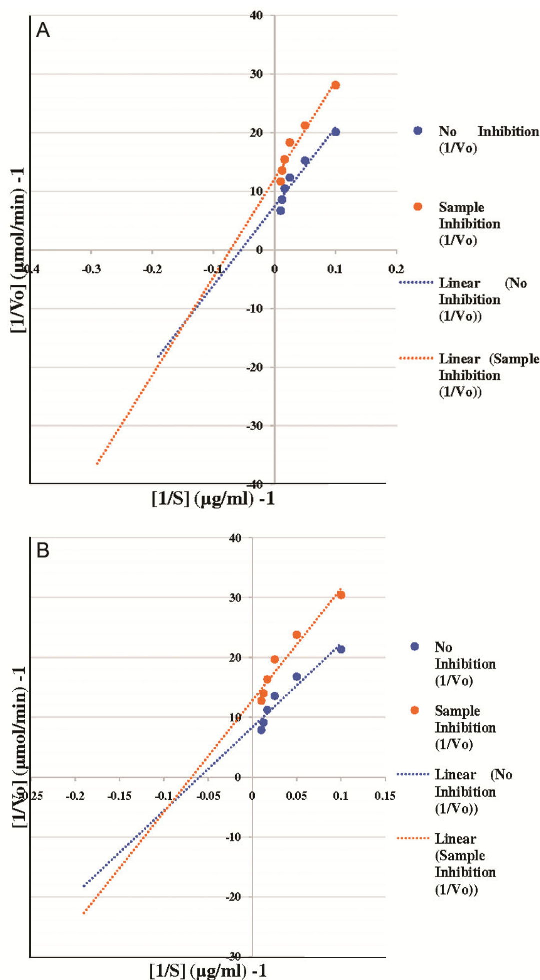

Fig. 6 — Mode of inhibition of a. α- amylase enzyme (competitive) and b. α- -glucosidase enzyme (competitive) by *Cajanus cajan* extracts.

Antioxidant capacity and mitigation of diabetic oxidative stress

Oxidative stress is a central pathogenic factor in Diabetes Mellitus (DM), linking chronic hyperglycemia to vascular complications. The autoxidation of glucose and the formation of Advanced Glycation End-products (AGEs) generate Reactive Oxygen Species (ROS), which deplete endogenous antioxidant defenses and cause pancreatic β-cell dysfunction. In this context, the antioxidant capacity of a phytomedicine is not merely a secondary property but a primary therapeutic mechanism. The present study establishes that *C. cajan* extracts possess a consistently superior antioxidant profile compared to *A. augustum* across DPPH, FRAP, and ABTS assays. The pronounced antioxidant activity exhibited by both *A. augustum* and *C. cajan* extracts underscores their potential role in mitigating oxidative stress, a critical factor in the pathogenesis and progression of diabetes mellitus[28]. The ability of plant-derived antioxidants to neutralize these ROS is therefore crucial in preventing cellular damage and reducing the inflammatory processes that exacerbate diabetic complications. This aligns with the findings of Dinore *et al.*[11], who attributed the strong antioxidant properties of *C. cajan* leaves to its diverse array of phenolic and flavonoid constituents identified through sophisticated analytical techniques. The strong reducing power (FRAP) and radical scavenging activity (DPPH/ABTS) of *C. cajan* are likely attributable to its high flavonoid content, specifically quercetin and rutin derivatives, which act as hydrogen donors to stabilize free radicals. This aligns with recent findings by Megha *et al.*[7], who

Table 5 — Cytotoxicity (IC_{50}) and cell viability of selected plant extracts at 500 µg/mL

Sl. No.	Sample species	Cell viability % (against control*) ± SE	Cytotoxic activity (IC_{50}) (mg/mL)± SE
1	*Abroma augustum* (L.) L.f.	26.67±0.037	115.79±0.044
2	*Cajanus cajan* (L.) Huth.	47.84±0.014	139.98±0.031

Note: Data represents mean of three replicates ± Standard error from mean.

*Control: Cells did not receive plant extract treatment, and their viability is assumed to be 100%. The test samples are compared relative to that 100% baseline. Values represent percentage viability relative to control.

reported a strong correlation between the antioxidant activity of pigeonpea germplasm and its phenolic composition.

While *A. augustum* also demonstrated measurable antioxidant activity, the variability in reported IC_{50} values and reducing power compared to previous studies[26,27] highlights the potential influence of factors such as the specific plant part used, the extraction solvent, and the geographical origin of the plant material on its antioxidant potential. The observed differences emphasize the need for standardized cultivation and extraction protocols to ensure consistent bioactivity. Interestingly, while *A. augustum* exhibited moderate antioxidant activity, recent independent investigations using zebrafish models have corroborated our findings. Sujaye et al.[8] reported that while *A. augustum* seed extracts possess measurable antioxidant potential (DPPH IC_{50} 105.57 µg/mL), they are less potent than standard antioxidants like BHT. Our data, showing an IC_{50} of 578.75 mg/mL for the ethanol extract of leaves, suggests that the antioxidant potency of *A. augustum* may vary significantly between plant parts (seeds vs. leaves) and extraction solvents, emphasizing the need for establishing standardized markers for this species[29].

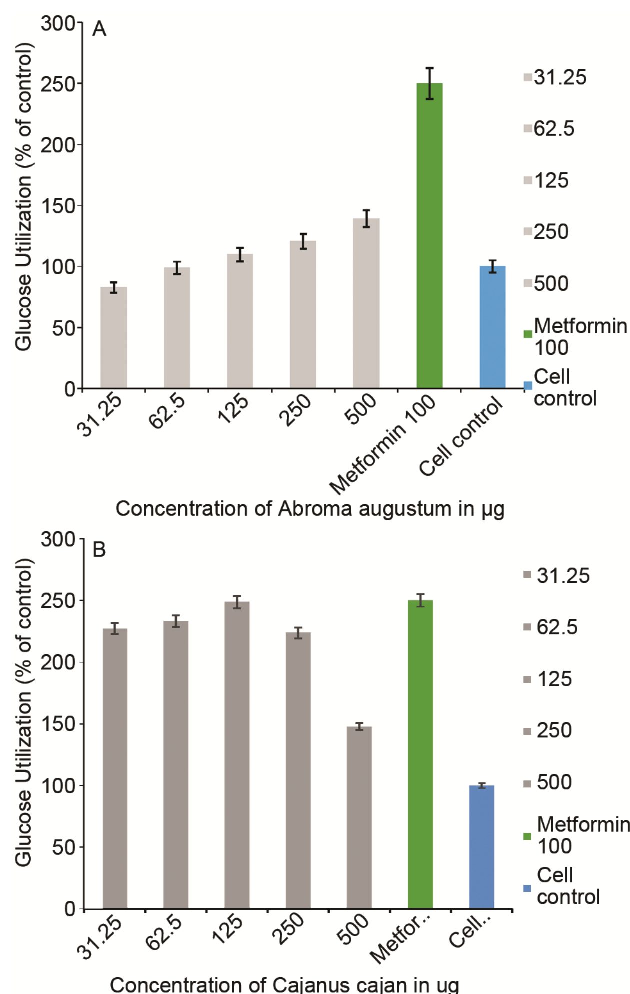

Fig. 7 — Percentage glucose uptake with a. *Abroma augustum* and b. *Cajanus cajan* extracts at 500µg, 250µg, 125µg, 62.5µg and 31.25 µg compared to Cell Control and Metformin.

Mechanistic insights into carbohydrate-hydrolyzing enzyme inhibition

The significant *in vitro* inhibitory activity of both *A. augustum* and *C. cajan* extracts against α-amylase and α-glucosidase (Table 4, Fig. 3 & 4) provides a strong rationale for their potential in managing postprandial hyperglycemia, a key therapeutic target in diabetes management. Management of Postprandial Hyperglycemia (PPHG) relies heavily on retarding carbohydrate digestion in the small intestine. The consistently stronger enzyme inhibitory activity observed with *C. cajan* extracts, particularly the ethanol extract, suggests that it may contain a higher concentration or more effective types of enzyme inhibitors compared to *A. augustum*. This finding is well-supported by existing literature on *C. cajan*, where various studies have identified specific compounds, including lignans and flavonoids, with potent α-glucosidase and α-amylase inhibitory activities[30,31]. While direct evidence for the enzyme inhibitory potential of *A. augustum* extracts is less extensive, our results contribute novel information in this area, suggesting its potential as a source of such inhibitors, possibly acting synergistically with other components in traditional polyherbal formulations[32].

Table 6 — Glucose concentration and percentage glucose uptake of plant extracts at 500µg compared to Cell Control and Metformin

Sl. No.	Samples	Glucose concentration (µg/mL)	% Glucose uptake against control treatment*
1	*Abroma augustum* (L.) L.f.	107.865±0.026	139.065±0.0035
2	*Cajanus cajan* (L.) Huth.	114.599±0.047	147.746±0.0134
3	Metformin (100 µg/mL)	194.558 ±0.011	250.833±0.0169
4	Cell control (Untreated)	77.565	100.00

Note: Data represents mean of three replicates ± Standard error from mean

* Cell control represents untreated cells and is considered as 100% baseline. Higher % glucose uptake indicates greater enhancement of glucose consumption by the cells. Metformin was tested at 100 µg/mL, while plant extracts were tested at 500µg/mL.

The current study reports that both plant extracts function as competitive inhibitors for both enzymes. The Lineweaver-Burk plots (Figs. 5 and 6) distinctively show that the Michaelis-Menten constant (K_m) increases while the maximum velocity (V_{max}) remains constant in the presence of the extracts. This kinetic signature indicates that the bioactive phytoconstituents—likely the flavonoids identified in Table 2 structurally mimic the natural substrates (starch and oligosaccharides), thereby competing for the active binding sites of the enzymes. This is a highly desirable characteristic for antidiabetic agents. Unlike non-competitive inhibitors, which may permanently alter enzyme structure, competitive inhibitors offer a reversible and concentration-dependent control of glucose release, potentially reducing the risk of hypoglycemia. This mode of action suggests that the active compounds in these plant extracts likely possess structural similarities to the natural substrates of α-amylase and α-glucosidase, allowing them to effectively compete for the active site. This mechanism is commonly observed with various plant-derived antidiabetic compounds[33] and provides a scientific basis for their potential in controlling postprandial glucose excursions[34-36].

Comparatively, *C. cajan* demonstrated superior inhibitory potency (lower IC_{50}) than *A. augustum*. This is consistent with the higher density of competitive ligands (flavonoids) in the *C. cajan* extract. These results are reinforced by a 2025 study by Aslam *et al*.[29] which utilized molecular docking to show that tannins and gallic acid from related medicinal species form stable complexes with the active site of α-glucosidase (PDB ID: 3W37), confirming the molecular basis of this competitive interaction. Furthermore, while our extracts were less potent than the synthetic inhibitor Acarbose, plant-based competitive inhibitors are often preferred due to fewer gastrointestinal side effects (e.g., flatulence, bloating) compared to synthetic alternatives, making *C. cajan* a promising candidate for nutraceutical development.

Modulation of glucose uptake in HepG2 cells

Beyond enzymatic inhibition in the gut, the ability to enhance glucose uptake in peripheral tissues is essential for overcoming insulin resistance, a key aspect of managing Type 2 diabetes[37]. The assessment of glucose uptake in HepG2 cells provides insights into the potential of these plant extracts to enhance glucose metabolism at the cellular level. Our results (Fig. 7a and 7b; Table 6) demonstrated that both *A. augustum* and *C. cajan* extracts promoted glucose uptake in a concentration-dependent manner. *C. cajan* exhibited a robust 147.75% increase in glucose uptake at 500 μg/mL. Although the precise molecular pathway was not mapped in this specific assay, recent literature on flavonoid-rich extracts suggests this effect is likely mediated through the upregulation of Glucose Transporter-2 (GLUT2) in liver cells or the translocation of GLUT4 via the PI3K/AKT signaling pathway. A 2024 study on similar phenolic-rich plant extracts in HepG2 cells proposed that such extracts mitigate hyperglycemic oxidative stress, which in turn restores the sensitivity of the insulin receptor substrate (IRS-1), facilitating glucose entry[38]. These findings align with previous animal models demonstrating the blood glucose-lowering effects of *C. cajan*, supporting its potential to improve glucose homeostasis[39,40].

The observation that *A. augustum* also significantly enhanced glucose uptake (139.06%) is particularly interesting given its lower antioxidant profile compared to *C. cajan*. This suggests *A. augustum* may contain specific non-phenolic bioactive moieties, such as sterols or triterpenes (e.g., taraxerol), which have been reported to independently activate AMPK pathways to promote glucose metabolism [32]. This finding complements past studies indicating the potential of *A. augustum* to lower postprandial glucose levels by reducing glucose absorption and inhibiting α-glucosidase[31].

However, neither extract matched the efficacy of the standard drug Metformin (250.83%), indicating that these herbal preparations might be most effective as adjunct therapies rather than monotherapies for severe insulin resistance. Further research is required to isolate the specific compounds responsible for this activity and to explore potential synergistic effects with existing antidiabetic medications.

Critical assessment of cytotoxicity and safety profiling

While efficacy is promising, the evaluation of cytotoxicity is a crucial differentiator in assessing the therapeutic viability of these plants[41]. Findings of the present study (Table 5) revealed that *C. cajan* displayed a relatively favourable safety margin (IC_{50} 139.98 μg/mL in VERO cells), whereas *A. augustum* exhibited higher cytotoxicity (IC_{50} 115.79 μg/mL).

The comparatively lower cytotoxicity of *C. cajan* supports its classification as a "functional food" with a wider therapeutic index. The traditional consumption of *C. cajan* leaves as a tea in Nagaland appears to be scientifically safer compared to the use of *A. augustum*.

This observation is consistent with studies indicating neuroprotective effects of *C. cajan* extracts[39], further highlighting its favourable safety profile.

In contrast, the higher cytotoxicity observed in *A. augustum* is of significant toxicological importance and aligns with emerging data. A recent 2024 study evaluating *A. augustum* seed extracts on zebrafish embryos reported clear teratogenic effects, including yolk sac edema and tail deformation, at concentrations as low as 100 µg/mL[8.] Furthermore, methanolic extracts of *A. augustum* bark have been shown to induce apoptosis in Ehrlich Ascites Carcinoma (EAC) cells via the upregulation of the p53 tumor suppressor gene[37]. While this suggests potential anticancer properties, it raises concerns regarding the chronic use of *A. augustum* for diabetes management without careful dosage regulation. Thus, while *A. augustum* possesses promising bioactivity, careful consideration of dosage and potential toxic effects on normal cells is necessary for its safe application.

Conclusion

This study scientifically validates the traditional use of both species while highlighting distinct pharmacological profiles. *Cajanus cajan* emerges as a superior candidate for further drug development due to its high phenolic content, potent antioxidant capacity, strong competitive enzyme inhibition, and favourable safety profile. *Abroma augustum*, while effective in glucose uptake and enzyme inhibition, warrants caution due to its higher cytotoxicity. Future research should focus on: (1) Bioassay-guided fractionation to isolate the specific competitive inhibitors from *C. cajan*; (2) *In vivo* chronic toxicity studies to establish the "No Observed Adverse Effect Level" (NOAEL) for *A. augustum*; and (3) Molecular docking studies to visualize the interaction between identified Nagaland-specific phytocompounds and the crystal structures of α-amylase and α-glucosidase.

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CRediT statement

CRD: Conceptualized, designed of the research, methodology, fund acquisition, project administration, Supervision, resources, validation, and editing the manuscript. TIS: Data curation, methodology, formal analysis, investigation, software, data analysis, software, validation, visualization, and original drafting of the paper.

Declaration of competing interest

The authors declare that no conflicts of interest exist.

Competing Interests

The authors declare that there are no conflicts of interest.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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