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Original Article

Antioxidant, antidiabetic, neuroprotective, and phytochemical evaluation of *Chenopodium ambrosioides* ethanol extract

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ABSTRACT

Aim: Our goal in this work was to investigate *Chenopodium ambrosioides* bioactive profile as well as its *in vitro* antidiabetic, neuroprotective, and antioxidant properties.

Methods: The antioxidant capacity of *Chenopodium ambrosioides* extracts (CAE) was assessed by using four complimentary tests: 2,2-diphenyl-1-(2,4,6-trinitrophenol) hydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and total antioxidant capacity (TAC) assays. α -Glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibition assays were used to assess the antidiabetic and neuroprotective potential of CAE.

Results: According to the assay findings, CAE exhibited considerable antioxidant activity and a high concentration of proanthocyanidins, flavonoids, tannins, and total phenols. CAE showed significant total antioxidant activity (EC_{50} 106.51 μ g/mL) in comparison to the ascorbic acid (EC_{50} 76.34 μ g/mL). There is no as such significant difference between the inhibitory effects of CAE towards α -glucosidase (IC_{50} 27.5 μ g/mL) in comparison to acarbose (IC_{50} 39.3 μ g/mL), however CAE showed considerable inhibition to acetylcholinesterase (IC_{50} 29.3 μ g/mL) and butyrylcholinesterase activity of (IC_{50} 57.8 μ g/mL) as compared to the standard drug galantamine (IC_{50} 53.6 μ g/mL).

Conclusion: These results suggest that based on the antioxidant and enzyme inhibitory potential, CAE could be used as a natural remedy for the treatment of diabetes and neurodegenerative disorders.

Keywords: *Chenopodium ambrosioides*, Antidiabetic, Neuroprotection, Phytomedicine, Natural product

INTRODUCTION

Bioactive natural products can be produced in the most concentrated and targeted manner in medicinal plants. One of the drug discovery and strategy investigation works in research organizations throughout the world is the search for excellent, real, inexpensive, and easily accessible natural pharmaceuticals or enzyme inhibitors.^[1] Preventing the body from breaking down carbohydrates is one strategy for controlling postprandial hyperglycemia in people with type 2 diabetes mellitus (T2DM). Dietary carbohydrates including starch are converted into simpler monosaccharides by the digestive enzyme pancreatic amylase. Polysaccharides are broken into dissolved glucose by α -glucosidases, and once absorbed, glucose penetrates the bloodstream. Therefore, inhibiting the α -glucosidase and α -amylase

enzymes can delay the assimilation of glucose, stop the decomposition of carbs, and reduce blood sugar levels.^[2]

The earliest and most extensively researched molecular processes in the pathophysiology of Alzheimer's disease (AD) were proposed to involve the cholinergic system. It is described as the main kind of neuronal degeneration that can harm cholinergic neurons and impair cognition. Cholinergic receptors are involved in the interaction between neurofibrillary changes and α -amyloid pathology, which quickens AD development.^[3] The hydrolase group of enzymes, which includes cholinesterase, controls cholinergic nerve and neuromuscular transmission and breaks down acetylcholine (ACh) into choline and acetate to stop its function.^[4,5]

Approximately 80% of the global population looks to medicinal plants, their derivatives, and fractions as a primary source for fulfilling their basic healthcare needs.^[6] These plants, known for their vast medicinal properties, serve as a substantial foundation for natural pharmaceuticals.^[7] There is an expectation that medicinal plants may contain secondary compounds with suitable properties for modern medical applications in addressing a range of disorders that are currently untreatable.^[8] Traditional medical practices have gained international attention throughout history. Acquiring approval of herbal medications is frequently required for matters of national security and other exigencies. Several individuals in developed nations have recently started seeking alternative or complementary therapies that include medicinal plants.^[9] Ayurvedic medicine is prepared from a wide variety of medicinal plants, and purified active compounds often fall short of expectations. There is a deficiency of pharmacological data concerning various medicinal plants and isolated compounds, making it improbable that these will regulate the potent molecules necessary for their intended biological functions. The significant role of manufacturing in the technological discipline is indispensable for the local manufacturing and production of materials that meet the desired quality standards.^[10] Enzymes serve as the molecular target for nearly 50% of the small-molecule drugs available in the market. Enzymes are attractive candidates for novel drug development due to their protein structure, which allows for effective drug interactions and robust target validation.^[11]

Beneficial therapies in the management of T2DM include inhibitors of the endogenous glycosidases i.e., α -glucosidase and α -amylase, which lower the postprandial hyperglycemia by delaying the digestion of dietary carbohydrates.^[12] Inhibiting glycosidases and pancreatic lipases results in caloric restriction, which might be helpful for weight control and possible therapy for obesity.^[13] Screening of medicinal plants for natural pharmaceuticals for enzyme inhibition is the more appropriate approach to discovering or developing novel antidiabetic, anti-obesity, and neuroprotective medicines for better treatment and control of metabolic disorders.^[14] *Chenopodium ambrosioides* (*C. ambrosioides*) has an abundance of phytochemicals with significant pharmacological properties including antioxidant, anti-inflammatory, anthelmintic, antileishmanial, antitumoral, respiratory problems, tuberculosis, enzyme inhibition, and aiding wound healing and cancer prevention.^[15-17]

The objective of the current study is to explore the potential of *C. ambrosioides* extract (CAE) as a natural remedy for diabetes and neurodegenerative disorders. We investigated CAE's antioxidant potential and ability to inhibit enzymes linked to the above conditions. The CAE contained higher levels of beneficial phytochemicals i.e., proanthocyanidins, flavonoids, tannins, and total phenols, and showed significant antioxidant potential. Moreover, it demonstrated comparable effects to standard drugs acarbose and galantamine in inhibiting α -glucosidase, acetylcholinesterase, and butyrylcholinesterase, respectively. These findings suggest that *C. ambrosioides* could offer therapeutic benefits for managing diabetes and addressing neurodegenerative diseases due to its potent antioxidant and enzyme-inhibitory properties.

MATERIALS AND METHODS

Plant material and extraction

The *C. ambrosioides* powder was purchased from the local market and subjected to a microwave-assisted extraction method.^[18,19] Household Microwave System with a digital control system allowing adjustment of microwave power ranging from 100 to 1000 W. The microwave oven was modified to condense vapors formed within the sample during extraction. One gram of *C. ambrosioides* powder was placed into a 250 mL volumetric flask containing ethanol as an extraction solvent. Post-extraction, the resulting extract was filtered using Whatman N°1 filter paper, and the total volume was adjusted to match the initial extraction volume. The CAE was then stored at 4 °C until subsequent analysis.

Quantification of bioactive compounds

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

TPC was determined using the Folin-Ciocalteu method. In a study referenced as [16], 100 µL of CAE was mixed with 500 µL of 0.2 N Folin-Ciocalteu reagent and left at room temperature. To stabilize the reaction, 400 µL of saturated Na₂CO₃ (75 g/L) was added after 5 minutes. After 90 minutes, the absorbance of the resulting blue color was measured at 765 nm against a blank reagent. Gallic acid was used to create a standard curve, and the results are presented as milligrams of GAE per gram of dry weight.^[20]

The TFC of the CAE was determined using the following method described by Chang et al^[21] 250 µL of CAE was combined with 50 µL of 10% aluminum chloride solution, 50 µL of 1M potassium acetate solution, and 1.4 mL of distilled water, followed by incubation at room temperature. The absorbance was measured at 415 nm after 40 minutes. A standard curve was established using quercetin, and the results are reported as milligrams of QE per gram of dry weight.

Determination of total tannin content (TTC) and proanthocyanidin content (PC)

The total tannin content in CAE was assessed following Pearson's method (1976).^[22] This involved mixing 200 µL of CAE with an equal volume of Folin-Denis reagent. After five minutes, 500 µL of a saturated Na₂CO₃ solution was added, followed by dilution with one milliliter of distilled water. The absorbance intensity was measured at 760 nm after 30 minutes. Tannic acid was used to create a standard curve, and the results are reported as milligrams of TAE per gram of dry weight.

Quantification of proanthocyanidins in CAE was done as described by.^[23] This method involved mixing 250 µL of the CAE with 600 µL of 1% methanol vanillin solution and 600 µL of 25% H₂SO₄. The absorbance was measured at 500 nm after 15 minutes. Catechin was used to establish a standard curve, and the findings are presented as milligrams of CE per gram of dry weight.

High-Performance Liquid Chromatography (HPLC) Analysis

High-Performance Liquid Chromatography analysis of CAE was performed using a Shimadzu LC-20AT HPLC system equipped with a diode array detector (SPD-M20A), column oven (CTO-20A), and auto-sampler (SIL-20A). For this analysis, the Purospher Star RP-18 end-capped 5 µm 100 Å column (250 x 4.60 mm, Merck, Germany) along with the KJO-4282 Phenomenex guard column were utilized.^[24] The mobile phase consisted of 0.1% acetic acid (A) and methanol (B)^[25] and the flow rate was maintained at 0.8 mL/min. By comparing retention times and UV-Vis spectra of chromatographic peaks with those of known reference standards at 280 nm, phenolic compounds were identified.^[26]

Antioxidant activity of CAE

DPPH assay

CAE antioxidant capacity was measured using the DPPH test.^[27] A 1:1 mixture of plant bioactive extract and DPPH reagent (100 μ M) was created. Allow it to sit at ambient temperature for thirty minutes in the dark. The samples' abundance of antioxidant chemicals suggested that the hues violet to pale yellow corresponded to varying levels of activity. After measuring the intensity of absorption at 515 nm, the formula as follows was used to determine the effectiveness of the DPPH radical regarding percentage inhibition:

$$I \% = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

The IC₅₀ values are used to express the results. Utilizing linear regression analysis, the amount of CAE needed to block the DPPH radical by fifty percent is known as the value of the IC₅₀.

ABTS assay

The ABTS reactive cation decolorization test was used to evaluate the CAE's antioxidant potential.^[28] A distilled water solution was used to make the ABTS reagent (7 mM). After combining 150 μ L of the CAE with 500 μ L of ABTS solution, this was left in darkness for ten minutes. At 734 nm, the blue-green chemical mixture's decolonization was measured in comparison to the solvent-containing blank. The subsequent formula was used to compute the percentage of inhibition (I%):

$$I \% = \text{Abs control} - \text{Abs sample} / \text{Abs control} \times 100$$

The values of the IC₅₀ are used to express the results. Employing linear regression evaluation, the IC₅₀ value- the amount of plant bioactive extract needed for 50% suppression of a particular ABTS radical was determined.

FRAP assay

Ferric reducing antioxidant power (FRAP) activity of CAE was examined using the Benzie & Strain method^[29] which determines the iron-reducing capacity of the CAE (Fe³⁺ to Fe²⁺). The sample bioactive extract was mixed with the FRAP mixture (10:1:1 ratio of TPTZ solution, 2.4 mL distilled water, acetate buffer, and FeCl₃ solution), and allowed to sit at room temperature for five minutes. At 593 nm, the absorbed energy of a detectable blue color was measured. Plant extracts FRAP effectiveness was calculated using the FeSO₄ standard, and the results are displayed per mMol Fe²⁺ g/DW. Results are expressed in terms of EC₅₀ values. The EC₅₀ value refers to the concentration of CAE in the solution, which shows the absorbance of 0.5 that was calculated using linear regression analysis.

Total antioxidant capacity using phosphomolybdate reagent

The total antioxidant capacity (TAC) of CAE was quantified by the method of Prieto et al.^[30] Ammonium molybdate tetrahydrate (at 4 mM concentration), sulfuric acid (0.6 M), and solution of sodium phosphate dibasic (28 mM) were added at a ratio of 1:1:1 to create the phosphomolybdate reagent. A bath of boiling water was used for heating 100 μ L of CAE from the sample and 1 mL of phosphomolybdate reagent for approximately 90 min. At 765 nm, absorption was measured upon cooling. Results are expressed in terms of EC₅₀ values. The EC₅₀ value refers to the concentration of CAE in the solution, which shows the absorbance of 0.5 that was calculated using linear regression analysis.

α -Glucosidase inhibition assay

The technique outlined by Rengasamy et al.^[31] was utilized to ascertain the inhibitory capacity of α -glucosidase. The total DMSO concentration used in the preparation of the samples was limited to 7%. The following formula was used to determine the percentage of inhibition.

$$\% \text{Inhibition} = (\text{Ac} - \text{As}) / \text{Ac}$$

Whereas Ac = Absorbance of control, As = Absorbance of the sample.

Cholinesterase inhibition assay

Employing butyryl thiocholine iodide and solution of acetylthiocholine iodide as substrates, BCh and ACh inhibition was measured spectrophotometrically using CAE, following the methodology used by Nazir et al. method.^[32]

Statistical Analysis

The collected data were presented as Mean \pm SD and subjected to statistical examination employing the one-way ANOVA test.^[33] Additionally, the pairwise comparison of each group was evaluated utilizing Tukey's and Fisher's tests, utilizing IBM SPSS Statistics (version 28.0.1.0) as a statistical program.

RESULTS

Phytochemical analysis of CAE

Medicinal plants serve an important role in society and individual healthcare as well. Medicinal applications of particular plant species can be attributed to the presence of natural pharmaceutical substances, including alkaloids, flavonoids, tannins, and saponins.^[34] In the current study, *C. ambrosioides* was screened for quantitative phytochemical profiling and represented as mean \pm SE (Standard error of the mean) in (Table 01). Quantitative analysis for phytochemicals constituents demonstrated the presence of a higher concentration of total phenolic content 56.98 ± 2.12 mg GAE/g dry plant material. Although total flavonoid content (28.57 ± 0.84 mg QE/g), total tannins (8.29 ± 0.70 mg TAE/g), and total proanthocyanidin (6.23 ± 0.07 mg CE/g) were determined in the current study.

Table 1: Polyphenolic compounds of *C. ambrosioides* extract (CAE)

Polyphenolic compounds	Quantity
Total phenols (mg GAE/g)	56.98 ± 2.12
Total flavonoids (mg QE/g)	28.57 ± 0.84
Total tannins (mg TAE/g)	8.29 ± 0.70
Total proanthocyanidin (mg CE/g)	6.23 ± 0.07

HPLC analysis of CAE

The HPLC analysis of CAE showed a considerable amount of polyphenols (Table 02). The relevant peaks of phenolic contents of CAE were detected by using standard compounds and are presented in (Figure 01). The peaks of detected compounds were aligned with catechol, caffeic acid, ferulic acid, gallic acid, kaempferol, and quercetin, and all compounds were observed to be present in considerably high concentrations (mg/g dry weight) with retention time (Rt, mins) such as gallic acid (9.52 ± 0.27 at $Rt=11.39$), while catechol (4.28 ± 0.09 at $Rt=13.47$), Caffeic acid (2.75 ± 0.03 at $Rt=17.75$), Ferulic acid (3.38 ± 0.02 at $Rt=23.87$), Quercetin (4.63 ± 0.04 at $Rt=35.02$), and Kaempferol (1.21 ± 0.01 at 41.68), their retention times and concentration are presented with appropriate units in (Table 02).

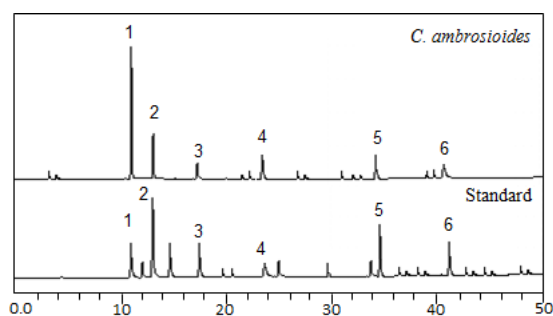


Figure 01: HPLC chromatograms showing the phenolic profile of CAE and standard compounds *i.e.*, Gallic acid (1), Catechol (2), Caffeic acid (3), Ferulic acid (4), Quercetin (5), and Kaempferol (6).

Table 02: Phenolic composition (mg/g dry weight) of *C. ambrosioides* extract (CAE)

Sr. No.	Compounds	Retention time (minutes)	Concentration (mg/g dry weight \pm SE)
1	Gallic acid	11.39	9.52 \pm 0.27
2	Catechol	13.47	4.28 \pm 0.09
3	Caffeic acid	17.75	2.75 \pm 0.03
4	Ferulic acid	23.87	3.38 \pm 0.02
5	Quercetin	35.02	4.63 \pm 0.04
6	Kaempferol	41.68	1.21 \pm 0.01

Antioxidant activity of CAE

The potential of CAE to detoxify free radicals, such as 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl radical (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS+), in addition to their capacity to reduce ferric (III) iron into ferrous (II) iron along with total capacity for antioxidants, were evaluated using in vitro antioxidant activity analyses. Figure 02 demonstrates the comparison between CAE and ascorbic acid, highlighting that CAE exhibits greater free radical scavenging capabilities across various assays, including DPPH, ABTS, free-reducing antioxidative power, and total antioxidative capacity. The analysis reveals a significant disparity in the total antioxidant content between *C. ambrosioides* and ascorbic acid. For instance, the DPPH concentration (IC₅₀ μ g/mL) for CAE was 37.04, while ascorbic acid, a standard, exhibited a value of 28.65. Similarly, the ABTS concentration (IC₅₀ μ g/mL) for CAE was 55.49, in contrast to the ascorbic acid standard at 43.36. The FRAP level (EC₅₀ μ g/mL) for CAE was 50.46, whereas the ascorbic acid level was 61.86. Additionally, the TAC level (EC₅₀ μ g/mL) for CAE was 106.51, surpassing the ascorbic acid level of 76.34. These findings highlight the profound antioxidant potential of *C. ambrosioides*, primarily due to its abundant phytochemicals, outperforming the standard represented by ascorbic acid (Table 03).

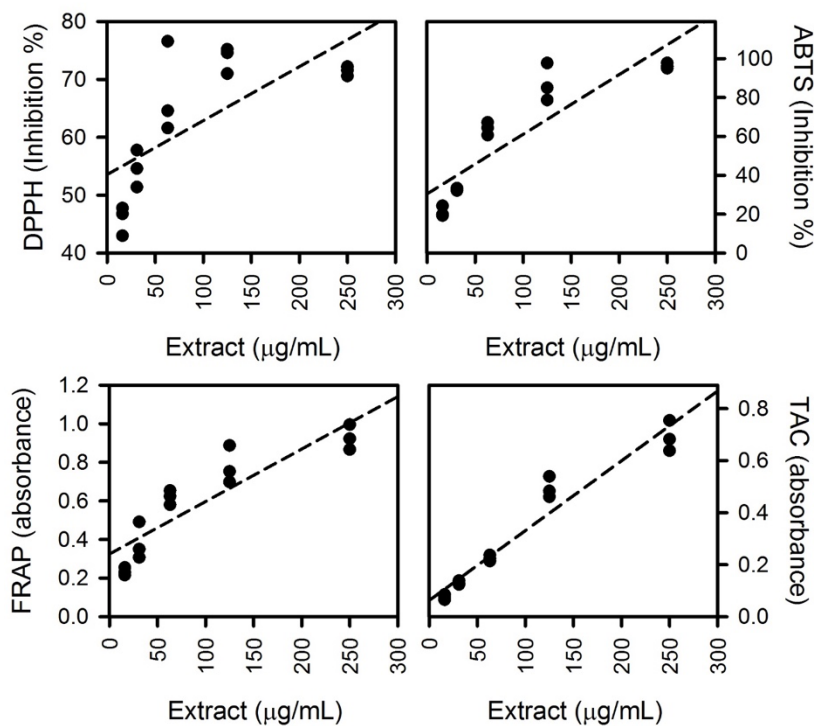


Figure 02: Dose-dependent response of *C. ambrosioides* extracts (CAE) under different antioxidant measuring systems

Table 03: Comparison of antioxidant activities of CAE with ascorbic acid

Antioxidant activities	Values
DPPH (IC₅₀ µg/mL)	
<i>C. ambrosioides</i> extract	37.04
Ascorbic acid	28.65
ABTS (IC₅₀ µg/mL)	
<i>C. ambrosioides</i> extract	55.49
Ascorbic acid	43.36
FRAP (EC₅₀ µg/mL)	
<i>C. ambrosioides</i> extract	50.46
Ascorbic acid	61.86
TAC (EC₅₀ µg/mL)	
<i>C. ambrosioides</i> extract	106.51
Ascorbic acid	76.34

Enzymes inhibitory activity of CAE

One of the major strategies to combat hyperglycemia in people with T2DM has been to suppress the digestive system's enzymes that hydrolyze carbohydrates, particularly α -glucosidase. The hydrolase group of enzymes, which includes cholinesterase, controls cholinergic nerve and neuromuscular transmission. Table 04 presents the inhibitory effects of CAE on different enzymes, specifically α -glucosidase, AChE, and BChE. This study investigated the inhibitory activity of CAE in comparison to established standards. The results revealed a considerable contrast in α -glucosidase activity when compared to acarbose, with α -glucosidase inhibition (IC₅₀ 27.5 µg/mL) astonishingly more effective than that of acarbose (IC₅₀ 397.3 µg/mL). Moreover, CAE exhibited AChE inhibitory activity (IC₅₀ 29.3 µg/mL) comparable to galantamine (IC₅₀ 34.2 µg/mL). While *C. ambrosioides* showed BChE inhibition (IC₅₀ 57.8 µg/mL), nearly identically effective as galantamine (IC₅₀ 53.6 µg/mL) as presented in (Table 4).

Table 04: Enzymes inhibitory activity of CAE

Enzyme inhibition assay	Sample	IC ₅₀ value (µg/mL)
α -Glucosidase	CAE	27.5 ± 0.3
	Acarbose	39.3 ± 0.8
Acetylcholinesterase (AChE)	CAE	29.3 ± 0.5
	Galantamine	34.2 ± 0.4
Butyrylcholinesterase (BChE)	CAE	57.8 ± 0.6
	Galantamine	53.6 ± 0.5

DISCUSSION

The abundance of phytochemicals such as tannins, flavonoids, phenolics, and proanthocyanidins in *C. ambrosioides* has led to the use of a variety of medicinal plants for screening potential drugs, each with numerous therapeutic capabilities. Both natural alkaloids and their synthetic analogs have shown a plethora of healing actions, encompassing antibacterial, antispasmodic, and effects that relieve pain.^[35] Moreover, it is imperative to note that the abundance of phenolic compounds in herbal treatments contributes significantly to their antioxidant efficacy.^[36] In a research, Munir et al.^[37] determined the quantitative phytochemicals of *Epimedium grandiflorum* and concluded that total flavonoid (241.8 ± 11.18 mg CE/g dry plants material) and phenolic contents (1010.72 ± 12.49 mg GAE/g dry plants material) were present in *E. grandiflorum*. In another study,^[17] *C. ambrosioides* methanol extracts from seeds revealed the highest phenolic (64.6±0.6µg gallic acid equivalent/mg) and flavonoid (50.9±0.5µg quercetin equivalent/mg) levels. This extract displayed potent antioxidant properties, showing strong free radical

scavenging (IC_{50} of $110.7 \pm 5 \mu\text{g/ml}$), total antioxidant capacity ($110.6 \pm 2.2 \mu\text{g AAE/mg}$), and total reducing power ($94.30 \pm 0.46 \mu\text{g AAE/mg}$). The ethanol extract demonstrated notable protein kinase inhibition that is consistent with results of this study. Our results were also in line with various studies including [37-40] suggesting ethanolic extracts of *C. ambrosioides* leaves contain potential phytochemicals that revealed different medicinal, anti-oxidant, and free radical scavenging properties proficiently.

It's been widely accepted that flavonoids, a class of naturally occurring phenolic compounds, stand out as a prominent class among natural products. Methanol and ethanol have been proven to be efficient solvents for the purification of phenolic antioxidants.[6,35] The present study delves into the investigation of antioxidant, anti-diabetic, and cytotoxic effects of kaempferol, quercetin, ferulic acid, and caffeic acid. The antioxidant activity of CAE can be attributed to the synergist effect of the presence of antioxidant compounds and other phytochemicals (Table 02). Our results in the present investigation are also in line with a study conducted by Houngnimassoun et al.[41] which found alkaloids, catechin, tannins, flavonoids, and saponosides after CAE characterization. Moreover in another study, similar tests were conducted in Benin.[42] Gallic acid, catechol, caffeic acid, ferulic acid, quercetin, and kaempferol were shown to be present in *C. ambrosioides* L. and saponosides were not present in phytochemical screening using HPLC-DAD screening for antioxidant activity.[42] In our study, the antioxidative activities included DPPH, ABTS, FRAP, and TAC of CAE were compared with ascorbic acid, as a standard, and the values observed were better for CAE's free radical scavenging using DPPH, ABTS, free reducing antioxidative power, and total antioxidative capacity (37.04, 55.49, 50.46, 106.51, respectively) than ascorbic acid (28.56, 43.36, 61.86, 76.34, respective of the order of assay) (Table 3). Reyes-Becerril et al.[43] evaluated the antioxidant activity and chemical content of CAE to determine its application as a nutraceutical. The extract showed strong antioxidant effects, improving cell viability by 88% and boosting beneficial compound production. Overall, it suggests this extract is a valuable addition to diets for its antioxidant and potential anti-inflammatory benefits.[43] The aqueous extract of the two portions of the *C. ambrosioides* L plant showed a substantial reaction for the TPC, TFC, and metal chelating assays, while the methanol extract showed a strong response to the ABTS assay. The results showed that *C. ambrosioides* L had stronger antibacterial and antioxidant activity, which corroborates with our current findings.[44]

In our study, we observed the enzyme inhibitory activity of CAE against different enzymes along with their respective standard inhibitors. The findings revealed a prominent difference in α -glucosidase activity in contrast to the standard drug, acarbose. Acetylcholinesterase activity was inhibited by CAE with the same capacity as its standard drug, galantamine. Inhibition of butyrylcholinesterase was observed by CAE with almost equivalent capacity of galantamine (Table 4). Phenolic compounds like vitamin C, gallic acid, quercetin, epigallocatechin gallate, ellagic acid, morin, rutin, and catechin were reported to exhibit strong antioxidant potential and linked to memory-improving and neuroprotective effects.[32] Another study investigated carvacrol and p-cymene, monoterpenes found in aromatic plants, for their potential enzyme inhibition and neuroprotective effects. Carvacrol showed significant inhibition of acetylcholinesterase ($IC_{50} = 3.8 \mu\text{g/mL}$) and butyrylcholinesterase ($IC_{50} = 32.7 \mu\text{g/mL}$), alongside anti- α -amylase activity ($IC_{50} = 171.2 \mu\text{g/mL}$). These compounds demonstrated promising in vitro enzyme inhibition and neuroprotective potential against oxidative stress.[45] The present findings concurred with earlier research indicating that phenolic compounds might be employed as enzyme inhibitors of α -glucosidase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE).

The prevalence of T2DM has increased over the past years, and experts predict that this trend will continue owing to obesity and sedentary lifestyles.[46] The rapid breakdown of oligosaccharides carried out by pancreatic α -amylase and α -glucosidases causes a rapid blood sugar increase, worsening hyperglycemia in T2DM, and inhibition of α -glucosidase can help control T2DM.[47, 48] After a two-week

treatment period, the crude leaf extract of *C. ambrosioides* (given at doses of 100–300 mg/kg body weight) significantly decreased blood glucose levels in mice exposed to both low-dose STZ and a high-fat diet. Furthermore, the root hexane extract displayed potential as an antidiabetic agent, demonstrating a substantial inhibition of α -amylase ($50.24 \pm 0.9\%$) at a concentration of 20 $\mu\text{g}/\text{m}$, reviewed in.^[49,50] In our study, CAE showed α -glucosidase inhibition IC₅₀ at $27.5 \pm 0.3 \text{ mg}/\mu\text{L}$ when compared to standard Acarbose $39.3 \pm 0.8 \text{ mg}/\mu\text{L}$; showing greater inhibition potential of CA extract (Table 4). Our results are in line with the above-mentioned studies which suggest CAE is a stronger candidate for glucose regulation which means better diabetic control.

Alteration in the enzyme activity, specifically acetylcholinesterase, which has been associated with a rise in reactive species in the neural system, might be responsible for the adverse effects of ambrosioides species. This change would promptly enhance the levels of antioxidant-producing enzymes like glutathione S-transferase, catalase, and the overall enzyme activity.^[51,52] However, the etiology of AD is uncertain, enhancing acetylcholine levels through AChE enzyme inhibition has been identified as the most successful therapeutic approach. The inhibition of AChE and BChE has emerged as a promising approach for the treatment of AD.^[53] Nevertheless, AChE inhibitory drugs like tacrine, rivastigmine, and donepezil come with specific adverse effects and are only effective in mild AD cases, and there are currently no medications available with BChE inhibitory activity.^[50] Therefore, it is essential to screen and find potential novel pharmaceuticals to battle neurodegradative diseases in which CAE might play a role as a potential herbal remedy for AD. In another study, Ahmed et al.^[54] used *in vitro* and *in vivo* methods to examine the antidiabetic, antioxidant, and anticholinesterase beneficial effects of *C. murale L.* extracts. They found that *C. murale* treatment significantly protected the brain of the STZ-induced diabetic rats compared to neuronal damage caused by oxidative stress and improved cognitive dysfunction in behavioral studies which is consistent with our results of this study.^[38] Nazir et al.^[54] investigated the antioxidant and cholinesterase inhibitory properties of *Rosa moschata* Herrm leaves and fruit methanolic extracts. Leaf extracts showed significantly higher anticholinesterase effects with IC₅₀ values of 40 $\mu\text{g}/\text{ml}$ (AChE) and 110 $\mu\text{g}/\text{ml}$ (BChE), along with strong DPPH (IC₅₀ = 360 $\mu\text{g}/\text{ml}$) and ABTS (IC₅₀ = 105 $\mu\text{g}/\text{ml}$) radical scavenging capabilities than fruit extracts. These findings are also in line with our findings and highlight the potential for neurodegenerative disorder treatment (Table 4).

Based on the discussions above, it can be suggested that CAE is a promising option for the inhibition of α -glucosidase, AChE, and BChE enzymes associated with T2DM and Alzheimer's disease, respectively. In addition to Gallic acid, Catechol, Caffeic acid, Ferulic acid, Quercetin, and Kaempferol other flavonoids, phenolics, flavones, and other phytochemicals might also present in Leaf extract from *C. ambrosioides*.^[35] Phytochemicals from different classes of compounds i.e., quercetin - a flavonoid, and chrysin - a flavone, from different plant extracts, also improve STZ-induced memory impairment by reducing the time spent in the target quadrant during the Morris water maze test and increasing the escape latency in the elevated plus maze, which is an example of amelioration of cognitive impairment.^[55-57] As per the above discussion, the inhibition of α -glucosidase, AChE, and BChE enzymes cannot be attributed to a single phytochemical alone suggesting a synergistic action of multiple compounds present within CAE may contribute to its full inhibitory potential. Therefore, CAE may offer a more comprehensive and potentially effective approach to addressing oxidative stress and chronic disease management i.e., T2DM, and AD by targeting both of these enzymes simultaneously. Further, *in vivo* studies are suggested to better understand the specific mechanisms and compounds responsible for these inhibitory effects and to develop potential CAE-based T2DM and AD treatments.

CONCLUSION

The current investigation into *C. ambrosioides* extract (CAE) revealed a comparable potential in inhibiting α -glucosidase, AChE, and BChE enzymes linked to type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD). The presence of various phytochemicals, including flavonoids and phenolics, in CAE, suggests a collective action contributing to its inhibitory effects. This synergy hints at CAE's comprehensive approach to managing oxidative stress and chronic conditions like T2DM and AD by targeting multiple enzymes simultaneously. However, further *in vivo* studies are suggested to find active pharmaceutical compounds and elucidate specific molecular mechanisms responsible for these effects, paving the way for potential CAE-based treatments for T2DM and AD management.

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Nil

Conflict of Interest

The authors declare that there is no conflict of interest relevant to this article.

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