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ORIGINAL ARTICLE

Phenolic composition, antioxidant and enzyme inhibitory activities of *Parkia biglobosa* (Jacq.) Benth., *Tithonia diversifolia* (Hemsl) A. Gray, and *Crossopteryx febrifuga* (Afzel.) Benth

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Abstract Medicinal plants from Chad grow under special climatic conditions in between the equatorial forest of Central Africa and the desert of North Africa and are understudied. Three medicinal plants from Chad (*T. diversifolia, P. Biglobosa* and *C. Febrifuga*) were evaluated for their phenolic composition, antioxidant and enzyme inhibition activities. The total phenolic composition varied from 203.19 ± 0.58 mg GAE/g DW in the ethyl acetate extract of *P. biglobosa*, to 56.41 ± 0.89 mg GAE/g DW in the methanol extract of *C. febrifuga* while the total flavonoid content varied from 51.85 ± 0.91 mg QE/g DW in the methanol extract of *P. biglobosa* to 08.56 ± 0.25 mg

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α-Glucosidase inhibition; Antidiabetic activity

QE/g DW in the methanol extract of *C. febrifuga*. HPLC-DAD revealed that rutin, gallic acid and protocatechuic acid were the most abundant phenolics in *T. diversifolia, P. Biglobosa* and *C. Febrifuga* respectively. The antioxidant activity assayed by five different methods revealed very good activity especially in the $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and CUPRAC assays where the extracts were more active than the standard compounds used. Good inhibition was exhibited against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with methanol (IC_{50}: 15.63 ± 0.72 μg/mL), ethyl acetate (IC_{50}: 16.20 ± 0.67 μg/mL) extracts of *P. biglobosa*, and methanol (IC_{50}: 21.53 ± 0.65 μg/mL) and ethyl acetate (IC_{50}: 30.81 ± 0.48 μg/mL) extracts of *T. diversifolia* showing higher inhibition than galantamine (IC_{50}: 42.20 ± 0.44 μg/mL) against BChE. Equally, good inhibition was shown on α-amylase and α-glucosidase. On the α-glucosidase, the ethyl acetate (IC_{50} = 12.47 ± 0.61 μg/mL) and methanol extracts (IC_{50} = 16.51 ± 0.18 μg/mL) of *P. biglobosa* showed higher activity compared to the standard acarbose (IC_{50} = 17.35 ± 0.71 μg/mL) and on α-amylase, the ethyl acetate (IC_{50} = 13.50 ± 0.90 μg/mL) and methanol (IC_{50} = 18.12 ± 0.33 μg/mL) extracts of *P. biglobosa* showed higher activity compared to acarbose (IC_{50} = 23.84 ± 0.25 μg/mL). The results indicate that these plants are good sources of antioxidant phenolics and can be used to manage oxidative stress linked illnesses such as Alzheimer's disease and diabetes.

1. Introduction

Reactive oxygen species (ROS) are at the origin of many human diseases and health complications and this occurs as a result of an imbalance between ROS generation and antioxidants (disequilibrium between generation of oxidants and antioxidants) in the body that spreads over most cell targets (Talla et al., 2014; Talla et al., 2017; Forman and Zhang, 2021). This unfavorable imbalance in pro-oxidant and antioxidant creates dangerous reactive nitrogen and oxygen species such as nitric oxide, hydroxyl radicals, superoxide anion, organic and hydrogen peroxides which are notorious for cellular damage with undesirable effects on metabolic enzymes and lipids, proteins and DNA (Hassan et al., 2017). This effect of overproduction of free radicals can cause disorders such as diabetes, atherosclerosis, myocardial infarction, cardiovascular diseases, arthritis, post-ischemic injury, aging, inflammatory disorders, neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, Huntington's disorder), pancreatic and liver diseases in humans due to the oxidative damage on biomolecules by the radicals and oxidative species (Fang et al., 2002; Uttara et al., 2009; Hassan et al., 2017). However, ROS also play important role as cell signaling molecules for usual physiological processes but only in situations of disproportion between generation of ROS and body's ability of detoxification that they are harmful and this leads to oxidative stress (Asraoui et al., 2021). Some free radicals are useful, and the body has several enzymatic mechanisms of antioxidant action which helps in maintaining a delicate balance and equilibrium between antioxidants and oxidants such as removal, suppression or inactivation of ROS and up-regulation of antioxidant defenses (Talla et al., 2014; Sharifi-Rad et al., 2020). Antioxidants are exogenous or endogenous compounds from natural or synthetic origin, capable of removing free radicals, scavenging and inhibiting formation of ROS and their precursors and chelating metals which intervene in the catalysis of ROS generation (Gilgun-Sherki et al., 2001). As mentioned earlier, oxidative stress involves several pathological conditions such as obesity, hypertension and atherosclerosis besides enzymatic disorders notably neurodegenerative diseases like Alzheimer's disease (AD) and diabetes (Liguori et al., 2018; Forman and Zhang, 2021). One of the most common types of dementia is the Alzheimer's disease (AD) characterized by oxidative stress, progressive degeneration of neurons and is commonly accompanied by observable deposits of amyloid-β in the brain and low levels of acetylcholine (Rahman and Choudhary, 2015; Tamfu et al., 2019). The unusual deposits within the central nervous system of β-sheets of amyloid plaques has a strong correlation with memory loss and dementia, and contributes to decrease in the uptake or release of choline thereby affecting cholinergic neurotransmission (Breijyeh and Karaman, 2020). Acetylcholine and butyrylcholine are usually hydrolyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) respectively, and this leads to the decrease in the neurotransmitter levels in cholinergic synapses within certain regions of the brain and therefore the inhibition of both AChE and BChE is a beneficial therapeutic strategy to remedy AD (Uddin et al., 2021; Birsan et al., 2021). AD, dementia, cognitive impairment resulting from increasing amyloid-beta accumulation and neuroinflammation can also be induced by oxidative stress and type 2 diabetes (impaired glucose tolerance) and hyperglycemia (Breijyeh and Karaman, 2020). Since acute hyperglycemia occurs in all diabetic patients, a major strategy to remedy diabetic conditions and achieve normal and effective blood glucose levels is to control postprandial hyperglycemia by slowing down the absorption of carbohydrate hydrolysis and uptake using voglibose, miglitol and acarbose which work by inhibiting digestive enzymes (Nguelefack et al., 2020). The most probable therapeutic strategy to reduce post-prandial glucose levels is by delaying glucose absorption through the inhibition of α-amylase and α-glucosidase which are major enzymes responsible for the hydrolysis of oligosaccharides and disaccharides into monosaccharides (Salar et al., 2017).

Polyphenols and flavonoids function as chemical entities that prevents ROS induced oxidative damage and equally capable of reducing postprandial hyperglycemia by inhibiting the enzymes involved in carbohydrate hydrolysis (Ibrahima et al., 2017). Medicinal plants extracts have been a valuable source of natural antioxidants and have demonstrated their usefulness as

medicinal supplements and alternative drugs that can prevent or protect the body against oxidative stress diseases and also as food additives by preventing lipid peroxidation and most recently, in the green synthetic process of metallic nanomaterials (Flieger et al., 2021). Plants provide sustainable, safer and more effective antioxidant alternatives mostly attributed to flavonoids, phenolic acids, tannins, stilbenes, and anthocyanins and these phenolic compounds are employed as a remarkable strategy to reduce pathological conditions such as diabetes, cardiovascular diseases, aging, cancer, and neurodegenerative disorders (Yu et al., 2021).

Tithonia diversifolia (Hemsl) A. Gray, also known as Mexican sunflower or tree marigold, is distributed in Africa, Asia, Central and South America (Hiransai et al., 2016). *Tithonia diversifolia* has been used for treatment of malaria, parasitic infections, fever, diarrhea, dermatitis, hepatitis in traditional medicine (Maregesi et al., 2007; Hiransai et al., 2016). Previous reports showed that *Tithonia diversifolia* contains alkaloids, tannins, flavonoids and saponins (Olayinka et al., 2015; Obayomi et al., 2021). In previous reports, antidiabetic, antiplasmodial, cytotoxic and antioxidant activities of *T. diversifolia* have been reported (Goffin et al., 2002; Miura et al., 2005; Hiransai et al., 2016). *Parkia biglobosa* (Jacq.) Benth., is commonly found in Western African Region and known as 'African locust bean'. *P. biglobosa* has been used traditionally for various medicinal purposes such as, antidiabetic, anti-hypertensive, anti-inflammatory, wound healing, antihelmintic, etc. (Musara et al., 2020). Previous reports indicated that *P. biglobosa* is rich in phytochemicals including saponins, tannins, flavonoids, terpenoids, alkaloids, cardiac glycosides and coumarins (Yakubu et al., 2021). Antidiabetic, antimalarial, antioxidant, antibacterial, antihypertensive, antiinflammatory, anti-carcinogenic, and analgesic activities of *P. biglobosa* have been reported in earlier studies (Kouadio et al., 2000; Lamien-Meda et al., 2008; Builders et al., 2011; Musara et al., 2020; Oyedemi et al., 2021). *Crossopteryx febrifuga* (Afzel.) Benth. is largely distributed in West to Central and East Africa. It is used in traditional and indigenous medicine in African in treating inflammatory diseases, fever, malaria, diarrhoea and dysentery (Chouna et al., 2014). Reports of phytochemical studies show that *C. febrifuga* contains saponins and flavonoids (Kayangar et al., 2019). Also, analgesic, anti-inflammatory, antipyretic, hypoglycemic, hypolipidemic, antimicrobial, antimalarial and antiplasmodial potential of C. *febrifuga* have been reported (Elufioye and Agbedahunsi (2004); Salawu et al., 2008; Halilu et al., 2012; Ojewale et al., 2013).

As a contribution to the search of natural and alternative medicines to cure oxidative damage related ailments, precisely Alzheimer's disease and diabetes, three medicinal plants from Chad were investigated for their potential activity using bioassays. Phenolic extracts were prepared from *Parkia biglobosa* (Jacq.) Benth., *Tithonia diversifolia* (Hemsl) A. Gray, and *Crossopteryx febrifuga* (Afzel.) Benth and evaluated for their antioxidant, acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase and α-glucosidase inhibitory activites.

2. Experimental

2.1. Plant material and extraction

The plants were collected during the month of October 2020 from Moundou locality, Lac Wey Division, Logone Occidental Region of Chad. The plants *T. diversifolia, P. biglobosa* and *C. febrifuga* were identified and voucher specimens prepared by the botanist Mr. Baroua Abouna of the Farcha Laboratory where they were deposited under the specimen numbers 1762, 1386 and 010 for *T. diversifolia, P. biglobosa*, and *C. febrifuga* respectively. The plant parts, stems for *T. diversifolia,* and stem barks for *P. biglobosa* and *C. febrifuga* were collected, cut into pieces, dried and then ground into powder. 200 g of each powdered plant material was extracted with 70% ethanol/water (v/v) solution. The supernatant was filtered and the solvent evaporated on a rotavapor. This was performed repeatedly three times to obtain a crude paste of extract for each plant. The crude extract was dissolved in water and re-extracted using liquid–liquid extraction with ethyl acetate and then methanol in order of increasing polarity to yield the ethyl acetate extract and the methanol extract of each plant. These extracts were freeze dried and stored at 4 °C prior to analyses.

2.2. Determination of total phenolic and total flavonoid contents

Folin-Ciocalteu reagent was employed in the determination of the total phenolic content (TPC) of the extracts according to the method described previously (Chandra et al., 2014) with little modifications. 0.2 mL of test sample was added to 0.6 mL of distilled water and 0.2 mL of Folin-Ciocalteu's reagent (1:1). The mixture was stirred for 5 min after which 1 mL of saturated Na_2CO_3 solution (8% w/v in H_2O) was added and the final volume made up to 3 mL using distilled water. The reaction mixture from each sample was incubated in the absence of light for 30 min giving rise to a blue colored mixture which was subjected to centrifuging and its absorbance of was measured at 765 nm. Gallic acid was used to establish a standard curve from which the phenolic content was deduced as gallic acid equivalents GAE/g of dry plant material. Each experiment was done in triplicate.

The $AlCl_3$ colorimetric method was used in determining the total flavonoids content (TFC) of the extracts as described elsewhere (Chandra et al., 2014) with minor modifications. Quercetin standard was used in establishing the calibration curve. 5.0 mg of quercetin was dissolved in 1.0 mL methanol as a stock solution from which pure methanol was added in different amounts to give serial dilutions (5–200 μg/mL). 0.6 mL of 2% $AlCl_3$ was added separately to 0.6 mL diluted standard quercetin solutions or extracts and mixed and the resulting solution incubated at room temperature for 1 h after which the absorbance of each mixture was read against a blank at a wavelength of 420 nm. The calibration curve was used to deduce the total flavonoid content in each test sample expressed as mg quercetin equivalent (QE)/g of dried plant material. Each experiment was done in triplicate.

2.3. Determination of HPLC–DAD phenolic profiles

The phenolic compounds in the plant extracts were detected and quantified using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with diode array detector (DAD) as described previously (Çayan et al., 2020; Tamfu et al., 2021a). Briefly, known weights of each extract were dissolved in water:methanol (80:20) then filtered on sterile 0.20 μm disposable filter disk for liquid chro-

matography disk and an Intertsil ODS-3 reverse phase C18 column was used for the separation employing a 1.0 mL/min solvent flow rate and 20 µL injection volume. Two mobile phases A (0.5% acetic acid H_2O) and B (0.5% acetic acid in CH_3OH). A gradient elution was applied as follows: 0–10% B (0–0.01 min); 10–20% B (0.01–5 min); 20–30% B (5–15 min); 30–50% B (15–25 min); 50–65% B (25–30 min); 65–75% B (30–40 min); 75–90% B (40–50 min) 90–10% B (50–55 min). A photodiode array detector set at 280 nm wavelength was employed in the detection and the UV data together with retention times were compared with authentic standards. Each analysis was performed three times. A calibration plot established through the elution of known concentrations (0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0 ppm) of authentic compounds was used in the identification and quantification of the constituent phenolic compounds. 26 phenolic standards (gallic, *p*-hydroxy benzoic, protocatechuic, ellagic, chlorogenic, *trans*-cinnamic, 3-hydroxy benzoic, vanillic, syringic, *p*-coumaric, rosmarinic and ferulic acids; catechin, kaempferol, hesperetin, pyrocatechol vanillin, 6,7-dihydroxy coumarin, coumarin, rutin, myricetin, chrysin, luteolin, apigenin taxifolin and quercetin) were used. The results were expressed as µg per g dry weight of extract.

2.4. Antioxidant activity

Five different methods namely: $DPPH^{\bullet}$ (2,2-diphenyl-1-picryl hydrazylhydrazyl) radical scavenging assay, $ABTS^{\bullet+}$ (2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) radical cation, CUPRAC (cupric reducing antioxidant capacity), metal chelation and β-carotene-linoleic acid assays were used to measure the antioxidant potential of the plant extracts. Inhibition of lipid peroxidation was evaluated using the β-carotene-linoleic acid assay as decribed in a previous study (Tel-Çayan and Duru, 2019). Radical scavenging potentials on $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ were measure by spectrophotometric means as previously described (Tel et al., 2012; Tamfu et al., 2020b). CUPRAC was determined in accordance to a method described elswhere (Apak et al., 2004). In the above assays, α-tocopherol and BHA (Butylated Hydroxyanisole) were employed as antioxidant standards against which the activities of the extracts were compared. EDTA was used as standard in the metal chelation assay performed on Fe^{2+} (Decker and Welch, 1990).

2.5. Anticholinesterase activity

Cholinesterases (AChE and BChE) inhibitory activity was measured using the Ellman's method with minor changes (Ellman et al., 1961; Tamfu et al., 2020a). Two cholinesterases, AChE and BChE from electric eel and horse serum respectively were used, and acetyl- and butyryl thiocholine iodides were employed as substrates. Cholinesterase inhibitory activity was assayed using DTNB (5,5′-Dithio-bis(2-nitrobenzoic)acid) in which the reference compound used was galantamine. 96 well plates were used for the tests and the absorbances were measured on a microplate reader (SpectraMax, Molecular Devices, California, USA). The results were given as 50% inhibitory concentration (IC_{50}).

2.6. Anti-tyrosinase activity

The inhibition of tyrosinase enzyme (mushroom source) was determined through the method published elsewhere (Masuda et al., 2005) using L-DOPA as the substrate. In a 96-well microplate, 10 µL solution of sample or kojic acid (reference) were added to 150 µL of sodium phosphate buffer (pH 6.8, 100 mM) followed by 20 µL of tyrosinase enzyme and the resulting mixture incubated at 37 °C for 10 min. 20 µL of L-DOPA were added after incubation and the absorbances of the resulting solutions were taken at 475 nm and the results expressed as concentration at which 50% inhibition was observed (IC_{50}).

2.7. In vitro α-amylase inhibition assay

The 3,5-dinitrosalicylic acid (DNSA) method was used to evaluate α-amylase inhibition (Wickramaratne et al., 2016) with little modifications. Each sample was dissolved in minimal amounts of 10% DMSO and diluted in buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) to obtain 6.25, 12.5, 25, 50 and 100 µg/mL. 200 µL of α-amylase (2 units/mL) were added to 200 µL of extract or acarbose and the mixture incubated 30 °C for10 minutes after which 200 µL of starch (1% in water (w/v)) were added and further incubated for 3 min. 200 µL DNSA reagent (12 g of sodium potassium tartratetetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 Mm of 3.5-dinitrosalicylic acid solution) were added and boiled about 85–90 °C for 10 min in a water bath after which the resulting mixture was cooled and diluted using 5 mL of distilled water. The absorbance was read at 540 nm. The control experiment was that in which acarbose was used. The percentage inhibition of α-amylase inhibition was calculated according to the equation given below and IC_{50} values were also determined.

$$\%\text{inhibition of } \alpha-\text{amylase} = \frac{Abs\ \ control - Abs\ \ sample}{Abs\ \ control} X100$$

2.8. In vitro α-Glucosidase inhibition assay

The inhibition of α-Glucosidase was assayed using a previously described procedure (Chokki et al., 2020) with minor changes. To 20 µL sodium phosphate buffer (pH 5.0) in a 96-well plate was added 20 µL of p-nitrophenyl-β-D-glucopyranoside (1 mg/mL) and 10 µL of extract or acarbose at different concentrations (6.25, 12.5, 25, 50 and 100 µg/mL in DMSO) and the mixture incubated for 10 min at 37 °C after which 10 µL of α-Glucosidase (from almonds) solution (5 mg/mL) were introduced into each well and further incubated for 30 min at 37 °C. 140 µL of sodium carbonate buffer pH = 10 was added to quench the reaction and absorbance was read at 410 nm using a microplate reader (iTecan Microplate reader). The system without α-glucosidase was used as blank. The percentage of inhibition of α-glucosidase was deduced according to the equation below as well as the IC_{50}.

$$\%\text{inhibition of } \alpha-\text{Glucosidase} = 100X\left(1 - \frac{ODsample - ODblank/sample}{ODcontrol - ODblank}\right)$$

2.9. Statistical analysis

Each measurement and absorbance for bioassays was done in triplicate. The results were recorded as the means ± standard

error of the mean for three parallel measurements. Statistical analysis was performed with MINITAB 16 and ANOVA (analysis of variance) procedure was employed to determine the significant differences between means and the level of $p < 0.05$ were regarded as significant.

3. Results

3.1. Total phenolic and flavonoid contents

Phenolic and flavonoids compounds are very important classes of bioactive constituents that occur in plants and are famous for their antioxidant activity. Phenolic content measured with the use of Folin-Ciocalteu reagent and gallic acid (0–250 μg/mL) and expressed in terms milligrams of gallic acid equivalent per gram dry weight of extract (GAE/g DW) are shown on Table 1. The total phenolic composition varied from 203.19 ± 0.58 mg GAE/g DW in the ethyl acetate extract of *P. biglobosa*, to 56.41 ± 0.89 mg GAE/g DW in the methanol extract of *C. febrifuga*. For *T. diversifolia,* TPC were 112.16 ± 0.33 and 105.74 ± 0.25 mg GAE/g DW respectively for ethylacetate extract and MeOH extracts. In *P. biglobosa*, 203.19 ± 0.58 and 158.07 ± 1.34 mg GAE/g DW in the ethyl acetate and MeOH extracts respectively and in the *C. febrifuga* TPC was 69.82 ± 0.65 and 56.41 ± 0.89 mg GAE/g DW respectively in the ethyl acetate and MeOH extracts. Colorimetric method was used with quercetin as standard to evaluate the total flavonoid content (TFC) of the plant extracts and the results reported in milligrams of quercetin equivalents per gram dry extract weight (mg QE/g DW) on Table 1. The TFC varied from 51.85 ± 0.91 mg QE/g DW in the methanol extract of *P. biglobosa* to 08.56 ± 0.25 mg QE/g DW in the methanol extract of *C. febrifuga*. Significant variation ($p < 0.05$) in the total content of phenolic and flavonoids was observed between the different plants extracts.

3.2. HPLC-DAD phenolic composition

Target phenolic constituents of extracts were evaluated and reported as μg/g extract using HPLC-DAD (Table 2). Out of the twenty-six standard and authentic phenolic compounds used in the analyses, ten phenolic compounds were detected in ethyl acetate extract of *T. diversifolia,* seven in methanol extract of *T. diversifolia*, ten in ethyl acetate and butanol extracts of *P. biglobosa*, eight in extracts of *C. febrifuga*. The chemical structures of the component compounds detected and quantified by HPLC-DAD are presented on Fig. 1. Amongst the detected compounds, rutin (113.2 ± 1.25 μg/g) was the most abundant compound in the ethyl acetate extract of the *T. diversifolia*, followed by ferulic acid (35.84 ± 0.29 μg/g) and caffeic acid (32.51 ± 0.40 μg/g). Rutin (13.84 ± 0.63 μg/g), coumarin (11.24 ± 0.45 μg/g) and apigenin (10.61 ± 0.52) were found as the most dominant phenolic compounds in the methanol extract of *T. diversifolia*. Gallic acid was revealed as the major phenolic constituent compound of ethyl acetate (65.10 ± 0.61 μg/g) and methanol (23.85 ± 0.49 μg/g) extracts of *P. biglobosa*. The other major compounds were found as *p*-coumaric acid (16.83 ± 0.33 μg/g) in ethyl acetate extract of *P. biglobosa* and ferulic acid (15.28 ± 0.37 μg/g) in the methanol extract of *P. biglobosa*. Protocatechuic acid was the most abundant compound in ethyl acetate (36.78 ± 0.24 μg/g) and butanol (9.84 ± 0.31 μg/g) extracts of *C. febrifuga*. Gallic acid, caffeic acid, ferulic acid and rutin were identified in all studied extracts with different amounts.

Table 1 Total phenolic content (TPC) and total flavonoid content (TFC) of extracts.

Plant	Extract	TPC (mg GAE/g dry extract weight)	TFC (mg QE/g dry extract weight)
T. diversifolia	AcOEt	112.16 ± 0.33	36.10 ± 0.58
	MeOH	105.74 ± 0.25	25.49 ± 0.31
P. biglobosa	AcOEt	203.19 ± 0.58	51.85 ± 0.91
	MeOH	158.07 ± 1.34	44.27 ± 1.03
C. febrifuga	AcOET	69.82 ± 0.65	19.08 ± 0.71
	MeOH	56.41 ± 0.89	08.56 ± 0.25

Values expressed are means ± S.E.M. of three parallel measurements ($p < 0.05$).

3.3. Antioxidant activity

The results of the antioxidant effects of the ethyl acetate and MeOH extracts of *T. diversifolia, P. biglobosa* and *C. Febrifuga,* measured according to the β-carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{+\bullet}$, CUPRAC and metal chelation models are presented on Table 3. Inhibition of lipid peroxidation capacity of the extracts was determined by β-carotene-linoleic acid assay. The ethyl acetate and methanol extracts of *P. biglobosa* exhibited the strongest inhibition activity of lipid peroxidation with IC_{50} values of 4.05 ± 0.15 and 4.46 ± 0.18 μg/mL, respectively. Also, ethyl acetate and MeOH extracts of *T. diversifolia* exhibited good activities with IC_{50} values of 9.05 ± 0.34 and 12.42 ± 0.25 μg/mL, respectively in β-carotene-linoleic acid assay while extracts of *C. febrifuga* were shown to possess low activity when compared to the other tested extracts.

The ability of the extracts to scavenge radical species was performed using a radical cation $ABTS^{\bullet+}$ and radical $DPPH^{\bullet}$. In scavenging assay on $DPPH^{\bullet}$, the ethyl acetate and MeOH extracts of *P. biglobosa* revealed the best scavenging capacity with IC_{50} values of 5.15 ± 0.20 and 5.83 ± 0.14 μg/mL, respectively when the compared to the standards α-tocopherol (IC_{50}: 38.70 ± 0.25 μg/mL) and BHA (IC_{50}: 19.70 ± 0.20 μg/mL). Also, Ethyl acetate extract of *T. diversifolia* possessed better activity than reference compounds with IC_{50} value of 16.51 ± 0.15 μg/mL. Similar results were observed in $ABTS^{\bullet+}$ assay where the ethyl acetate, MeOH extracts of *P. biglobosa* and ethyl acetate extract of *T. diversifolia* exhibited better activity than reference compounds with IC_{50} values of 3.45 ± 0.19, 4.18 ± 0.27 and 9.74 ± 0.21 μg/mL, respectively.

The Cu^{2+} reduction ability of the plant extracts was evaluated via the CUPRAC assay. Ethyl acetate, MeOH extracts of *P. biglobosa* and *T. diversifolia* were found to possess higher potent activity when the compared BHA (IC_{50}: 25.17 ± 0.01) and α-tocopherol (IC_{50}: 85.41 ± 0.01) with IC_{50} values of

Table 2 Phenolic composition of the extracts determined by HPLC-DAD (μg/g)[a].

No	Phenolic compounds	RT (min)	*T. diversifolia* AcOEt extract	*T. diversifolia* MeOH extract	*P. biglobosa* AcOEt extract	*P. biglobosa* MeOH extract	*C. febrifuga* AcOEt extract	*C. febrifuga* MeOH extract
1	Gallic acid	5.70	20.44 ± 0.48	6.58 ± 0.21	65.10 ± 0.61	23.85 ± 0.49	9.67 ± 0.35	6.33 ± 0.20
2	Protocatechuic acid	8.85	7.15 ± 0.27	–	8.58 ± 0.32	5.51 ± 0.27	36.78 ± 0.24	9.84 ± 0.31
3	Catechin	10.68	6.79 ± 0.31	–	4.42 ± 0.24	8.68 ± 0.41	1.78 ± 0.17	–
4	Pyrocatechol	11.04	6.92 ± 0.25	–	–	–	–	–
5	Chlorogenic acid	12.35	6.57 ± 0.42	–	3.43 ± 0.36	5.78 ± 0.20	4.65 ± 0.49	–
6	p-hydroxy benzoic acid	12.77	-[b]	–	–	–	–	–
7	6.7-Dihydroxy coumarin	14.10	–	–	–	–	–	–
8	Caffeic acid	15.09	32.51 ± 0.40	4.24 ± 0.15	5.86 ± 0.29	5.52 ± 0.33	15.38 ± 0.41	5.88 ± 0.36
9	3- hydroxy benzoic acid	15.98	–	–	–	–	–	–
10	Syringic acid	16.56	–	–	–	–	–	–
11	Vanillin	17.78	–	–	–	–	–	–
12	p-Coumaric acid	20.56	7.35 ± 0.18	–	16.83 ± 0.33	9.31 ± 0.52	–	–
13	Taxifolin	21.26	–	–	8.73 ± 0.21	6.41 ± 0.45	–	–
14	Ferulic acid	22.14	35.84 ± 0.29	3.49 ± 0.22	8.53 ± 0.16	15.28 ± 0.37	5.36 ± 0.14	2.58 ± 0.24
15	Coumarin	24.49	–	11.24 ± 0.45	–	–	–	–
16	Rutin	25.30	113.2 ± 1.25	13.84 ± 0.63	7.82 ± 0.25	4.95 ± 0.42	8.66 ± 0.37	2.04 ± 0.18
17	Ellagic acid	26.11	–	–	5.45 ± 0.31	6.33 ± 0.38	–	–
18	Rosmarinic acid	26.77	–	–	–	–	6.75 ± 0.25	3.74 ± 0.42
19	Myricetin	27.35	–	–	–	–	–	–
20	Quercetin	30.83	–	–	–	–	–	–
21	trans-cinnamic acid	31.34	–	–	–	–	–	–
22	Luteolin	31.70	–	–	–	–	–	–
23	Hesperetin	32.14	–	7.86 ± 0.41	–	–	–	–
24	Kaempferol	33.21	–	–	–	–	–	–
25	Apigenin	33.57	12.49 ± 0.35	10.61 ± 0.52	–	–	–	–
26	Chrysin	38.43	–	–	–	–	–	–

[a] Values expressed are means ± S.E.M. of three parallel measurements ($p < 0.05$). [b] -: not detected.

5.80 ± 0.30, 6.21 ± 0.42, 13.37 ± 0.16 and 19.69 ± 0.35 μg/mL, respectively

In metal chelating assay, ethyl acetate extract of *P. biglobosa* and *T. diversifolia* displayed highest activity amongst the tested extracts with IC_{50} values of 41.70 ± 1.22 and 45.61 ± 1.05 μg/mL, respectively

3.4. Anticholinesterase activity

Cholinesterases (AChE and BChE) are the major enzymes responsible for the hydrolysis of acetylcholine (ACh), thereby reducing the levels of Ach which is necessary for neurotransmission and causing neurodegenerative disorders notably AD. Therefore inhibition of these enzymes is beneficial as remedy to AD. The AChE and BChE inhibitory activity of *T. diversifolia, P. biglobosa* and *C. febrifuga* ethyl acetate extracts and MeOH extracts were evaluated and reported on Table 4. The MeOH and ethyl acetate extracts of *P. biglobosa* exhibited the strongest AChE and BChE activities amongst all the tested extracts. The methanol (IC_{50}: 15.63 ± 0.72 μg/mL), ethyl acetate (IC_{50}: 16.20 ± 0.67 μg/mL) extracts of *P. biglobosa*, methanol (IC_{50}: 21.53 ± 0.65 μg/mL) and ethyl acetate (IC_{50}: 30.81 ± 0.48 μg/mL) extracts of *T. diversifolia* showed higher activity than galantamine (IC_{50}: 42.20 ± 0.44 μg/mL) against BChE. Similarly, MeOH and ethyl acetate extracts of *P. biglobosa* and methanol extract of *T. diversifolia* displayed highest activities against AChE with IC_{50} values of 20.43 ± 0.81, 23.85 ± 0.46 and 28.75 ± 0.92 μg/mL, respectively.

3.5. Anti-tyrosinase activity

The antityrosinase activity of *T. diversifolia, P. biglobosa* and *C. febrifuga* ethyl acetate extracts and MeOH extracts are shown on Table 4. The MeOH and ethyl acetate extracts of *P. biglobosa* showed significant tyrosinase inhibitory activities with IC_{50} values of 51.28 ± 0.77 and 76.34 ± 1.24 μg/mL respectively while kojic acid had IC_{50} value of 23.75 ± 0.24 μg/mL. Extracts *of T. diversifolia* and *C. febrifuga* exhibited lower inhibitions against tyrosinase when the compared the extracts of *P. biglobosa* and the reference compound kojic acid used in the study.

3.6. α-amylase and α-glucosidase inhibitory activities

The α-amylase as well as α-glucosidase are key enzymes found in the digestive system which intervene in the hydrolysis of carbohydrate to glucose and inhibiting these enzymes will reduce the amounts of glucose that enters the body. The ethyl acetate and MeOH extracts of *T. diversifolia, P. biglobosa* and *C. febrifuga* were evaluated for α-amylase as well as α-glucosidase

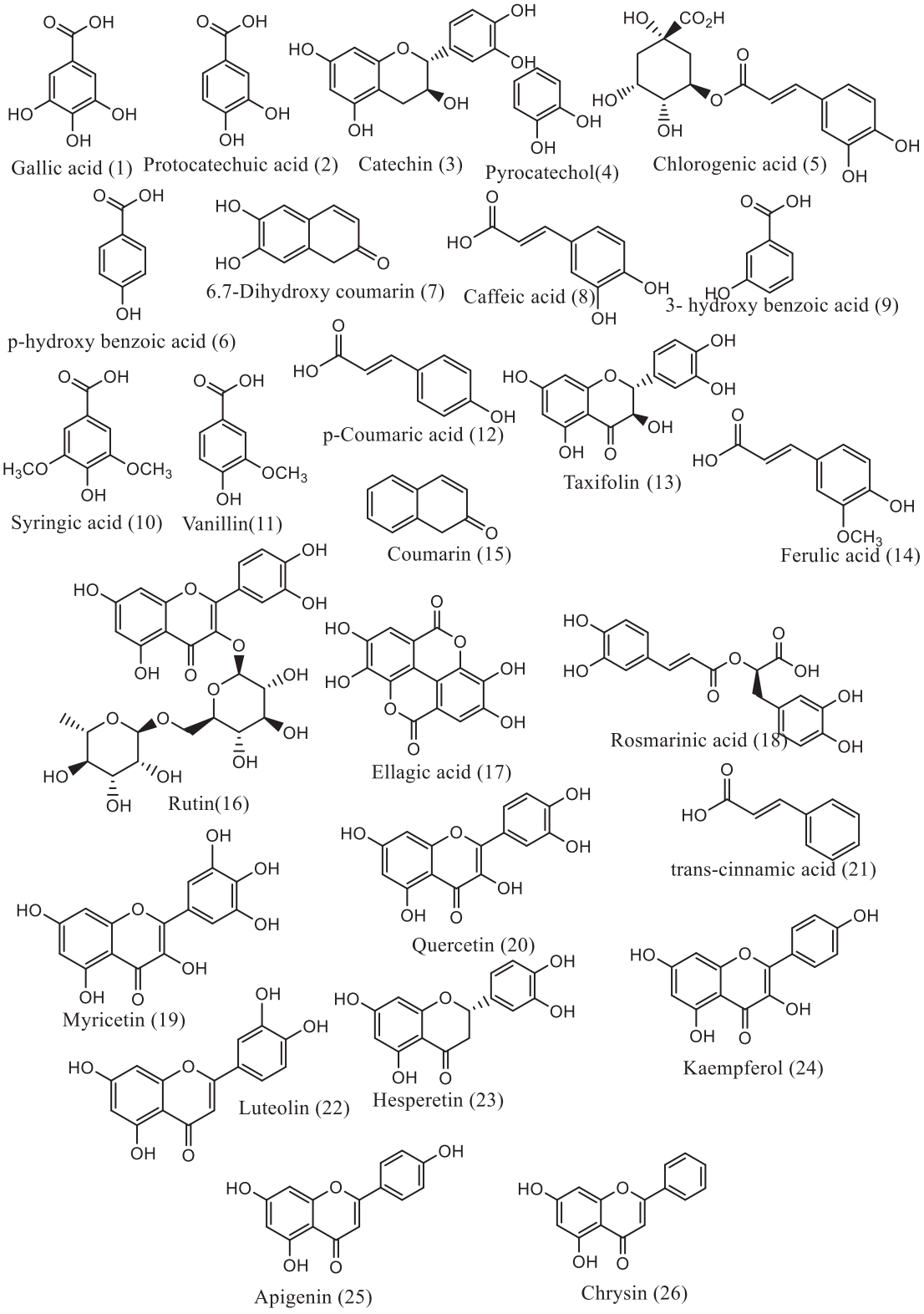

Fig. 1 Structures of identified phenolic compounds in the plant extracts.

inhibitory ability and the results are presented on Table 4. On the α-glucosidase inhibition, the ethyl acetate and MeOH extracts of *P. biglobosa* exhibited greater activity with IC_{50} values of 12.47 ± 0.61 µg/mL and 16.51 ± 0.18 µg/mL when compared to the standard acarbose whose IC_{50} value was 17.35 ± 0.71 µg/mL. For *T. diversifolia,* the ethyl acetate

Table 3 Antioxidant activity of the extracts.

Antioxidant activity						
Plants	Extracts	β-carotene-linoleic acid assay IC_{50} (μg/mL)[a]	DPPH• assay IC_{50} (μg/mL)	$ABTS^{\bullet+}$ assay IC_{50} (μg/mL)	CUPRAC assay $A_{0.5}$ (μg/mL)[b]	Metal chelating assay IC_{50} (μg/mL)
T. diversifolia	AcOEt	9.05 ± 0.34	16.51 ± 0.15	9.74 ± 0.21	13.37 ± 0.16	45.61 ± 1.05
	MeOH	12.42 ± 0.25	25.80 ± 0.21	15.40 ± 0.28	19.69 ± 0.35	68.21 ± 1.36
P. biglobosa	AcOEt	4.05 ± 0.15	5.15 ± 0.20	3.45 ± 0.19	5.80 ± 0.30	41.70 ± 1.22
	MeOH	4.46 ± 0.18	5.83 ± 0.14	4.18 ± 0.27	6.21 ± 0.42	53.25 ± 1.48
C. febrifuga	AcOEt	43.57 ± 0.35	37.52 ± 0.51	28.47 ± 0.74	35.41 ± 0.88	> 100
	MeOH	70.25 ± 0.49	96.44 ± 0.89	65.61 ± 1.32	79.33 ± 1.15	> 100
Standards	BHA[c]	1.45 ± 0.02	19.70 ± 0.20	12.80 ± 0.50	25.17 ± 0.01	*NT*
	α-tocopherol[c]	2.23 ± 0.03	38.70 ± 0.25	34.50 ± 0.47	85.41 ± 0.01	*NT*
	EDTA[c]	*NT*	*NT*	*NT*	*NT*	5.45 ± 0.65

[a] IC_{50} values represent the means ± SEM of three parallel measurements ($p < 0.05$).
[b] : $A_{0.50}$ values represent the means ± SEM of three parallel measurements ($p < 0.05$).
[c] :Reference compounds, NT: not tested.

extract (IC_{50} = 26.30 ± 1.21 μg/mL) and the MeOH extract (IC_{50} = 24.59 ± 0.73 μg/mL) equally showed good activity while the extracts of *C. febrifuga* had moderate activity. On the α-amylase, the ethyl acetate and MeOH extracts of *P. biglobosa* exhibited better activity with IC_{50} values of 13.50 ± 0.90 μg/mL and 18.12 ± 0.33 μg/mL with respect to the standard acarbose whose IC_{50} value was 23.84 ± 0.25 μg/mL. For *T. diversifolia,* the the MeOH extract (IC_{50} = 28.35 ± 0.25 μg/mL) and the ethyl acetate extract (IC_{50} = 31.22 ± 0.41 μg/mL) equally showed good activity and the ethyl extract of *C. febrifuga* had moderate inhibitory potential with IC_{50} of 91.58 ± 2.10 μg/mL.

4. Discussion

Phenolic compounds are ubiquitous in plant kingdom and chemically have a single or multiple aromatic rings with at least a hydroxyl group directly bonded to the aromatic ring.

Table 4 Cholinesterase, tyrosinase, α-amylase and α-glucosidase inhibitory activities of the extracts.

		Cholinesterase inhibition									
		AChE		BChE		Tyrosinase inhibition		α-amylase inhibition		α-glucosidase inhibition	
Plants	Extracts	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)[a]	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)
T. diversifolia	AcOEt	70.48 ± 0.87	46.43 ± 0.77	78.91 ± 0.68	30.81 ± 0.48	22.46 ± 0.31	> 100	70.90 ± 0.84	31.22 ± 0.41	77.52 ± 1.80	26.30 ± 1.21
	MeOH	80.21 ± 1.46	28.75 ± 0.92	82.07 ± 1.55	21.53 ± 0.65	15.62 ± 0.79	> 100	76.63 ± 1.10	28.35 ± 0.25	83.65 ± 1.25	24.59 ± 0.73
P. biglobosa	AcOEt	81.35 ± 1.15	23.85 ± 0.46	84.11 ± 1.44	16.20 ± 0.67	65.39 ± 0.97	76.34 ± 1.24	87.73 ± 1.52	13.50 ± 0.90	91.31 ± 0.79	12.47 ± 0.61
	MeOH	82.58 ± 0.89	20.43 ± 0.81	84.63 ± 1.02	15.63 ± 0.72	70.22 ± 1.30	51.28 ± 0.77	85.91 ± 0.26	18.12 ± 0.33	88.74 ± 0.16	16.51 ± 0.18
C. febrifuga	AcOEt	20.72 ± 0.60	> 100	58.81 ± 0.75	82.31 ± 0.94	14.74 ± 0.87	> 100	69.44 ± 1.73	91.58 ± 2.10	72.38 ± 0.42	84.21 ± 1.75
	MeOH	17.63 ± 1.34	> 100	32.17 ± 0.43	> 100	8.51 ± 0.74	> 100	45.31 ± 0.11	> 100	55.64 ± 1.11	93.10 ± 0.29
Standards	Galantamine	85.40 ± 0.52	5.50 ± 0.20	74.63 ± 0.25	42.20 ± 0.44	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*
	Kojic acid	*NT*	*NT*	*NT*	*NT*	79.48 ± 0.32	23.75 ± 0.24	*NT*	*NT*	*NT*	*NT*
	Acarbose	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	82.59 ± 1.10	23.84 ± 0.25	88.14 ± 0.43	17.35 ± 0.71

Values (inhibition % and IC_{50}) represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
NT: not tested.

Phenolic compounds possess interesting bioactivities, according to many scientific studies and more research is still needed on them so as to understand their main actions in organisms and improve their bioavailability, sustainable extraction methods, refine modification, and procedures of stability to increase the fields of application (Albuquerque et al., 2021). Flavonoids constitute one of the largest groups of phenolic metabolites that has attracted great attention for research (Su et al., 2021). Ethyl acetate and methanol were used in the extraction because it is generally believed that solvents of high polarity easily dissolve and remove phenolic compounds from plant material and hence phenolic constituents are easily extractable in these solvents (Alara et al., 2021). TPC and TFC from three medicinal plants *T. diversifolia, P. biglobosa* and *C. febrifuga* showed variation in composition between the different species which might be explained from a genetic perspective. Within the same plant species, the amounts varied between the ethyl acetate and methanol solvents indicating that phenolic compounds and flavonoids in the plants have different affinities for methanol and ethyl acetate. In *P. biglobosa* the ethyl acetate extract had higher TPC than the methanol extract meanwhile the methanol extract had higher TFC than the ethyl acetate extract. The TPC and TFC in this study are higher those reported for *P. biglobosa* collected from Burkina Faso (Windmi et al., 2021). In another study, TPC and TFC were found to be 225.2 ± 18.25 mg GAE/g and 99.28 ± 12.3 mg QE/g respectively (Oyedemi et al., 2021) and these values confirm the high contents determined in this study. As reported in this study, for *P. biglobosa* the ethyl acetate extract had lesser phenolic content than the methanol extract and a similar trend was reported in another study in which ethanol extract had higher TPC than the ethyl acetate extract (Ibrahim et al., 2013). For *T. diversifolia* and *C. febrifuga* the ethyl acetate extracts had more TPC and TFC than their corresponding methanol extracts. Similar trend was also observed for *T. diversifolia* collected from Phillipines where TPC was found to be 15.20 mg GAE/g dry sample and the TFC where 12.50 mg QE/g dry sample (Aileen et al., 2019). Our contents were higher than these but lower than those detected in *T. diversifolia* from Nigeria which showed TPC of 251.63 mg. g^{-1} GAE and TFC of 98.21 mg. g^{-1} QE (Ojo et al., 2020). The TPC and TFC were low in *C. febrifuga* and probably because this plant is usually rich in triterpene and saponins instead (Kayangar et al., 2019). The low amounts of phenolic compounds and flavonoids detected in this work for *C. febrifuga* is in agreement with the works of some researchers who determined low contents of phenolics and flavonoids in this plant (Ouedraogo, et al., 2019).

The detection and quantification of specific phenolic compounds were carried out using HPLC-DAD against 26 standard phenolic compounds. Since HPLC-DAD analysis can provide precisely useful information about the nature of individual compounds, it is more advantageous over total phenolics content as determined by the Folin-Ciocalteu method (Komolafe et al., 2014). In the *T. diversifolia,* ten compounds were detected in the ethyl acetate extract while seven compounds were identified and subsequently quantified in the methanol extracts. Our results corroborates with some studies in which RP-HPLC in which this method was used to identify phenolic compounds in *T. diversifolia* previously (da Costa Inácio et al., 2020) and most especially, some identified major compounds were detected in *T. diversifolia* collected from Nigeria notably phenolic acids and flavonoids like p-coumaric, gallic, caffeic and chlorogenic acids and apigenin (Ojo et al., 2020) some of which are also identified and quantified in our extracts, though in different amounts. Phenolic compounds and flavonoids have also been reported in *T. diversifolia* using UPLC/MS (Omokhua et al., 2018). Flavonoids and phenolic compounds are known to be contained in this plant as one of the major bioactive constituents (Bouberte et al., 2006; Ambrósio et al., 2008; Pantoja Pulido et al., 2017; Chagas-Paula et al., 2011). In *P. biglobosa*, ten compounds were identified and quantified in both ethyl acetate and methanol extracts. The phenolic compounds identified in our study corroborates well with one previous study in which gallic acid, quercetin, catechin, rutin, chlorogenic acid, epigallocatechin, epigallocatechin gallate, caffeic acid and kaempferol were identified and quantified in *P. biglobosa* (Komolafe et al., 2017). Previously, phenolic compounds such as quercetin, caffeic acid, gallic acid, rutin, catechin and epigallocatechin have been equally identified in the extract from *P. biglobosa* stem bark using an HPLC analysis (Oyedemi et al., 2021). Bioactive compounds including phenolic compounds such as trans-ferulic acid and 4-O-methyl-epi-gallocatechin have been isolated from the stem bark (Tringali et al., 2000). The identified phenolic compounds in this study therefore are in agreement with the reports in literature. In *C. febrifuga*, a total of eight and six compounds were identified and quantified in the ethyl acetate and methanol extracts respectively. Previously, some phenolic compounds were reported in *C. febrifuga* using GC-MS (Nma et al., 2018). This supports the identification of phenolics in this study. However, the class of phenolic compounds that have been isolated from *C. febrifuga* are mostly flavonoids (Tomás-Barberán and Hostettmann, 1988).

There is an increasing interest and attention towards medicinal plant and their phenolic content and those rich in phenolic compounds because of their beneficial therapeutic applications. The most famous bioactivity of phenolic compounds is antioxidant activity and capacity to prevent or reduce oxidative stress associated diseases. After determining the TPC and TFC of the different plant extracts, their antioxidant capacities were determined using five. In the β-carotene-linoleic acid assay, no extract was more active than the standards BHA and α-tocopherol but the activities of *T. diversifolia* and *P. biglobosa* were good as their IC_{50} values remained close to those of standards while the activity of *C. febrifuga* was low. For the radical scavenging of $DPPH^{\bullet}$, the ethyl acetate and MeOH extracts of *P. biglobosa* and equally the ethyl acetate extract of *T. diversifolia* were more active than the standard compounds α-tocopherol and BHA used. In the CUPRAC and $ABTS^{\bullet+}$ assays, all extracts of *T. diversifolia* and *P. biglobosa* were more active than the standards α-tocopherol and BHA as their IC_{50} values were lower than those of the standards used. For *C. febrifuga* only the ethyl acetate extract was more active than α-tocopherol which was used as one of the standards but BHA was more active than this extract. In the metal chelating assay, the activity of the extracts were low to moderate and the standard used was EDTA and it was more active than all the extracts. The high antioxidant activity observed in this study can confirm the one reported in another study in which extracts of *T. diversifolia* exhibited competitive activity with the standard compounds in the capacity to scavenge free radicals and this was attributed

to the phenolic compounds present in the extracts since the phenolic compounds possess hydrogen donating ability which helps to reduce the radicals (Ojo et al., 2010; Hiransai et al., 2016). In another study, n-butanol and ethyl acetate extracts of *T. diversifolia* were shown to possess good antioxidant activity in DPPH and metal chelating assays (Pulido et al., 2017). It was shown through DPPH scavenging mechanism of free radicals that antioxidant activity of *T. diversifolia* could reduce oxidative stress and improve cytoprotection (Juang et al., 2014). *T. diversifolia* has exhibited properties that are important in health and which are attributable to both its free radical scavenging capacity and ability to induce cellular protective systems involved in stress defences in the cells (Di Giacomo et al., 2015). The antioxidant activity is in agreement with the findings reported elsewhere in which the extracts of *P. biglobosa* showed better antioxidant activities that the standards trolox and quercetin used in the assay (Windmi et al., 2021). This high antioxidant activity of *P. biglobosa* is attributable to its phenolic and flavonoid contents since these classes of compounds are known to confer antioxidant activity on plant extracts. The results in this study agrees with some work in which *P. biglobosa* showed significant inhibition of lipid peroxidation, reactive oxygen species and scavenging capacities on $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (Komolafe et al., 2014; Oyedemi et al., 2021). Various extracts of *P. biglobosa* possess promising antioxidative properties in several experiments demonstrated as free radical scavengers, electron donors and nitric oxide inhibitors and the stem bark extract as well as the ethanol extracts of leaves and roots exhibited the most powerful activity (Ibrahim et al., 2013). *P. biglobosa* can therefore be used as antioxidant substance in living systems to overcome free radical species and consequently reducing the risk of ROS related ailments such as cancer, arthritis and diabetes (Nwahujor et al., 2011). Antioxidant activity was also exhibited by *C. febrifuga* although its activity was moderate with respect to other tested plant extracts and higher than that of α-tocopherol in the CUPRAC, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. In some study, the extract of *C. febrifuga* showed higher antioxidant activity than quercetin though with relatively low phenolic and flavonoid contents for the extracts (Ouedraogo et al., 2019).

The plant extracts showed good inhibition on AChE and BChE except the methanol extract of *C. febrifuga*. In both AChE and BChE inhibitory assays, the activity of each extract was higher in the BChE than in the AChE inhibition. No extract was more active than galantamine in the AChE assay but the ethyl acetate and methanol extracts of *T. diversifolia* and *P. biglobosa* were more active than galantamine in the BChE assay. The anticholinesterase activity observed here is in accordance with previous experimental report in which extracts of *T. diversifolia* showed higher BChE and AChE inhibitory activities than the drug reference prostigmine used in the assay and the activity was attributed to high amounts of caffeic and chlorogenic acids and this was considered a promising mechanism for the treatment of AD (Ojo et al., 2018). In another study, BChE and AChE inhibition of *T. diversifolia* were good but lower than our results and AChE inhibition had IC_{50} of 73.9 ± 11.06 μg/mL attributable to terpenes and sesquiterpene lactones found in *T. diversifolia* (Pantoja-Pulido et al., 2020). Though reports on the anticholinesterase activities of this plant are scarce, it has been shown to possess oxidative stress and to have effect on some illnesses related to the central nervous system and many enzymes that intervene in neuro-metabolic processes (Saleh et al., 2021; Oyedemi et al., 2021). *P. biglobosa* extracts exhibited good AChE and BChE inhibitions. However, in one study, the inhibition of cerebral acetylcholinesterases by phenolic extracts of *P. biglobosa* were found to be low compared to the standard (Ramipril) used in the assay (Komolafe et al., 2017). *C. febrifuga* extracts showed no activity on AChE as the IC_{50} was not found within tested concentration range and only its ethyl acetate extract exhibited very weak inhibition on BChE. Although no previous works described anticholinesterase activity of *C. febrifuga*, it is one of the plants used traditionally as memory enhancer to manage neurodegenerative disorders like Alzheimer's disease and Parkinson disease (Babawale et al., 2016). Plant extracts particularly those rich in phenolic compounds and flavonoids possess good inhibition of angiotensin-converting enzymes including AChE and BChE and the plausible mechanism is the generation of chelating substrates capable of forming complexes within the active sites of the angiotensin-converting enzymes thereby inactivating them (Guerrero et al., 2012; Oboh et al., 2014; Komolafe et al., 2017). Acetylcholine is known to be involved in many physiological processes in the brain and is necessary in neurotransmission but the enzymes AChE and BChE reduce its levels in the brain leading to neurological problems such as loss of memory. Therefore, simultaneous inhibition of both BChE and AChE will reduce the rate of the hydrolysis of acetylcholine and therefore is an effective strategy for the improvement of the pathological conditions in Alzheimer's disease patients (Khoobi et al., 2013). Amongst the plant extracts tested for anti-tyrosinase activity, only the extracts of *P. biglobosa* showed activity, though weak when compared to the standard thiourea and the methanol extract was more potent than the ethyl acetate extract. Phenolic compounds are amongst the good cholinesterase inhibitors and therefore estracts which are rich in phenolic compounds can possess justified AChE and BChE inhibitory activities (Tamfu et al., 2021b). Inhibitors of tyrosinase constitute an attraction to the cosmetic industry because they can find applications as agents of depigmentation and also in the food industry where they are used for enzymatic browning and phenolic compounds are considered as being capable of mimicking the tyrosinase substrate and competitively inhibiting the enzyme as well (Zolghadri et al., 2019).

Inhibition of α-glucosidase and α-amylase is a widely accepted approach for controlling levels of glucose found in the blood of diabetes mellitus patients (Quan et al., 2019). The plant extracts showed significant inhibitory activities against the enzymes α-amylase and α-glucosidase. Particularly, extracts of *P. biglobosa* exhibited higher activities than acarbose which used as reference compound against both enzymes. *P. biglobosa* is used in the management and treatment of diabetes in folk medicine by local people in African countries (Ekperikpe et al., 2019). According to Sunmonu and Lewu (2019), extract of *P. biglobosa* demonstrated strong α-amylase inhibitory activity. In another study, phenolic rich extract of *P. biglobosa* showed significant inhibitory potential against both enzymes (Ademiluyi and Oboh, 2012). The present study is in agreement previous reports and showed that great potential of extracts from *P. biglobosa* as antidiabetic agents. Also, on α-glucosidase and α-amylase, extracts of *T. diversifolia* possessed good inhibitory activity. Although, there is no study about α-glucosidase and α-amylase inhibition activities of *T. diversifolia*, antidiabetic potentials have been inves-

tigated in animal studies (Miura et al., 2005; Kadima et al., 2016; Fauziyah et al., 2018; Yazid et al., 2021). Extracts of *C. febrifuga* demonstrated relatively lower activities when the compared acarbose and other tested plant extracts. To the best of our knowledge, α-glucosidase and α-amylase potential inhibitory activities of extracts from *C. febrifuga* were investigated first time in this study. Plants extracts and compounds are possible alternatives for α-glucosidase and α-amylase inhibition and hence can find applications in combatting diabetes through their capacity of reducing blood glucose levels (Feunaing et al., 2021; Tchuente Djoko et al., 2021).

5. Conclusion

Though reactive oxygen species can have some physiological relevance in the body, their over-production can lead to tissue damage and oxidative stress whose ultimate effect can lead to some neurodegenerative diseases and diabetes amongst others. The body has mechanisms of maintaining a healthy balance between oxidants and antioxidants but oxidative stress occurs as a result of an imbalance between the pro-oxidants and antioxidants. Several antioxidants from sources external to the organism can restore the balance and they are believed to produce beneficial health effects in terms of disease prevention especially diseases that result from or are linked to oxidative stress. Phenolic compounds are one of the most powerful and commonly used antioxidants and they are widely distributed in plants. Medicinal plants provide a cheap, safe and effective source of bioactive phenolic compounds whose extraction, characterization and application have become a subject of intense research. In this study, ethylacetate and methanol extracts of three plants *T. diversifolia, P. biglobosa* and *C. febrifuga* collected from Chad showed high contents of phenolic and flavonoids and certain target phenolic compounds were identified and quantified by HPLC-DAD method. The plants displayed good antioxidant activity assayed through five different methods with the extracts of *P. biglobosa* showing good activity. The anticholinesterase activity results revealed good activity of *T. diversifolia* and *P. biglobosa* extracts indicating that these plants could be used to remedy Alzheimer's disease. The capacity of these plant extracts to inhibit the key enzymes, α-amylase and α-glucosidase, which are responsible for hydrolyses of starch to glucose shows that the plants can be used to reduce postprandial blood glucose levels and manage diabetic conditions. This study ascertains the usage of these medicinal plants popularly used in African traditional medicine in the treatment of various diseases and also in other cultures in developing countries where modern medicine is inaccessible to a large population. Proper and documented knowledge on chemical composition and bioactivities of medicinal plants is very crucial for the lives of the 80% of the world's population who rely on them for their health care.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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