Research Article

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Bioactive agents from *Parkia biglobosa* (Jacq.) R.Br. ex G. Don bark extracts for health promotion and nutraceutical uses

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Abstract

BACKGROUND: *Parkia biglobosa* stem bark extracts were prepared using methanol, methanol 80%, water and ethyl acetate to investigate their phytochemical contents, as well as antioxidant and enzyme inhibitory properties.

RESULTS: Liquid chromatography (LC) quadrupole time-of-flight mass spectrometry (MS) and LC-MS^n revealed the presence of flavonoids, hydroxycinnamic acid derivatives and gallotannins. Particularly, the water extract contained rutin (480 μg per 100 mg) and 3-caffeoylquinic acid (1109 μg per 100 mg) in higher amounts, whereas the 80% methanol extract contains methoxyluteolin-7-*O*-rutinoside and catechin derivatives as major compounds. Total phenolic and flavonoid contents of the extracts were yielded in the range of 32.26–119.88 mg gallic acid equivalents g^{-1} and 0.60–2.39 mg rutin equivalents g^{-1}, respectively. Total antioxidant capacity was also displayed in the range of 0.53–6.34 mmol Trolox equivalents (TE) g^{-1}. Both the methanolic extracts showed higher total antioxidant capacity that could be related to the total phenolic contents. Radical scavenging capacity in DPPH (2,2-diphenyl-2-picryl-hydrazyl) (37.21–508.30 mg TE g^{-1}) and ABTS [2,2-azinobis(3-ethylbenzothiazoline- 6-sulfonic acid)] (60.95–1068.06 mg TE g^{-1}) assays, reducing power in cupric ion reducing antioxidant capacity (54.23–1002.78 mg TE g^{-1}) and ferric ion reducing antioxidant power (33.18–558.68 mg TE g^{-1}) assays, as well as metal chelating activity (2.45–11.28 mg EDTA equivalents g^{-1}), were exhibited by all extracts. All extracts were found to inhibit acetylcholinesterase [0.23–2.47 mg galanthamine equivalents (GALAE) g^{-1}], tyrosinase [27.20–83.33 mg kojic acid equivalents g^{-1}], amylase [mmol acarbose equivalents (ACAE) g^{-1}]. On the other hand, all extracts, except the water extract, inhibited butyrylcholinesterase (5.38–6.56 mg GALAE g^{-1}), whereas only the water and ethyl acetate extract showed glucosidase inhibitory potential (1.96 and 1.82 mmol ACAE g^{-1}). In general, the water extract was found to be a weaker enzyme inhibitor suggesting that water is not the preferrable extraction solvent to obtain active products.

CONCLUSION: The present study demonstrated that the stem bark extracts of *P. biglobosa* contains good amount of phytochemical and extracts present significant antioxidant, as well as reasonable enzyme inhibitory effects. Hence, these findings suggest that further studies can be performed on more specific biological targets and models of bioactivity to determine their safe usage as a nutraceutical or for the preparation functional foods.

Supporting information may be found in the online version of this article.

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Keywords: *Parkia biglobosa*; polyphenolic contents; antioxidant; antienzyme

INTRODUCTION

The plant kingdom has been an excellent source of bioactive compounds and drugs as demonstrated by the numerous registered medicinal compounds that have been indirectly or directly derived from them.[1] Furthermore, in more recent years, the attention of the scientist has been also dedicated to food plants because of their health promoting properties, and also the edible species have been successfully studied as sources of important bioactive compounds. Many examples have been published such as herbal teas for hyperuricemia,[2] herbal products containing phenols, terpenes and other phytochemical for obesity and diabetes,[3] or plants containing saturated fatty acid useful for cosmeceutical applications.[4] Also, some significant studies have considered the role as antioxidants of specific compounds as glucosinolates in *Brassica* species[5] and, in the recent years, new extraction approaches have been proposed,[6] allowing the valorization of plant materials. Another good example can be observed from a berry fruit source of powerful health promoting compounds.[7] In this regard, a large part of the plant biodiversity is still unexplored and many vegetables and plant foosd have still not been investigated with respect to their chemical composition and health promoting properties.

In African countries especially, *Parkia biglobosa*, also referred to as the African locust bean, a perennial tree belonging to the Fabaceae family, displays important socio-economic, cultural and medicinal values. Moreover, it is a food species for which the significance is well established.[8] Phytochemical investigations of *P. biglobosa* have elucidated the presence of tannins, saponins, flavonoids, resins, terpenoids, phenols, sterols, carbohydrates and cardiac glycosides. Indeed, the pharmacological and nutritional virtues of *P. biglobosa* have been related to its physicochemical properties.[9] The plant is a versatile tree legume found in many African countries. The seeds, pulp and leaves are used to prepare numerous foods and drinks, as well as to feed livestock and poultry.[10]

Different parts (roots, stem bark and leaves) of the plant have also been utilized in various forms (decoction, paste and infusion) for a variety of conditions such as dental disorder, infertility, diarrhea, leprosy, hypertension and malaria, and are also used as antisnake venom, for wound healing and against fever and hepatitis in African countries such as the Ivory coast, Nigeria Gambia and Senegal.[11-13] Along with their renowned status in traditional medicine, their actual pharmacological effects validated by a panoply of studies have also been widely elaborated. For exampel, it has been found to possess analgesic, anti-inflammatory, antimicrobial, antiepileptogenic, anti-amnesic and anxiolytic-like activities.[14-16] Additionally, phenolic compounds were found to be the major antimalarial components of *P. biglobosa* and have shown antimalarial activity against *Plasmodium berghei* and clinical isolate of *Plasmodium falciparum*.[17] Furthemore, extracts of fermented seeds of *P. biglobosa* were reported to exert a hypoglycaemic effect, thus demonstrating an antidiabetic effect.[18]

Therefore, given its well-established reputation in African traditional medicine, further investigations of this plant can help to unveil other interesting chemical and biological properties that could aid to elaborate more on its pharmacological potency. Hence, the present study aimed to determine the chemical characterization and assess the biological properties of four extracts of *P. biglobosa* stem bark.

MATERIALS AND METHODS

Plant materials and extraction

Stem bark of the plants (*Parkia biglobosa* (Jacq.) R.Br. ex G. Don) was collected in Yamoussoukro City of Ivory Coast in the summer season of 2020. The plant was identified by one botanist (Dr Ouattara Katinan Etienne). Voucher specimens were deposited at the herbarium in the Selcuk University.

For the extraction of compounds with different polarities from plant extracts, four solvents (ethyl acetate, methanol, methanol/water (80%) and water) were utilized. A maceration technique was selected for ethyl acetate, methanol and methanol/water extracts and, for this purpose, plant materials (10 g) were stirred with the 200 mL of methanol for 24 h at room temperature. After that, the mixtures were filtered using Whatman filter paper (Cytivia, Marlborough, MA, USA) and the solvents were removed using rotary-evaporator. Regarding water extraction, the extract was prepared as a traditional infusion and the plant materials (10 g) were kept in the boiled water (200 mL) for 15 min. Then, the mixture was filtered and lyophilized for 48 h. All extracts were stored at 4 °C until analysis.

Profile of bioactive compounds

Folin–Ciocalteu and $AlCl_3$ assays, respectively, were utilized to determine the total phenolic and flavonoid contents.[19] For respective assays, the results were expressed as gallic acid equivalents (mg GAEs g^{-1} extract) and rutin equivalents (mg REs g^{-1} extract).

LC-DAD-MS^n and LC-HR-QTOF

To establish the composition of the plant extract data obtained coupling multiple stage mass spectrometry (MS^n) and liquid chromatography high-resolution quadrupole-time-of-flight (LC-HR-QTOF) measurements were combined. The extracts were dissolved in dimethyl sulfoxide, preparing a concentrated starting solution of 50 mg mL^{-1}, then samples were diluted 1:10 with water. In volumetric flask, solutions were centrifuged at 16,000 x g prior to transfer to 2-mL vials for chromatographic analysis.

As equipment, an Agilent 1260 (Agilent Technologies, Santa Clara, CA, USA) chromatography system comprising an autosampler, column oven, diode array detector and quaternary pump system was used. A 'T' connector was used to exactly divide the flow coming out from column in two, with one part connected to diode array detector (DAD) to collect UV-visible spectra and chromatographic traces, and the other fixed to the electrospray ionization (ESI) source of an ion trap mass spectrometer (model 500 MS; Varian, Palo Alto, CA, USA). The spectrometer was operating in negative ion mode. Spectra were acquired in the range *m/z* 100–2000 using the turbo data depending scanning (tdds) function, which allows the recording of the fragmentation of the most abundant ionic species. MS parameters were: needle voltage: 4800 V, capillary voltage: 70 V, drying gas pressure: 30 psi,

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nebulizer pressure:35 psi, drying gas temperature: 250 °C, nebulizer temperature: 250 °C. The quantification of compounds was accomplished by utilizing specified reference standards for each classified component. Chlorogenic acid was used as reference standard for the hydroxycinnamic derivatives; benzoic acid was used for the protocathecuic acid derivatives; phosphatidilethanolamine was used for the lipids and phospholipids; triolein was used for triglyceride; 7-hydroxy-coumarin was used for coumarins; rutin was used for the flavonoids; catechin, epicatechin, gallocatechin and epigallocatechin were used for the specific compounds, procyanidin B2 was used for the procyanidins. The calibration curves were obtained for the reference compounds and all sample runs and reference compound runs were obtained in triplicate. Calibration curves revealed that the method for the considered reference compounds present linearity. The SD calculated on the retention time of the detected reference compounds demonstrates the accuracy of the method. The curves were as follows for flavonoid: rutin (detected in MS, negative ion mode, and in DAD at 350 nm, linear response from 100 to 0.5 μg mL^{-1}, $y = 41\,418\,x + 12$) LOQ = 1.0 μg mL^{-1} LOD = 0.30 μg mL^{-1}; chlorogenic acid (detected in MS, negative ion mode, and in DAD at 330 nm, linear response from 100 to 0.5 μg mL^{-1}, $y = 29\,319\,x + 33$) LOQ = 0.9 μg mL^{-1}, LOD = 0.25 μg mL^{-1}; epigallocatechin gallate (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from 120 to 0.3 μg mL^{-1}, $y = 8136\,x + 9$) LOQ = 0.9 μg mL^{-1}, LOD = 0.3 μg mL^{-1}; catechin (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from 100 to 1.0 μg mL^{-1}, $y = 4536\,x + 12$) LOQ = 0.9 μg mL^{-1}, LOD = 0.3 μg mL^{-1}; epicatechin (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from 100 to 1.0 μg mL^{-1}, $y = 4916\,x + 8$) LOQ = 0.9 μg mL^{-1}, LOD = 0.3 μg mL^{-1}; benzoic acid (detected in MS, negative ion mode, and in DAD at 254 nm, linear response from 105 to 1.0 μg mL^{-1}, $y = 2403\,x + 13$) LOQ = 1.9 μg mL^{-1}, LOD = 0.6 μg mL^{-1}; procyanidin B2 (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from 150 to 1.0 μg mL^{-1}, $y = 3521\,x + 15$) LOQ = 1.5 μg mL^{-1}, LOD = 0.65 μg mL^{-1}; phosphatidyl ethanolamine (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from from 250 to 20 μg mL^{-1}, $y = 1205\,x + 31$) LOQ = 2.5 μg mL^{-1}, LOD = 1.0 μg mL^{-1}; triolein (detected in MS, negative ion mode, and in DAD at 280 nm, linear response from 250 to 2.5 μg mL^{-1}, $y = 542\,x + 14$) LOQ = 12.5 μg mL^{-1}, LOD = 3.0 μg mL^{-1}.

For the LC-QTOF analysis, a Waters Acquity UPLC system was coupled with a Xevo G2 QTOF (Waters Corp., Waters, Milford, MA, USA) mass spectrometer. As stationary phase, an Zorbax Eclipse Plus C18 (2.1 × 50 mm, 1.8 μm) column (Agilent Technologies) was used, and column temperature was maintained at 40 °C. A mixture of water + 1% formic acid (A) and methanol + 1% formic acid (B) was used as the mobile phase. The elution gradient was as follows: 0–1 min, 98% A; 11 min, 15% A; 16 min, 0% A; 20 min, 0% A; 21 min, 98% A; 24 min, 98% A. The flow rate was 0.3 mL min^{-1} and the injection volume was 2 μL. MS data were acquired in negative ionization mode (ESI–) in the mass range 50–2000 Da. The sampling cone voltage was adjusted at 40 V and the source offset at 80 V. The capillary voltage was adjusted to 3.5 kV. The nebulizer gas used was N_2 at a flow rate of 800 L h^{-1}. The desolvation temperature was 450 °C. The mass accuracy and reproducibility were maintained by infusing lock mass (leucine–enkephalin, $[M - H]^- = m/z$ 554.2620) thorough Lockspray at a flow rate of 20 μL min^{-1}. The *m/z* value of all acquired spectra was automatically corrected during acquisition based on lock mass. An MS^e experiment was simultaneously performed to collect structural information, setting the collision energy to 30 V.

For the identification of compounds, MS^n spectra and HR-MS^e spectra were acquired, and the results were compared with literature and available online databases [Human Metabolome Database (HMDB) (https://hmdb.ca) and Food Metabolome Database (FoodB) (https://foodb.ca)]. Furthermore, MS data were compared with those of reference compounds available in the laboratory or acquired by commercial sources. Chromatograms are given in the Supporting information (Figs S1–S4).

Determination of antioxidant and enzyme inhibitory effects

The antioxidant and enzyme inhibitory activity of comfrey root extracts was determined in accordance with previously described methods.[20] DPPH (2,2-diphenyl-2-picryl-hydrazyl) and ABTS [2,2-azinobis(3-ethylbenzothiazoline- 6-sulfonic acid)] radical scavenging activity, cupric ion reducing antioxidant capacity (CUPRAC) and ferric ion reducing antioxidant power (FRAP) were expressed as mg Trolox equivalents (TE)/g extract. The metal chelating ability (MCA) was reported as mg EDTA equivalents (EDTAE)/g extract, whereas the total antioxidant activity (phosphomolybdenum assay, PBD) was expressed as mmol TE g^{-1} extract. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were given as mg galanthamine equivalents (GALAE)/g extract; tyrosinase inhibitory activity was expressed as mg kojic acid equivalents (KAE)/g extract; amylase and glucosidase inhibitory activities were presented as mmol acarbose equivalents (ACAE)/g extract.

Molecular modeling

The crystal structures of human AChE (PDB ID: 6O52),[21] human BChE (PDB ID: 6EQP),[22] human pancreatic alpha-amylase (PDB ID: 1B2Y)[23] and were retrieved the protein data bank (PDB) (https://www.rcsb.org). *Priestia megaterium* tyrosinase (PDB ID: 6QXD)[24] and *Mus musculus* alpha-glucosidase (PDB ID: 7KBJ),[25] also retrieved from the PDB, were used to build the homology models of human tyrosine and glucosidase using their respective human sequences, UniProt IDs P14679 and P0DUB6. The details of the model building have been described previously.[26]

To prepare proteins for docking, the 'Playmolecule ProteinPrepare' module was used (https://playmolecule.com/proteinPrepare).[27] The pKa of titratable residues in each protein was predicted, which was then used to prepare the proteins at physiological pH of 7.4. The 3D structures of each ligand were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and optimized using Frog2.[28]

Docking grid files were generated based on the size of each protein and according to the binding (*x*, *y*, *z*) coordinates of the cocrystal ligand in each complex using AutoDockTools 1.5.6 and docking was done using AutoDock 4.2.6 (https://autodock.scripts.edu).[29] A similar docking protocol was applied previously.[30-32] The binding energy of each ligand pose was predicted and protein-ligand interaction was analyzed using Biovia DS Visualizer (Dassault Systèmes Biovia Software Inc., San Diego, CA, USA).

Statistical analysis

Data are presented as the mean ± SD of the number (n = 3) of replicates. A Kruskal–Wallis with Dunn post-hoc test was conducted. $P < 0.05$ was considered statistically significant. The statistical evaluation was performed using XLSTAT, version 2016

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(Addinsoft, Paris, France). The relationship between molecules and antioxidant activities as well as enzyme inhibitory activities was assessed by calculating the Pearson correlation coefficient. Afterwards, principal component analysis (PCA) was performed. Before analysis, chemical and bioactivities datasets were scaled. The statistical analysis was performed using R, version 4.1.2 (R Foundation, Vienna, Austria).

RESULTS AND DISCUSSION

Chemical profiling

Many medicinal plants are rich in antioxidants, mostly belonging to the chemical family of polyphenols. These compounds have various biological effects, such as anti-inflammatory, anti-atherosclerosis, antiaging and anticancer properties.[33] The effective extraction and suitable assessment of antioxidants from plants are crucial to explore their potential antioxidant sources and encourage their applications as functional foods, food additives and pharmaceuticals.[34,35]

Polar solvents are commonly used to recover polyphenols from plant matrices; the most suitable solvents being aqueous mixtures containing methanol, ethanol, ethyl acetate and acetone. In general, methanol has been found to be more efficient for extracting lower molecular weight polyphenols.[36] This research made use of methanol (100%), methanol (80%), water and ethyl acetate to measure any possible variation in the chemical composition of the extracted samples.

Parkia bark is renowned for its high levels of procyanidins and epigallocatechin derivatives,[37] and other compounds that can influence bioactivity and extraction operations. In the present study, samples from Ivory Coast were put through liquid chromatography to investigate the composition of the various extracts. HR-QTOF measurements and MS^n by ion trap multiple stage mass spectrometry fragmentation were employed to corroborate the data. The combination of the two datasets allowed identification of the presence of more than 30 different constituents that were then quantified using LC-DAD, and the results are summarized in Table 1.

At first, we could observe that the chemical composition of the Ivory cost Parkia bark is characterized by four classes of constituents. A series of hydroxycinnamic derivatives are observed in the extracts and HR-QTOF and MS^n fragmentation help to discriminate various compounds not only on the basis of the *m/z* values, but also on the basis of MS2 and MS3 data to discriminate isobaric species such as the 3,5 and 3,4 dicaffeoyl quinic acids.[38,39] Other esters are coumaroyl quinic esters and shikimic acid esters with caffeic acid and caffeoyl malic acid (Table 1). The bark extract contains mono-caffeoyl and mono-coumaroyl quinic acids, as well as 3,5 and 3,4 dicaffeoyl quinic acid. The most abundant compound is caffeoyl shikimic acid. Furthermore, several flavanol derivatives were identified such as catechin, epicatechin and some dimeric procyanidins. Compared to previous studies, a lower abundance of procyanidin was observed in this specific sample.[37] Nevertheless, dimeric epicatechin/gallocatechin as well as gallocatechin epigallocatechin dimers have been identified, as previously reported.[37] Furthermore, some flavonoid glycosides were also reported, showing difference composition compared to previous studies;[13] in our Ivory coast sample the most abundant are rutin and isorhamnetin glycosides. Other classes of compounds that have been identified are coumarins mainly as the glycosylated form. In the analysis of the extracts, we also observed phosphatidylethanolamine (PE) derivatives and PE is a major non-bilayer lipid that is widely distributed in extra-plastid membranes of plant cells. In the present study, the plant material was subjected to maceration with different solvents systems; namely, methanol, 80% methanol water, water and a lipophilic solvent ethyl acetate. In terms of extraction yields, the highest yield was found in 80% methanol (17.80%), followed by methanol (14.92%), water (8.17%) and ethyl acetate (1.22%).

The quali-quantitative results summarized in Table 1 allow evaluation of the different amounts of extracted compounds on the basis of the used solvents.

As second point, we can consider the amount of compounds extracted and grouped in four classes of constituents. As we can observe, the water and water methanol mixtures were more efficient in the extraction of compounds from plant materials.

This may reflect the ability of solvent to penetrate the plant material, and clearly suggests that most of the extracted compounds are typically polar rather than lipophilic. Considering the hydroxycinnamic derivatives, methanol 80% appears to be the more favorable solvent, whereas, for the coumarins and flavonoids, water extraction obtained the major content (Fig. 1).

There is large difference in the extraction yields of several compounds considering the different used solvents. As an example, dicaffeoyl quinic acid is efficiently extracted with water and to a lower extent with the other solvents. Surprisingly, rutin, a flavonoid glycoside is efficiently extracted in water but only poorly extracted in methanol and methanol 80 and, furthermore, is better extracted in ethyl acetate compared to the latter two. This suggest that the overall composition of the extract and possibly the structure of the bark tissue can influence the extraction efficiency. Taking into consideration only the solubility of pure compounds in the used extraction media, we could expect that a limited amount of rutin can be extracted because of the limited water solubility of rutin. Thus, the obtained data indicate that different behaviors can be observed as a result of the presence of other phytochemicals. The behavior in this case may be influenced by the phospholipids that have been tentatively identified; possibly, these compounds are able to act in water as surfactants increasing and improving the extraction of the phytochemicals; on the other hand, in alcohol-based solvents, this behavior is does not occurr and thus the extraction efficiency is reduced. In ethyl acetate, possibly the more lipophilic nature of the solvent is able to dissolve the surfactant because of their lipophilic nature and also in this case there is an increase in the extraction of compounds.

These results highlight the need to carefully evaluate the medium of extraction and also the constituents of plant material with relevant importance to those classes of compounds that can play a role in the modification of solubility of the phytoconstituents.

In the present study, spectrophotometric assays were also performed to determine the total phenolic and flavonoid contents. The extracts yielded total phenolic content (TPC) in the range of 32.26–119.88 mg GAE g^{-1}. The methanolic extracts were found to have better TPC yields compared to the other extracts. In particular, the water extract TPC yield was significantly lower relative to the methanolic extracts. On the other hand, the total flavonoid contents (TFC) of the extracts were yielded in much lower quantities compared to that of the TPC. The ethyl acetate and methanolic extracts yielded TFC 2.39 and 2.01 mg RE g^{-1}, respectively, whereas lower TFC yields were obtained for the water and methanolic (80%) extracts (0.65 and 0.60 mg RE g^{-1}, respectively) (Table 2). As for the total antioxidant capacity (TAC) determined by the phosphomolybdenum assay, the methanolic extracts

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Table 1. Compounds identified by LC-MS in studied extracts (mg per 100 g extract)

rt	*m/z*	Molecular formula	Fragments	Compound	MeOH	EA	Water	MeOH80
				Hydroxycinnamic acids				
8.37	353.0873	C16H17O9	191	3-CQA*	3.93 ± 0.11	4.78 ± 0.13	1108.92 ± 12.5	365.61 ± 2.10
8.82	353.0873	C16H17O9	191 179	5-CQA*	1.76 ± 0.06	2.14 ± 0.14	58.21 ± 0.43	1.86 ± 0.30
9.78	337.0908	C16H18O8	191	3-Coumaroyl quinic acid	2.29 ± 0.08	2.78 ± 0.13	78.48 ± 0.24	45.87 ± 0.90
10.01	337.0908	C16H18O8		Coumaroyl quinic acid isomer	2.60 ± 0.10	3.15 ± 0.18	156.11 ± 0.40	8.13 ± 0.30
10.2	295.0546	C13H11O8	163	Caffeoyl malic acid	26.46 ± 0.23	32.14 ± 0.41	7.76 ± 0.12	0.12 ± 0.03
11.6	335.0343	C16H15O8	179	Caffeoyl shikimic acid isomer 1	4.16 ± 0.11	5.05 ± 0.13	10.18 ± 0.51	6.00 ± 0.2
12.1	335.0343	C16H15O8	179	Caffeoyl shikimic acid isomer 2	97.50 ± 0.45	118.45 ± 0.85	10.20 ± 0.71	2.27 ± 0.10
12.2	515.1189	C25H23O12	353 191	3,5-DCQ*	0.98 ± 0.06	0.45 ± 0.06	292.64 ± 1.02	418.48 ± 0.90
12.47	515.1192	C25H23O12	353 191 173	3,4-DCQ*	0.88 ± 0.06	0.36 ± 0.08	236.81 ± 1.10	303.16 ± 1.04
				Total	141	170	1722	2875
				Other phenolics				
8.5	315.0732	C13H15O9		Protocatecuic acid hexoside	11.32 ± 0.11	12.50 ± 0.10	126.80 ± 0.90	128.20 ± 0.90
				Lipids and phospholipids				
13.58	273.1764	C14H25O5		3-Hydroxytetradecanedioic acid	16.50 ± 0.43	20.05 ± 0.22	11.5 ± 0.95	11.3 ± 0.75
25.5	295.2929	C20H40O		Eicosen-1-ol	18.96 ± 0.31	12.35 ± 0.12	151.47 ± 0.95	261.00 ± 0.93
16.5	329.2722	C19H38O4		MG (0:0/i-16:0/0:0)	53.72 ± 0.14	65.26 ± 0.66	855.80 ± 1.20	95.28 ± 1.85
15.8	201.0665	C11H21O3		Fatty acid derivative	33.42 ± 0.31	40.60 ± 0.31	280.29 ± 0.99	0.04 ± 0.03
13.03	187.0985	C9H15O4		Azelaic acid	10.2 ± 0.99	13.8 ± 0.87	0.55 ± 0.06	4.83 ± 0.21
13.4	273.1764	C14H26O5		3-Hydroxytetradecanedioic acid	16.09 ± 0.64	19.55 ± 0.34	0.56 ± 0.11	0.04 ± 0.02
19.8	670.4811	C37H69NO7P		Phosphatidylethanolamine derivative	9.00 ± 0.19	10.90 ± 0.11	35.26 ± 0.20	380.33 ± 0.90
20.4	670.4811	C37H69NO7P		Phosphatidylethanolamine derivative	19.45 ± 0.46	23.63 ± 0.13	109.06 ± 0.90	273.02 ± 0.60
21.6	670.4811	C37H69NO7P		Phosphatidylethanolamine derivative	52.60 ± 1.11	64.26 ± 0.16	6.43 ± 0.10	251.03 ± 0.91
14.9	267.233	C17H31O2		Heptadecenoic acid	6.79 ± 0.11	8.25 ± 0.12	0.71 ± 0.30	0.16 ± 0.05
19–23	999–1100			Triacylglycerol lipids	-	22 120 ± 25	—	-
				Total	237	277	1450	1412
				Coumarins				
11.9	323.0688	C15H16O8	177	7-Hydroxycoumarin-glucoside isomer	4.70 ± 0.11	5.70 ± 0.15	605.74 ± 1.25	134.61 ± 0.80
12.3	323.0688	C15H16O8	177	7-Hydroxycoumarin-7-glucoside	27.64 ± 0.23	33.58 ± 0.22	24.59 ± 0.81	45.19 ± 0.78
				Total	250	319	2068	1444
				Flavonols				
10.7	609.1455	C27H29O16	301 179 151	Rutin*	20.79 ± 0.21	25.26 ± 0.23	479.5 ± 41.0	719.36 ± 2.10
11.5	623.1631	C28H31O16	315 300 271 255	Isorhamnetin-3-*O*-rutinoside	8.53 ± 0.10	10.36 ± 0.13	198.05 ± 0.99	21.45 ± 0.23
11.82	607.1102	C28H31O15	299 284 256	Methoxyluteolin-7-*O*-rutinoside	9.26 ± 0.10	11.25 ± 0.35	659.90 ± 3.55	0.13 ± 0.03
11.92	593.12145	C30H26O13	467 441	Isorhamnetin-3-*O*-diglucoside	34.05 ± 0.31	41.37 ± 0.35	498.96 ± 1.75	148.06 ± 1.31
				Total	72	58	1835	889
				Flavan-3-ol derivatives				
11.7	593.1263	C30H25O13	467 441 289	Epicatechin-epigallocatechin	28.04 ± 0.34	34.06 ± 0.85	571.12 ± 1.22	161.78 ± 0.99
12.52	319.0816	C16H15O7		4′-Methyl-epigallocatechin	104.87 ± 1.05	127.40 ± 0.92	151.06 ± 1.01	21.30 ± 0.80

Table 1. Continued

rt	*m/z*	Molecular formula	Fragments	Compound	MeOH	EA	Water	MeOH80
12.8	289.0725	C15H13O7		Catechin	7.27 ± 0.11	8.83 ± 0.18	20.60 ± 0.34	1.10 ± 0.03
13.5	577.1352	C30H25O12	451 425 407 289	Procyanidin dimer B type epicatechin	16.12 ± 0.41	19.59 ± 0.30	46.90 ± 1.02	1.68 ± 0.30
14.6	305.1334	C15H13O7	331 305	Gallocatechin*	117.19 ± 0.85	142.36 ± 0.95	86.81 ± 0.61	1.11 ± 0.05
16.2	305.1334	C15H13O7	331 305	Epigallocatechin*	14.95 ± 0.11	18.16 ± 0.16	86.05 ± 0.56	1.22 ± 0.10
16.6	289.0725	C15H13O6		Epicatechin*	57.41 ± 0.31	69.75 ± 0.78	2.41 ± 0.10	1.05 ± 0.06
17.6	609.1328	C30H26O14	305	Gallocatechin (4–8)-epigallocatechin dimer 1	16.82 ± 0.15	20.43 ± 0.31	0.52 ± 0.11	0.12 ± 0.06
17.92	609.1328	C30H26O14	305	Gallocatechin (4–8)-epigallocatechin dimer 2	34.97 ± 0.19	42.48 ± 0.65	0.49 ± 0.10	0.07 ± 0.05
18	457.08125	C22H17O11	331 305 169	Epigallocatechin gallate	9.85 ± 0.11	11.97 ± 0.20	0.76 ± 0.10	0.15 ± 0.05
18.3	609.1328	C30H26O14	305	Gallocatechin (4–8)-epigallocatechin dimer 3	190.05 ± 1.20	230.88 ± 1.18	0.76 ± 0.10	0.17 ± 0.10
25.1	481.08956	C21H21O13	463 305 313	Epigallocatechin glucuronide	10.89 ± 0.56	13.24 ± 0.12	78.60 ± 1.10	126.19 ± 0.87
				Total	609	738	1047	317

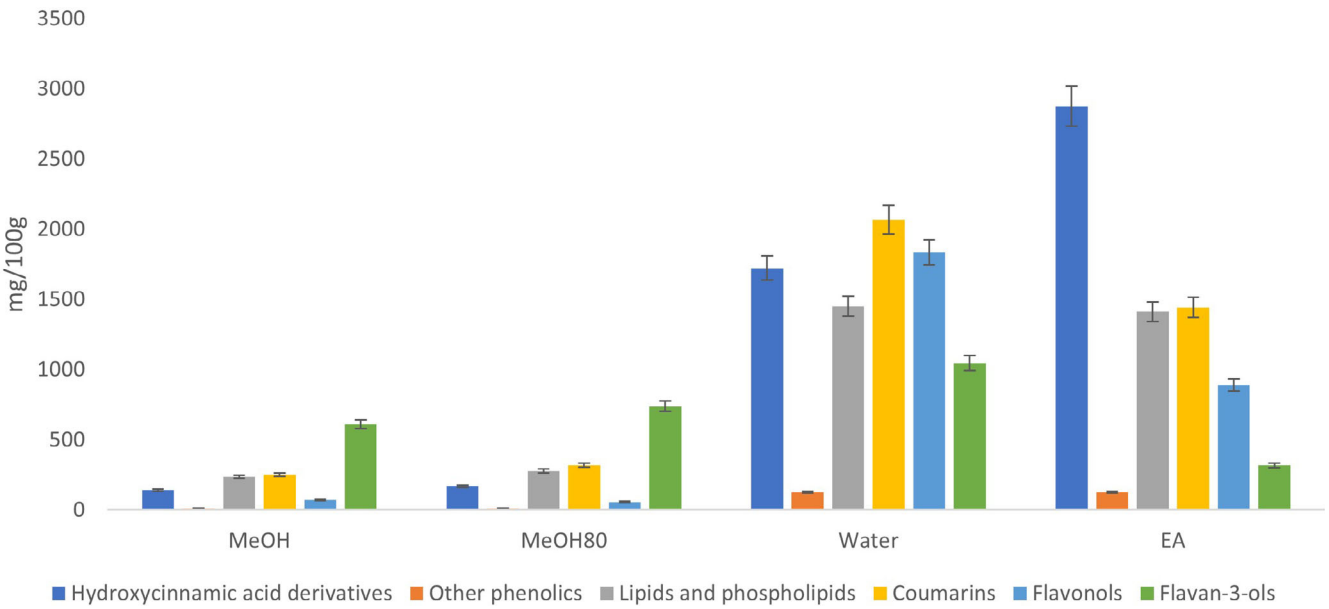

Figure 1. Variations of different groups of phytochemicals in the tested extracts.

showed higher TAC (6.34 and 6.15 mmol TE g^{-1}), whereas only half the TAC was displayed by the ethyl acetate extract (3.09 mmol TE g^{-1}). However, the lowest TAC was demonstrated by water extract (0.53 mmol TE g^{-1}) (Table 2). This could be related to the TPC of the extracts because they followed the same trend.

Antioxidant properties

Oxidative stress has been recognized to be the source of the development of several ailments. Indeed, a promising way to combat the unwanted effects of reactive oxygen species (ROS)-induced oxidative damage is by augmenting exogenous antioxidants or enhancing endogenous antioxidant defenses of the body. In this context, plants have an innate ability to biosynthesize a broad variety of non-enzymatic antioxidants that assist in the diminution of ROS-induced oxidative damage.[40] Indeed, the role of plant-derived natural antioxidants in decreasing oxidative stress is well documented.[41]

In the present study, both methanolic extracts displayed strong radical scavenging abilities in DPPH and ABTS assays (505.95–1068.06 mg TE g^{-1}), followed by the ethyl acetate extract (371.31 and 510.70 mg TE g^{-1}), whereas very low scavenging potential was exhibited by the water extract (37.21 and 60.95 mg TE g^{-1}). Remarkably, a similar pattern was obtained with the reducing power assays (CUPRAC and FRAP assays), with both methanolic extracts showing better reducing activities (543.05–1002.78 mg TE g^{-1}), followed by the ethyl acetate extract (260.44 and 462.40 mg TE g^{-1}) (Table 3). The least reducing

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potential was demonstrated by the water extract in the CUPRAC and FRAP assays (54.23 mg TE g^{-1} and 33.18 mg TE g^{-1}, respectively). Metal chelating activity was also displayed by all extracts (2.45–11.28 mg EDTAE g^{-1}); however, unlike the other antioxidant assays, the water extract showed better metal chelating activity compared to the other extracts (Table 3).

Previous studies on different solvent extracts and parts of *P. biglobosa* also revealed this plant to have potent antioxidant abilities, as well as phytochemical contents. For example, in the study by Komolafe and Oyelade,[42] the aqueous-methanolic leaf extract of *P. biglobosa* was found to yield total phenol and flavonoid contents of 144.18 mg gallic acid equivalent g^{-1} extract and 256.858 mg quercetin equivalent g^{-1} extract, respectively. Moreover, the extract exhibited significantly high and dose-dependent DPPH radical scavenging and ferric reducing potential comparable with the respective standards, quercetin and ascorbic acid. Furthermore, in another study,[42,43] crude methanol and ethanol-water extracts of *P. biglobosa* fruits were found to possess total phenolic contents 128.32 ± 0.49 and 127.23 ± 0.11 mg GAE g^{-1} dry weight, respectively, and the total antioxidant capacity of the extracts ranged from 160.44 ± 2.26 (methanol) to 157.31 ± 1.90 mg g^{-1} (ethanol/water). Surprisingly, the antioxidant activities evaluated by the DPPH and reducing power assays yielded concentration-dependent values comparable to that of ascorbic acid.[43]

Enzyme inhibitory effects

The progression of Alzheimer's disease (AD) involves the deterioration of neurons and synapses, chiefly characterized by cholinergic damage. This characteristic makes AChE inhibitors the main class of drugs currently in use for treating AD dementia phase, among which galantamine is the only naturally occurring substance. Nevertheless, several species of plants containing miscellaneous classes of coumarins, alkaloids, polyphenols and terpenes have been evaluated for their AChE inhibition properties, and many of them can be regarded as potential candidates for novel anti-AD drugs.[44,45] Furthermore, in the attempt to identify new drugs against AD, BChE inhibitors have also been considered. The increased BChE level in patients with advanced AD makes the latter a promising target and a variety of selective BChE inhibitors with high inhibitory activity have been reported.[46] Hence, both enzymes are likely to have involvement in the regulation of acetylcholine levels and represent valid therapeutic targets to amend the cholinergic deficit.

In the present study, all other extracts showed a higher AChE inhibitory effect (2.22–2.47 mg GALAE g^{-1}) in contrast to the water extract, which had an AChE inhibitory value of 0.23 mg GALAE g^{-1}. In the same way, the water extract did not display any anti-BChE activity, whereas the other extracts showed BChE inhibition in the range of 5.38–6.56 mg GALAE g^{-1} (Table 4).

As a key rate-limiting enzyme for melanogenesis, tyrosinase is considered an important target in the development of therapeutic agents against pigmentation disorders.[47] This has received notable focus by researchers with respect to the identification, isolation and synthesis of new potent inhibitors of this enzyme for various application in the cosmetics and pharmaceutical industries.[48] Interestingly, plants and their phytocompounds have offered promising scope as tyrosinase inhibitors.[49-51]

In the present study, although the water extract showed the least tyrosinase inhibitory potential (27.20 mg KAE g^{-1}), the ethyl acetate extract displayed the strongest inhibitory potency (83.33 mg KAE g^{-1}).

Diabetes is characterized by chronic hyperglycemia with disturbances in the macromolecules' metabolism as a result of impairments in insulin secretion, action or both. Although conventional antidiabetic drugs are known to be effective, they are also accompanied with inevitable side effects. In this context, medicinal plants are well regarded to act as an alternative source of antidiabetic agents. The valuable potential of each plant matrix is determined by the joint and concerted action of their profile of bioactive components. Indeed, the role of natural medicines, especially involving medicinal plants, for the treatment of diabetes has been extensively documented.[52-54] Moreover, it is acknowledged that the inhibition of α-amylase and α-glucosidase enzymes involved in carbohydrates digestion can significantly decrease the post-prandial surge of blood glucose level and

Table 2. Total bioactive compounds and total antioxidant capacity of the tested extracts

Extracts	TPC (mg GAE g^{-1})	TFC (mg RE g^{-1})	PBD (mmol TE g^{-1})
MeOH	119.88 ± 0.40 a	2.01 ± 0.18 ab	6.15 ± 0.24 a
MeOH (80%)	119.67 ± 0.42 a	0.60 ± 0.04 b	6.34 ± 0.23 a
Water	32.26 ± 0.19 b	0.65 ± 0.08 b	0.53 ± 0.01 b
Ethyl acetate	106.04 ± 2.14 ab	2.39 ± 0.05 a	3.09 ± 0.08 ab

Note: Values are reported as the mean ± SD of three parallel measurements. GAE, gallic acid equivalent; RE, rutin equivalent; TE, Trolox equivalent. TPC, total phenolic content; TFC, total flavonoid content; PBD, phosphomolybdenum. Different lowercase letters indicate significant differences between the tested extracts ($P < 0.05$).

Table 3. Antioxidant properties of the tested extracts

Solvents	DPPH (mg TE g^{-1})	ABTS (mg TE g^{-1})	CUPRAC (mg TE g^{-1})	FRAP (mg TE g^{-1})	MCA (mg EDTAE g^{-1})
MeOH	505.95 ± 0.60 ab	1007.33 ± 7.73 ab	1002.78 ± 15.28 a	558.68 ± 11.29 a	5.08 ± 0.49 ab
MeOH (80%)	508.30 ± 0.84 a	1068.06 ± 1.29 a	997.71 ± 28.83 a	543.05 ± 7.53 a	5.23 ± 0.17 ab
Water	37.21 ± 0.24 c	60.95 ± 0.73 c	54.23 ± 0.93 b	33.18 ± 0.21 b	11.28 ± 2.97 a
Ethyl acetate	371.31 ± 1.29 bc	510.70 ± 4.05 bc	462.40 ± 5.99 ab	260.44 ± 0.84 ab	2.45 ± 0.49 b

Note: Values are reported as the mean ± SD of three parallel measurements. TE, Trolox equivalent; EDTAE, EDTA equivalent. Different lowercase letters indicate significant differences between the tested extracts ($P < 0.05$).

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Table 4. Enzyme inhibitory properties of tested extracts

Solvents	AChE (mg GALAE g^{-1})	BChE (mg GALAE g^{-1})	Tyrosinase (mg KAE g^{-1})	Amylase (mmol ACAE g^{-1})	Glucosidase (mmol ACAE g^{-1})
MeOH	2.47 ± 0.06 a	5.38 ± 0.40 ab	82.23 ± 0.14 ab	0.57 ± 0.01 ab	NA
MeOH (80%)	2.41 ± 0.01 a	5.62 ± 0.37 ab	82.50 ± 0.24 ab	0.63 ± 0.03 a	NA
Water	0.23 ± 0.03 b	NA	27.20 ± 7.08 b	0.12 ± 0.01 b	1.96 ± 0.01 a
Ethyl acetate	2.22 ± 0.12 ab	6.56 ± 0.03 a	83.33 ± 0.03 a	0.57 ± 0.02 ab	1.82 ± 0.01 ab

Note: Values are reported as the mean ± SD of three parallel measurements. GALAE, galantamine equivalent; KAE, kojic acid equivalent; ACAE, acarbose equivalent. NA, not active. Different lowercase letters indicate significant differences between the tested extracts ($P < 0.05$).

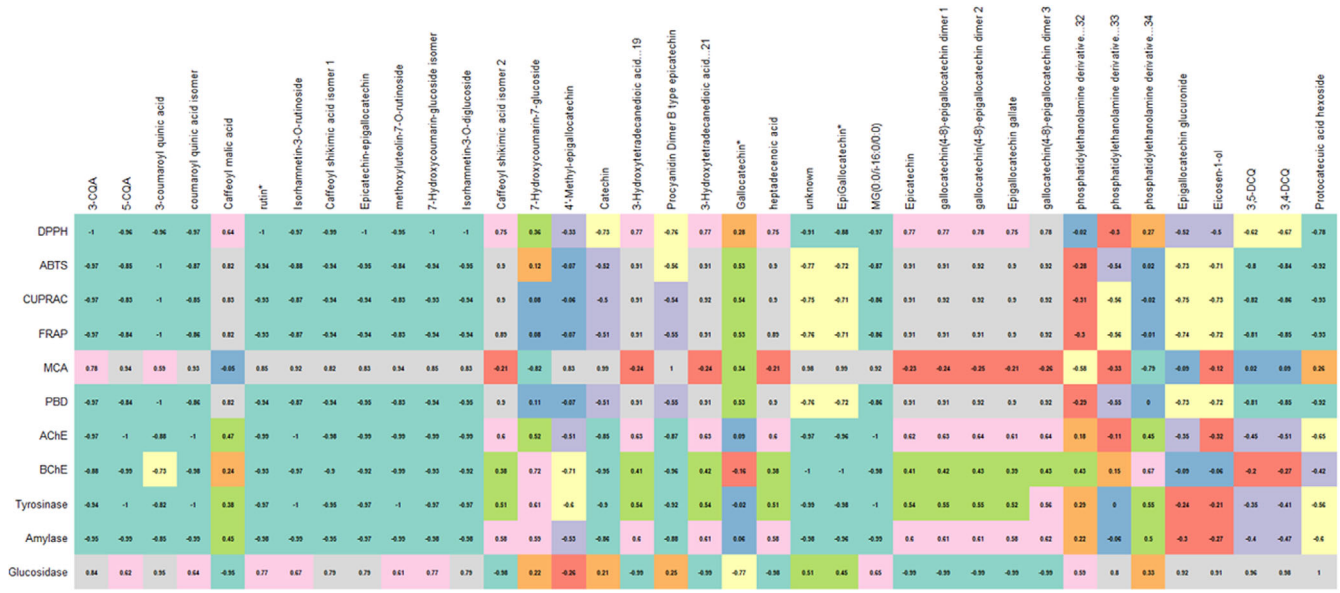

Figure 2. Pearson correlation between the identified compounds and biological activities.

consequently can be considered as an important approach in the management of blood glucose level in diabetic patients. In recent years, there has been a renewed research interest in plant-based diets and medicines that are able to modulate physiological effects in the prevention and cure of diabetes.[55,56]

In the present study, all extracts were found to possess anti-amylase activity (0.12–0.63 mmol ACAE g^{-1}), with water extract showing the least activity. By contrast, none of the methanol extracts displayed anti-glucosidase potential, whereas the water and ethyl acetate extracts had glucosidase inhibitory potential of 1.96 and 1.82 mmol ACAE g^{-1}, respectively, as shown in Table 4.

Interestingly, in a similar recent investigation, good inhibition was shown against cholinesterases with methanol (IC_{50} = 15.63 μg mL^{-1}) and ethyl acetate (IC_{50} = 16.20 μg mL^{-1}) extracts of *P. biglobosa*, showing higher inhibition than galantamine (IC_{50} = 42.20 μg mL^{-1}). Furthermore, α-glucosidase was inhibited by the ethyl acetate (IC_{50} = 12.47 μg mL^{-1}) and methanol extracts (IC_{50} = 16.51 μg mL^{-1}) of *P. biglobosa*, which has better activity compared to the standard acarbose (IC_{50} = 17.35 μg mL^{-1}) and also against α-amylase, and the ethyl acetate (IC_{50} = 13.50 ± 0.90 μg mL^{-1}) and methanol (IC_{50} = 18.12 μg mL^{-1}) extracts of *P. biglobosa* showed better potential compared to acarbose (IC_{50} = 23.84 μg mL^{-1}).[57] These reported findings on glucosidase inhibition partly differed from that obtained presently because the methanolic extracts in the present study showed no inhibition against glucosidase.

Data mining

In the last decades, multivariate analysis has gained interest to evaluate complex data and to understand the interactions between different parameters. Accordingly, we first performed a Pearson's correlation analysis between individual compounds (Table 2) and biological activities. The heat map is shown in Fig. 2. Clearly, several compounds were strongly correlated with antioxidant properties. For example, epicatechin had high correlation values ($R > 0.9$) for the observed antioxidant properties. In addition, gallocatechin (4–8)-epigallocatechin dimer 1, gallocatechin(4–8)-epigallocatechin dimer 2 and epigallocatechin gallate were highly connected with the antioxidant properties. Consistent with our results, the compounds were reported to exhibit significant properties in several other studies.[58-60] In the same way as antioxidant activities, a significant negative correlation was detected between different compounds and observed enzyme inhibitory activities. However, the maximum of positive and significant relationship was detected via anti-glucosidase activity, with compounds such as 3-CQA, epigallocatechin glucuronide, eicosen-1-ol, 3,5-DCQ, 3,4-DCQ and protocatecuic acid hexoside (Fig. 2). These differences for enzyme

inhibitory abilities could be explained by the different active sites of the tested enzymes and the complex nature of phytochemicals. Instead, given the observation of several negative correlations, the present results must be interpreted with caution. Despite the anti-enzymatic activity of some of these molecules being demonstrated by previous studies, our results are based on a statistical approach that has some limitations. Accordingly, we strongly suggest that the isolated compounds should be tested for enzyme inhibitory properties so we could understand which compounds contribute to the abilities.

Also, we performed PCA analysis to understand differences between tested solvents based on the biological activities. The results are presented in Fig. 3 and the first two dimension of PCA were sufficient to capture the maximum variance of the dataset (Fig. 3A). Clearly, the tested solvents were classified in three groups (Fig. 3B). Methanol and hydro-methanolic (80%) extracts were grouped in same group and were distributed in the positive side of the first dimension of PCA, such as ethyl acetate. However, both groups were separated along the second dimension. In addition, only the water was in negative side of the first and second dimensions. According to Fig. 2(C) methanol, hydro-methanolic (80%) and ethyl acetate were distinguished from water according to their highest antioxidant, amylase, AChE and tyrosinase activities. Based on the information, less polar solvents such as methanol or ethyl acetate could be considered as solvents for further applications with *P. biglibosa*. Subsequently, methanol and hydro-methanolic (80%) extracts were separated from ethyl acetate based on BChE, glucosidase and MCA assays.

Molecular docking

The computed binding energy of each ligand against each target protein is shown in Fig. 4. All of the compounds showed high preference for AChE. In particular, methoxyluteolin-7-*O*-rutinoside, rutin and 5-CQA bound to the AChE with remarkable affinity. Also, all of the compounds showed moderate binding to AChE, BChE, alpha amylase and glucosidase, as well as modest binding to tyrosinase. Hence, the protein–ligand interactions in some selected complexes were analyzed. Hydrogen bonds, several van der Waals interactions and a couple of π-π stacked

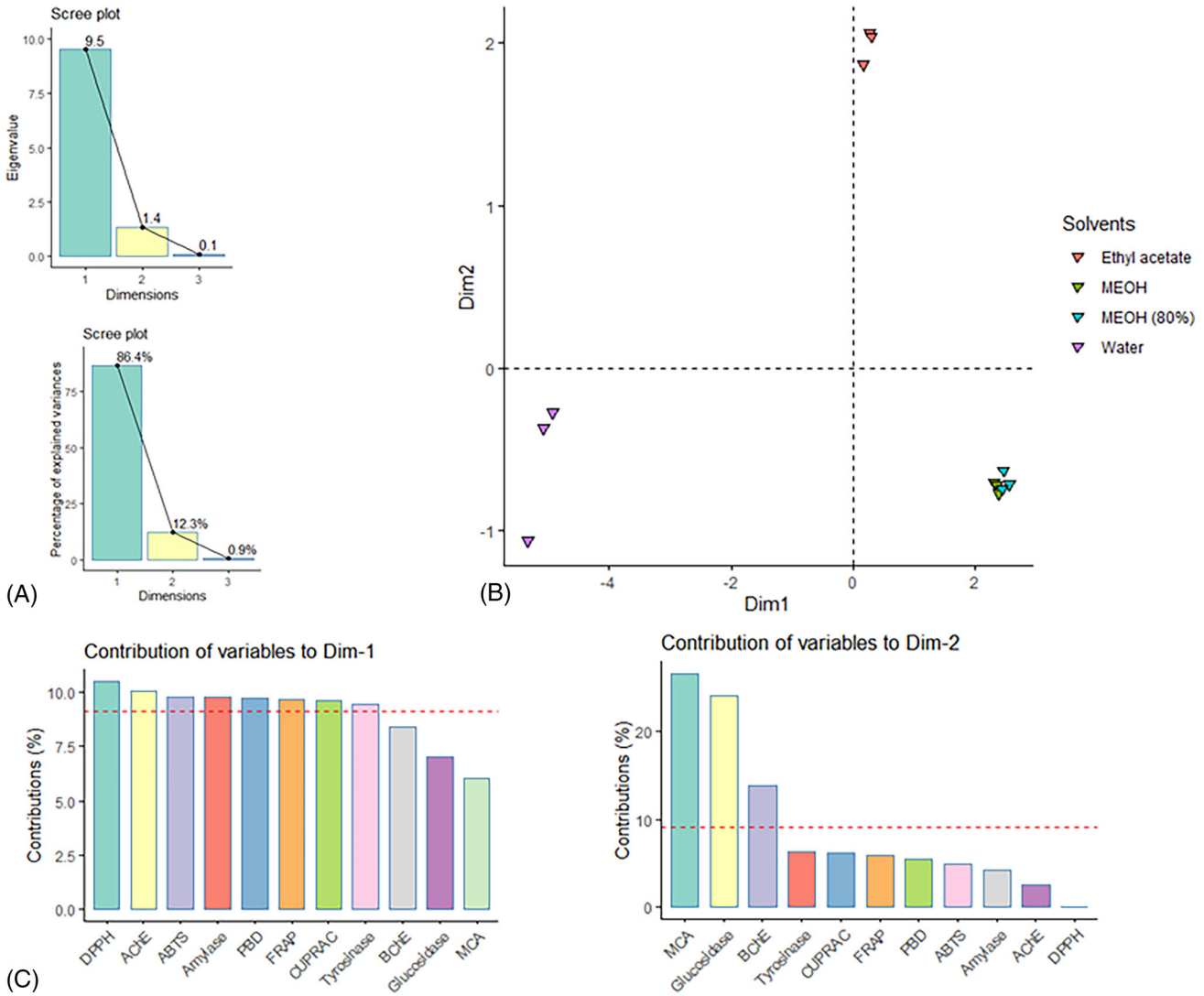

Figure 3. PCA on the biological activities of *P. biglibosa*. (A) Eigenvalue and percentage of explained variance of PCA dimensions. (B) Biplot showing the distribution of the solvents. (C) contribution of biological activities on the first two retained dimensions.

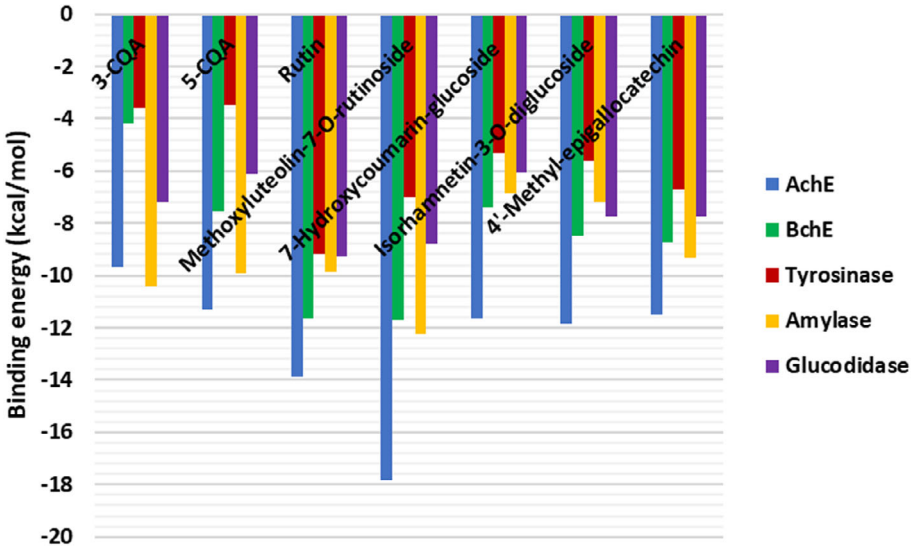

Figure 4. Binding energy (docking) scores of the bioactive compounds from the tested extracts.

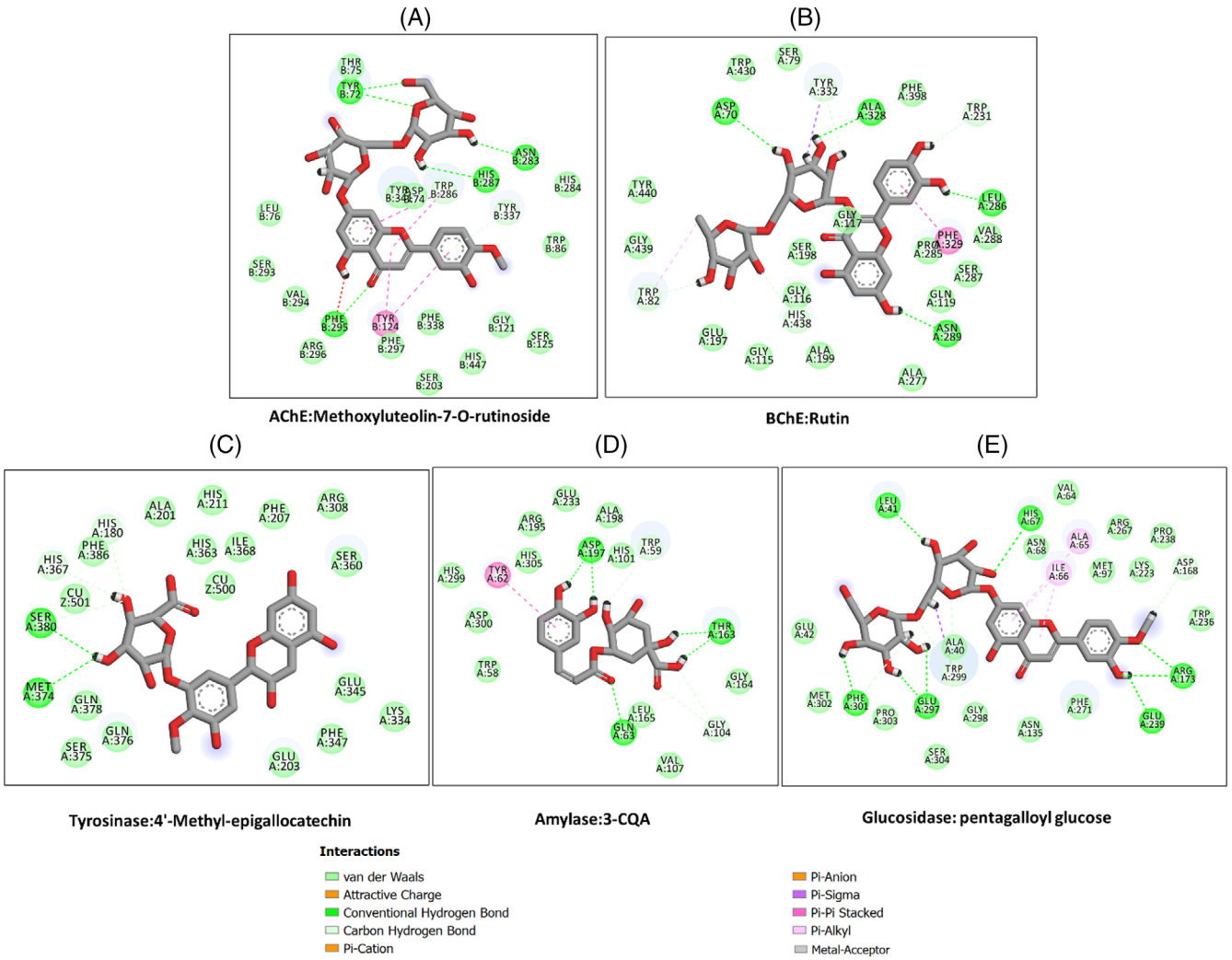

Figure 5. Protein–ligand interaction. (A) AChE and methoxyluteolin-7-O-rutinoside, (B) BChE and rutin, (C) tyrosinase and 4′-methyl-epigallocatechin, (D) amylase and 3-CQA, and (E) glucosidase and methoxyluteolin-7-*O*-rutinoside.

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interactions are the major contributors to the interaction between AChE and methoxyluteolin-7-*O*-rutinoside (Fig. 5A) and between rutin and BChE (Fig. 5B). The hydrogen bonds were formed between the polar amino acid residues in the active site of the enzymes and multiple hydroxyl groups on the phytochemical compounds. Also, 4′-methyl-epigallocatechin fitted in the narrow binding pocket of tyrosinase, forming similar interactions as described above (Fig. 5C). Interestingly, 3CQA, as a relatively smaller molecule, was completely buried in the catalytic channel of amylase, and interacted mainly via hydrogen bonds and van der Waals interactions (Fig. 5D). Likewise, methoxyluteolin-7-O-rutinoside spanned the catalytic pocket of glucosidase, forming less tight but similar interactions to those that it formed with AChE above (Fig. 5(E)). Together, these bioactive compounds likely exert biological activity by blocking the activity of these target enzymes.

CONCLUSIONS

In the present study, different stem bark extracts (methanol, methanol 80%, water and ethyl acetate) of *P. biglobosa* were investigated for their chemical composition and biological properties. Both methanolic extracts were revealed to possess higher TPC, which also correlated with the total antioxidant capacity of the extracts, whereas the water extract yielded the least TPC and TAC. LC-MS also revealed extracts rich in certain polyphenolic compounds. The methanolic extracts in particular were found to possess potent radical scavenging and reducing power compared to the other extracts. Although the water extract displayed the weakest activity in the antioxidant assays, it was nevertheless found to exhibit the highest metal chelating power. The extracts also exerted inhibitory properties against the studied enzymes, thus demonstrating their potential against AD, hyperpigmentation and diabetes. The presen study highlights the stimulating biological potential of the stem bark of the African locust bean, especially the methanolic extracts that could be further investigated and tested for their safe usage as phytomedicines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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