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Phenolic profiles, antioxidant, antiquorum sensing, antibiofilm and enzyme inhibitory activities of selected Acacia species collected from Benin

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ABSTRACT

Gum arabica is a local commercial nutraceutical and food preservative collected mostly from acacia plants. HPLC-DAD analysis identified gallic acid, syringic acid, ferulic acid, coumarin, rutin and rosmarinic acid in hydroethanol extracts of all three acacia species with ferulic acid being the most abundant component in *Acacia ataxacantha* (45.25 ± 0.33 mg/g) *Acacia sieberiana* (130.3 ± 0.65 mg/g) and *Acacia nilotica* (125.50 ± 0.51 mg/g). Extracts displayed good antioxidant capacities in β-carotene-linoleic acid, DPPH$^{\bullet}$, ABTS$^{\bullet+}$, CUPRAC and metal chelating assays and extracts were more active than the standards used in the DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and CUPRAC assays. Extracts inhibited violacein production in *Chromobacterium violaceum* CV12472 at MIC and sub-MIC concentrations as well as quorum sensing in *C. violaceum* CV026 under externally supplied acylhomoserine lactone. The extracts showed antimicrobial activity with MIC values ranging from 0.1562 mg/mL to 2.5 mg/mL on *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella Typhi, Escherichia coli* and *Candida albicans* with excellent biofilm inhibition at MIC and sub-MIC concentrations especially against *E. coli*. Extracts showed inhibitions on some enzymes with *A. Sieberiana* and *A. Nilotica* having very good anticholinesterase, antidiabetic (α-glucosidase and α-amylase) and antiurease activities. The results indicate the relevance of acacia in food applications.

1. Introduction

With time, viruses, bacteria and fungi become unsusceptible to the therapeutic effects of the drugs that were previously used to inhibit or kill them (Walusansa et al., 2022). Inappropriate and misuse of antimicrobials can contribute to the emergence of resistance in bacteria and it is worse in developing countries since patients can access antibiotics without prescription (Ayukekbong et al., 2017). Antibiotics which target the inhibition and death of bacteria and fungal cells are falling out of use since they are the faced with resistance, and antibiofilm and quorum sensing inhibition are new methods currently employed as suitable strategies to combat microbial resistance and reduce severity of infections (Ngenge et al., 2021). Most bacteria form extracellular polymeric protecting coat called biofilms on both biotic and abiotic surfaces, most especially on food surfaces with adequate nutrients, which protects them from the effects of antibiotics, disinfectants, detergents and the host defense system (Yum et al., 2022). Various food substances harbor bacteria that are responsible for contamination and food spoilage

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through the production of virulence factors, degrading enzymes and formation of biofilms which is trigerred by the cell density through a quorum sensing process (Machado et al., 2019). Quorum sensing (QS) is a communication system between microbial cells through synthesis, diffusion, detection and reaction to small signaling molecules called autoinducers.The biofilm formation is a quorum sensing mediated process that helps to increase microbial resistance and sessile microbial biofilm communities are approximately 10–1000 times more resistant to antibiotics than planktonic microbial communities (Abebe, 2020). Disrupting of biofilms and quorum sensing in bacteria can help to control human infection and food contamination and also reduces the expression of virulence factors in pathogenic bacteria. Some food substances are able to provide nutrients for human growth as well as bioactive molecules for human health. Such foods are a subject of investigation as strategic antimicrobials which can serve as food additives or nutraceuticals. Quorum-sensing inhibition is a suitable approach to target cell-to-cell communication aided by synthesized signal molecules, which takes place by blocking the production of the signal molecules thereby preventing food from spoilage and biofilm formation by food bacteria (Skandamis & Nychas, 2012). For this reason, most researchers are currently engaged in investigating plant products in the search of new therapeutic antibiotic agents that are capable of inhibiting quorum sensing (QS) processes and control infections without promoting the development of resistant microbial strains (Tamfu et al., 2020a, 2020b).

Nature provides a rich source of numerous bioactive compounds that have been extensively employed in traditional medicine since time immemorial (Cragg & Newman, 2001; Carol et al., 2017; Ngenge et al., 2019). In recent years,the Food and Drug Administration has approved an impressive number of modern drugs which are also natural products or directly derived therefrom (Newman & Cragg, 2016). For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use (Dar Refaz, Mohd, & Parvaiz, 2017; Talla et al., 2017; Eve et al., 2020). The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations (Hamburger & Hostettmann, 1991). Plants with therapeutic properties or beneficial pharmacological effects on the human body are generally designated as medicinal plants. It has been estimated that about 13 000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world (Yudharaj et al., 2016). Plant species contain active ingredients such as alkaloids, phenols, tannins, cryogenics, glycocides, terpenoids. These ingredients have been used and found effective as sweeteners, anti-infections and anti-bacterials (Akinyemi et al., 2018). Plant is an important source of medicine and plays a key role in world health (Sandberg & Corrigan, 2001). The genus *Acacia* belonging family Fabaceae is composed of several medicinal plants used for treating skin diseases. *Acacia*, mainly distributed in the tropical and subtropical regions, has been used in traditional medicines for the treatment of microbial infections, malaria, diarrhea, oedema and inflammation (Mounirou et al., 2019). *Acacia* includes a number of species that dominate the structure and floristic composition of extensive areas of African woodland, wooded grassland and bushland. Acacia plants represent a good source of gum arabica or acacia gum used in the food industry. The important members of this genus, *Acacia siberiana*, *A. nilotica* and *A. ataxacantha* are widely employed for their tremendous healing properties. The methanolic leaf extracts of *A. nilotica* and *A. ataxacantha* species showing the highest contents the total phenolic and flavonoid and equally exhibited high antioxidant potentials (Zheleva-Dimitrova et al., 2021). Some of the important phenolics detected in the studied extracts have been identified through LC-MS/MS studies in *A. nilotica* and *A. ataxacantha* alongside some of their glycosides. In acacia genus, the leaves and stem barks usually accumulate some terpenoids as well as phenolic acids which confers some medicinal properties including cytotoxic potentials, antimicrobial, anticancer, antioxidant and immunomodulatory activities (Amoussa et al., 2020).

Considering the significant health benefits and applications of gum arabica as an emulsifier, thickening-agent, soft candy, matrix and stabiliser, it is a suitable food additive even at local level and industrial level. Also, the low toxicity and low cost of gum arabica from acacia trees makes it a suitable nutraceutical. Hence, this present work aimed at determining the preliminary phenolic composition and *in vitro* biological activities such as antimicrobial, antibiofilm, anti-quorum sensing and antioxidant activities as well as enzyme inhibitory potential againts cholinesterase, α-glucosidase, α-amylase, urease and tyrosinase activities of ethanolic extracts of *Acacia siberiana*, *Acacia nilotica* and *Acacia ataxacantha*.

2. Materials and methods

2.1. Instrumentation

Ultra-sound device Bandelin (Sonorex Digitech Device, Germany) was used for extraction and Buchi Rotavapor (R215, Switzerland) was used to evaporate the solvents during extraction. Phenolic compounds were detected and quantified using a Shimadzu 20 AT series reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with diode array (HPLC–DAD, Shimadzu Cooperation, Japan). Multiskan Go microplate reader (Thermo Fischer Scientific, Waltham, MA, USA) was used to read optical densities during the MIC tests, biofilm assays and violacein inhibition studies and on a SpectraMax spectrophotometer (340 PC, Molecular Devices, USA) microplate reader for antioxidant and enzyme inhibitory studies.

2.2. Plant material and extraction

Acacia sieberiana **(AS),** *Acacia nilotica* **(AN)** and *Acacia ataxacantha* **(AA)** stem barks were collected during the month of January 2021 from North of Benin (Malanville). The plants were identified and voucher specimens prepared by the botanist Professor Hounnankpon Yedomonhan of the Benin National Herbarium where they were deposited under the specimen numbers YH 684/HNB, YH682/HNB and YH683/HNB for *Acacia sieberiana, Acacia nilotica* and *Acacia ataxacantha* respectively. The extraction was carried out using ultrasound-assisted extraction with ethanol as solvent. Briefly, 5 g of powdered plant material biomass from each plant were mixed with 100 mL solvent and sonicated for 2 h at 50 °C with Bandelin (Sonorex Digitech device). Further, all the extracts were filtered through Whatman No.1 filter paper and concentrated under vacuum (Buchi R215, heating bath B-491, rotation 280 rpm, vacuum controller V-850 of 290 mbar) at 50 °C. The residues were dried to constant weights and stored in the darkness at 4 °C to avoid the degradations until use (Agbangnan et al., 2012; Palayullaparambil et al., 2016).

2.3. HPLC–DAD phenolic profiles of plant extracts

The phenolic compounds in the acacia extracts were detected and quantified using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with diode array detector (DAD) as described previously (Tamfu, Kucukaydin, Ceylan, et al., 2021; Çayan et al., 2020). 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 chromatography 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

Lipid peroxidation inhibition activity was performed by β-carotene-linoleic acid test system with slight modifications (Tel-Çayan & Duru, 2019). The $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activity was evaluated spectrophotometrically as previously described (Tamfu et al., 2020b, 2022c; Tel et al., 2012; Öztürk et al., 2011). The cupric reducing antioxidant capacity (CUPRAC) was performed using the method reported earlier (Apak et al., 2004). BHA (Butylated Hydroxyanisole) and α-tocopherol were used as antioxidant standards for comparison of the β-carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and CUPRAC assays. The metal chelating activity of the extracts for Fe^{2+} was tested spectrophotometrically (Decker & Welch, 1990). EDTA was used as the reference compound comparison of the activity. Antioxidant activity results were given as 50% inhibition concentration (IC_{50}).

2.5. Microbial strains

The microorganisms used in this study were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, Salmonella Typhi ATCC 14028, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10239 and *Pseudomonas aeruginosa* PA01 for antimicrobial studies. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10239 and *Pseudomonas aeruginosa* PA01 for antibiofilm assays. *Chromobacterium violaceum* CV12472 and *Chromobacterium violaceum* CV026 for violacein and quorum sensing respectively. *Pseudomonas aeruginosa* PA01 for anti-swarming motility assay.

2.6. Determination of minimum inhibitory concentrations

Minimal inhibitory concentration (MIC) was determined by the broth dilution method described by the Clinical and Laboratory Standards Institute (Ceylan et al., 2020; CLSI, 2006). The MIC is the lowest extract concentration that yielded no visible microbial growth. The test medium was Mueller-Hinton broth and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (100 μL) were inoculated into the wells of 96-well microtitre plates in the presence of extracts with different final concentrations (5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.0781 mg/mL). The inoculated microplates were incubated at 37 °C for 24 h before being read.

2.7. Effect of extract on bacterial biofilm formation

The ability of the acacia extracts at MIC and sub-MIC concentrations including 1, ½, ¼, and 1/8 MIC to inhibit biofilm by test microorganisms were evaluated with a microplate biofilm assay (Merritt et al., 2005). Briefly, 1% of overnight grown cultures of isolates were added into 200 μL of fresh Tryptose-Soy Broth (TSB) supplemented with 0.25% glucose and cultivated in the presence and absence of extracts without agitation for 48 h at 37 °C. The wells containing TSB + cells only served as control. After incubation, remove planktonic bacteria were removed by gently washing with distilled water. The biofilm colonies were subsequently stained by filling wells with 200 μL of 0.1% crystal violet solution and then allowed for 10 min at room temperature. Wells were rinsed once more with distilled water using micro-pipette to remove the unabsorbed crystal violet. A volume of 200 μL of 33% glacial acetic acid (for Gram positive bacteria) or ethanol 70% (for Gram negative bacteria or fungi) were filled into the wells. After shaking 125 μL was pipetted from each of the wells into a sterile tube and volume was adjusted to 1 mL using distilled water. Finally, optical density (OD) of each well was measured at 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Percentage of inhibition of biofilm by the tested extracts was calculated using the formula:

$$\text{Biofilminhibition } (\%) = \frac{OD550_{Control} - OD550_{Sample}}{OD550_{Control}} \text{ x100}$$

2.8. Bioassay for quorum-sensing inhibition (QSI) activity using C. violaceum CV026

Inhibition of quorum sensing was determined as described elsewhere (Koh & Tham, 2011) with little modifications. 5 mL of lukewarm molten Soft Top Agar (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionized water) were seeded with 100 μL of an overnight grown culture of CV026, and 20 μL of 100 μg/mL acylhomoserine lactone (AHL) was added as exogenous hormone source. This was mixed gently and poured carefully over the surface of sterile solidified LBA plate as an overlay. 5 mm diameter wells were made on each plate after solidification of the overlay and each of the wells were filled with 50 μL of MIC and sub-MIC concentrations of filter sterilized acacia extracts. Each experiment was done in triplicate and the plates were incubated in upright position at 30 °C for 3 days after which the diameters of the quorum sensing inhibition zones were measured. A white or cream-colored halo around this well against a purple lawn of activated CV026 bacteria was an indication of QSI and its diameter was measured in millimeters.

2.9. Violacein inhibition assay using C. violaceum CV12472

Acacia extracts were subjected to qualitative analysis of QSI potentials for their ability to inhibit violacein production by *C. violaceum* ATCC 12472 (Tamfu, Ceylan, Fru, et al., 2020). Overnight grown cultures (10 μL) of *C. violaceum* (adjusted to 0.4 OD at 600 nm) were added into sterile microtiter plates containing 200 μL of Luria-Bertani (LB) broth and incubated in the presence and absence of MIC and sub-MICs of extracts. LB broth containing *C. violaceum* ATCC 12472 was used as a positive control. These plates were incubated at 30 °C for 24 h and observed for the reduction in violacein pigment production. The absorbance was read at 585 nm. The percentage of violacein inhibition was calculated by following the formula:

$$\text{Violacein inhibition } (\%) = \frac{OD\ 585\ control - OD585\ sample}{OD\ 585\ control} \text{ x100}$$

2.10. Swarming motility inhibition on Pseudomonas aeruginosa PA01

Swarming motility inhibition was evaluated according to a method previously described (Packiavathy et al., 2012). Briefly, overnight grown cultures of *P. aeruginosa* PA01 strain were point inoculated at the center of swarming plates consisting of 1% peptone, 0.5% NaCl, 0.5% agar and 0.5% of filter-sterilized D-glucose with various MIC and sub-MIC concentrations of acacia extracts (MIC, MIC/2 and MIC/4) and the plate without the extract was maintained as control. Plates were incubated at an appropriate temperature in an upright position for 18 h. The swarming migration was recorded by following swarm fronts of the bacterial cells.

2.11. Anticholinesterase activity

Anticholinesterase activity was measured spectrophotometrically by determining acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibitions described by Ellman with minor

modifications. (Ellman et al., 1961; Tamfu, Ceylan, Kucukaydin, & Duru, 2020). Electric eel AChE and horse serum BChE were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. The activity of the cholinesterase was monitored using DTNB (5,5′-Dithio-bis(2-nitrobenzoic) acid). Galantamine was used as a reference compound.

2.12. Anti-urease activity

The indophenol method was used to measure ammonia production in order to assess the inhibitory activity of the urease enzyme (Weatherburn, 1967). Urease solution was prepared with sodium phosphate buffer (pH 8.2, 100 mM). Following the addition of extracts (10 μL), urease enzyme (25 μL) and urea (100 mM, 50 μL) solutions were mixed and incubated at 30 °C for 15 min. After incubation, 45 μL of 1% (w/v) phenol reagent and 70 μL of 0.005% (w/v) alkali reagent was added to each well. Following 50 min of incubation, absorbance was recorded with the microplate reader at 630 nm. The reference compound was thiourea and results were expressed as percentage inhibition (%) at 100 μg/mL and 50% inhibition concentration (IC_{50}).

2.13. Anti-tyrosinase activity

Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described by Masuda et al., (2005). L-DOPA was utilized as substrate of the reaction. 150 μL of sodium phosphate buffer (pH 6.8, 100 mM), 10 μL of sample and 20 μL of tyrosinase enzyme solution in buffer were mixed and incubated for 10 min at 37 °C. Following incubation, 20 μL of L-DOPA was added. The absorbances in a 96-well microplate were monitored at 475 nm after 10 min of incubation at 37 °C. Kojic acid was used as a reference compound. Results were stated as percentage inhibition (%) at 100 μg/mL and 50% inhibition concentration (IC_{50}).

2.14. Antidiabetic activity: in vitro α-Amylase and α-glucosidase inhibition assay

The α-amylase inhibitory activity was evaluated by using starch-iodine method with some modifications (Küçükaydın et al., 2021; Quan et al., 2019). The enzyme α-amylase from porcine pancreas was used and enzyme solution was prepared with phosphate buffer (20 mM pH = 6.9 phosphate buffer prepared with 6 mM NaCl). Then, 50 μL of α-amylase and 25 μL of sample solutions were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. Then, 50 μL of starch solution (0.05%) was added and incubated for 10 min at 37 °C. Following incubation, the reaction was completed by adding HCl (0.1 M, 25 μL) and Lugol (100 μL) solutions, and the absorbance was recorded at 565 nm.

The α-glucosidase inhibitory activity was evaluated according to the method described previously (Kim et al., 2010; Tamfu, Munvera, et al., 2022). 50 μL of phosphate buffer (0.01 M pH 6.9), 10 μL of sample solution, 50 μL of α-glucosidase from *Saccharomyces cerevisiae* in phosphate buffer (0.01 M pH 6.0) and 25 μL of PNPG (4-N-nitrophenyl-α-D-glucopyranoside) in phosphate buffer (0.01 M pH 6.9) were mixed in a 96-well microplate. Then the solution was incubated for 20 min at 37 °C.

Acarbose was used as standard compound for both analyses. Results were given as percentage inhibition (%) at 100 μg/mL and 50% inhibition concentration (IC_{50}).

2.15. Statistical analysis

Activity assays were performed in triplicate analyses. The data were recorded as means ± Standard Error of the Means (SEM). Minitab 16 statistical software were used to determine the significant differences between means using one-way ANOVA (analysis of variance), in which $p < 0.05$ were regarded as significant.

3. Results and discussion

3.1. Phenolic profiles

Acacia trees are widely grown in tropical and subtropical areas and are famous for the production of gum arabic as sap from their tree barks which are usually used as food additives. Phenolic compounds are largely distributed in food products and their presence play a major role in the beneficial bioactivities of the food substance. Phenolic compounds and flavonoids present in food products are usually suitably characterized using HPLC system coupled with an UV–Vis diode array detector (Mizzi et al., 2020). Phenolic extracts of *Acacia sieberiana* **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA)** stem barks were prepared through ultra-sound assisted extraction with ethanol as solvent and the HPLC-DAD phenolic profiles determined and reported on Table 1. Gallic acid, syringic acid, ferulic acid, coumarin, rutin and rosmarinic acid were present in variable amounts in all three plants. Catechin was found only in AA and AS while ellagic acid was found only in AA and AS. Some compounds were detected exclusively in the extract of a single acacia species notably *p*-hydroxybenzoic acid, myricetin, luteolin and chrysin which were detected only in AA and chlorogenic acid which was

Table 1
Phenolic composition of the extracts determined by HPLC-DAD (mg/g)[a].

No	Phenolic compounds	RT (min)	AN	AA	AS
1	Gallic acid	5.70	7.83 ± 0.15	3.75 ± 0.12	8.20 ± 0.08
2	Protocatechuic acid	8.75	-[b]	–	–
3	Catechin	10.68	13.47 ± 0.27	–	22.50 ± 0.10
4	Pyrocatechol	11.04	–	–	–
5	Chlorogenic acid	12.35	–	–	10.87 ± 0.14
6	*p*-hydroxybenzoic acid	12.77	–	3.12 ± 0.07	–
7	6.7-Dihydroxy coumarin	14.10	–	–	–
8	Caffeic acid	15.09	–	–	–
9	3- hydroxy benzoic acid	15.98	–	–	–
10	Syringic acid	16.56	4.45 ± 0.11	3.14 ± 0.11	8.90 ± 0.13
11	Vanillin	17.78	–	–	–
12	*p*-Coumaric acid	20.56	–	–	–
13	Taxifolin	21.26	–	–	–
14	Ferulic acid	22.14	125.50 ± 0.51	45.25 ± 0.33	130.3 ± 0.65
15	Coumarin	24.49	25.11 ± 0.18	9.43 ± 0.24	23.51 ± 0.22
16	Rutin	25.30	4.64 ± 0.14	3.71 ± 0.18	4.41 ± 0.10
17	Ellagic acid	26.11	–	5.44 ± 0.12	4.20 ± 0.05
18	Rosmarinic acid	26.77	28.20 ± 0.25	11.50 ± 0.23	36.25 ± 0.34
19	Myricetin	27.35	–	3.20 ± 0.15	–
20	Quercetin	30.83	–	–	–
21	*trans*-Cinnamic acid	31.33	–	–	–
22	Luteolin	31.70	–	3.41 ± 0.09	–
23	Hesperetin	32.14	–	–	–
24	Kaempferol	33.21	–	–	–
25	Apigenin	33.77	–	–	–
26	Chrysin	38.40	–	9.20 ± 0.18	

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

detected exclusively in AS. The most abundant compound in the extracts is ferulic acid present in AN (125.50 ± 0.51 mg/g), AA (45.25 ± 0.33 mg/g) and AS (130.3 ± 0.65 mg/g) seconded by rosmarinic acid detected in AN (28.20 ± 0.25 mg/g), AA (11.50 ± 0.23 mg/g) and AS (36.25 ± 0.34 mg/g). The HPLC chromatograms of the extracts are given on Fig. 1. The hydro-ethanol extracts of the three acacia species were rich in significant bioactive phenolic compounds. Phenolic compounds are easily extractable with polar organic solvents usually in combination with water and ethanol has added advantage over other solvents since it is safe for consumption and therefore suitable for extraction of bioactive excipients (Lohvina et al., 2022). Ethanol extracts are therefore very important for food applications. The hydro-ethanol solution is more polar than absolute ethanol and this is believed to increase the extraction yields of phenolic compounds (Hikmawanti et al., 2021). In one study, the methanol and water extracts of acacia plants obtained by ultrasonication and maceration showed good yields of phenolic compounds and the yields varied with method of extraction as well as solvent of extraction (Zheleva-Dimitrova et al., 2021). Most, acacia species contain high amounts of anthocyanins, glycosides, various phenolics and flavonoids and these chemical components especially phenolics showed positive correlation with high antioxidant capacity (Abdel-Farid et al., 2014). The phenolic compounds identified in the three acacia species reported in milligrams per gram of extract were high and this indicates possibility of strong antioxidant activity since phenolic compounds are good antioxidants. The structures of the identified phenolic compounds are shown on Fig. 2. Hydroxyl groups bonded to the aromatic nucleus of phenolic compounds improve antioxidant activity of phenolics by various mechanisms (Rasouli et al., 2017; Tamfu, Roland, et al., 2022; Zeb, 2020). Several classes of phytoconstituents were detected in *A. sieberiana* including various phenolic compounds, phenolic glycosides, tannins, flavonoids and their glycosides, alkaloids, saponins, anthraquinones, triterpenoids, sterols and cardiac glycosides (Donkor et al., 2013; Mary-Ann et al., 2019; Ngaffo et al., 2020; Olapade et al., 2014; Sam et al., 2011; Toma et al., 2009; Traore et al., 2015b; Traore et al., 2017). The identification of phenolic compounds in *A. sieberiana* is compatible with some existing findings that described the presence of phenolics in these plants alongside other metabolites (Aba et al., 2015; Amoussa et al., 2014; Amoussa et al., 2014, 2014, 2015, 2016a, 2016b; Arise et al., 2016; Zheleva-Dimitrova et al., 2021).

3.2. Antioxidant activity

Various plants contain bioactive compounds such as phenolic compounds which are famous for their high antioxidant activity and it is necessay to evaluate antioxidant potentials of various plant species in order to reveal the value of the plants as antioxidant sources. The results of the antioxidant capacity of the three acacia apecies determined using five complementary methods namely β-carotene-linoleic acid, DPPH$^{\bullet}$, ABTS$^{\bullet+}$, CUPRAC and metal chelating assays and given on Table 2. In the β-carotene-linoleic acid assay, the most active extract was AS (IC_{50} = 4.75 ± 0.25 μg/mL) and no extract was more active than the standard

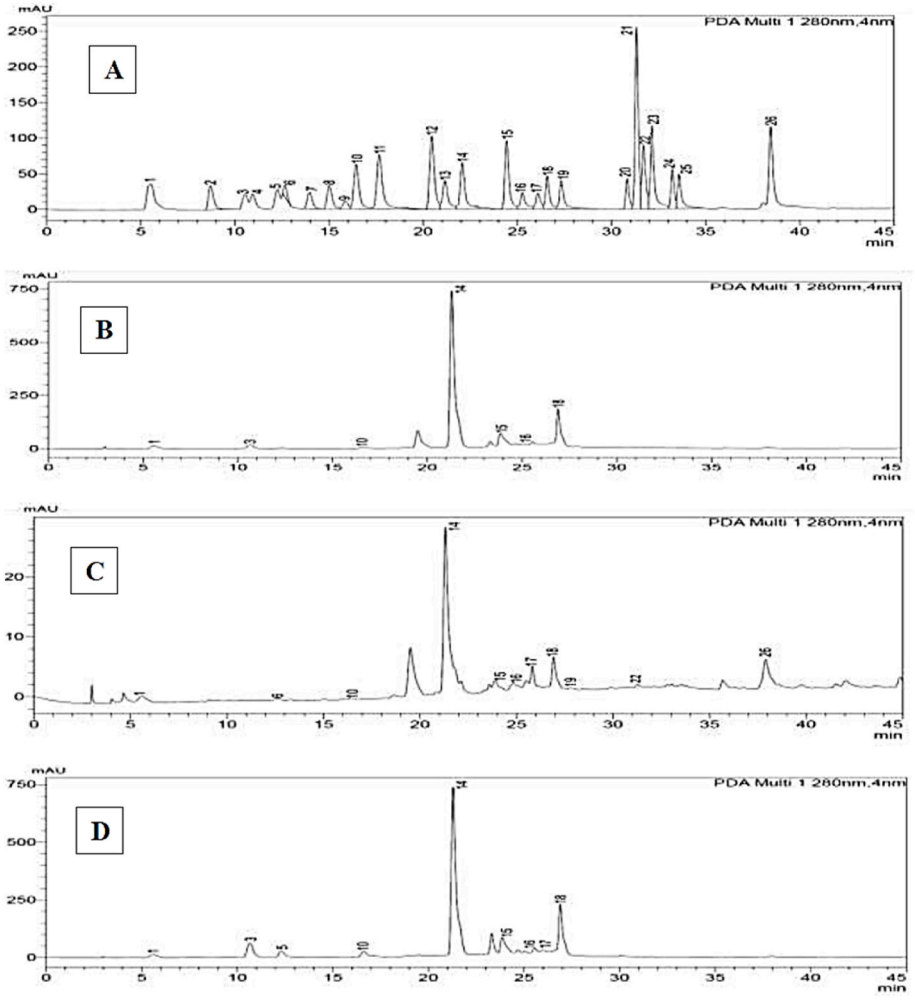

Fig. 1. A: HPLC chromatogram of standard phenolic Compounds; B: HPLC-DAD chromatogram of AN; C: HPLC-DAD chromatogram of AA; D: HPLC-DAD chromatogram of AS

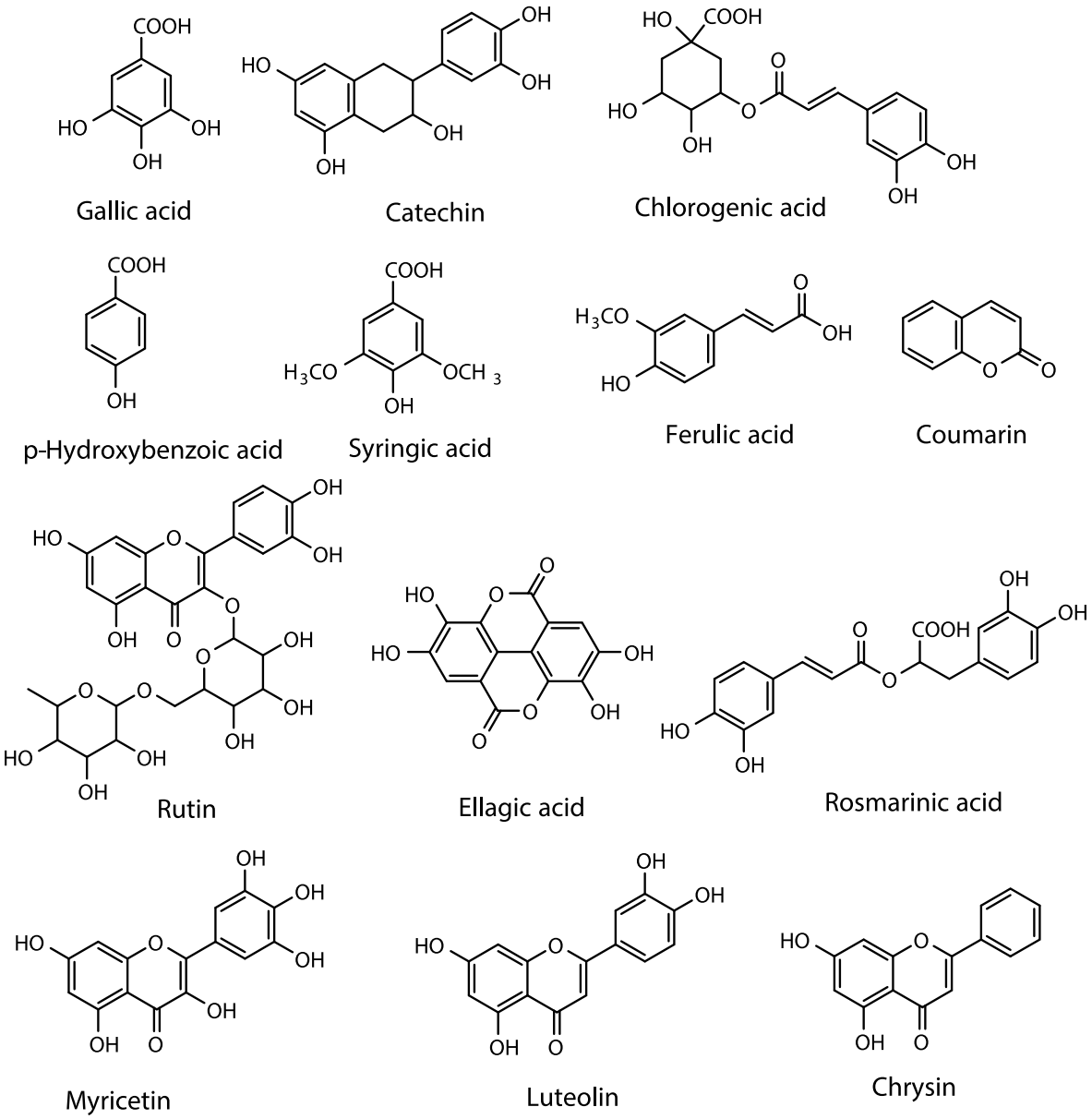

Fig. 2. Structures of phenolic compounds identified by HPLC-DAD in the three acacia extracts.

Table 2
Antioxidant activity of the extracts by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays.

Antioxidant Activity						
		β-Carotene-linoleic acid assay	DPPH• assay	ABTS•+ assay	CUPRAC assay	Metal chelating assay
		IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	$A_{0.50}$ (μg/mL)	IC_{50} (μg/mL)
Extracts	**AN**	5.93 ± 0.32[a]	13.15 ± 0.55[b]	7.88 ± 0.20[a]	11.21 ± 0.35[b]	18.05 ± 0.29[d]
	AA	12.51 ± 0.17[b]	34.40 ± 0.80[c]	23.63 ± 0.75[c]	27.37 ± 0.55[d]	40.25 ± 0.77[c]
	AS	4.75 ± 0.25[a]	12.33 ± 0.42[b]	7.12 ± 0.24[d]	10.54 ± 0.43[a]	19.57 ± 0.48[b]
Standards	**α-Tocopherol**	2.11 ± 0.07[a]	38.15 ± 0.45[a]	35.50 ± 0.56[b]	61.40 ± 0.75[a]	NT
	BHA	1.40 ± 0.03[a]	19.82 ± 0.33[b]	12.80 ± 0.08[a]	25.50 ± 0.4[b]	NT
	EDTA	NT	NT	NT	NT	5.60 ± 0.45[a]

Different subscripts in the same row indicate significant differences ($p < 0.05$).
NT: not tested.

used but their IC_{50} values were close to those of the standard antioxidants α-tocopherol ($IC_{50} = 2.11 \pm 0.07$ μg/mL) and BHA ($IC_{50} = 1.40 \pm 0.03$). In the DPPH• assay, AN ($IC_{50} = 13.15 \pm 0.55$ μg/mL) and AS ($IC_{50} = 12.33 \pm 0.42$ μg/mL) showed higher activity than the two standard antioxidants α-tocopherol ($IC_{50} = 38.15 \pm 0.45$ μg/mL) and BHA ($IC_{50} = 19.82 \pm 0.33$ μg/mL) while AA ($IC_{50} = 34.40 \pm 0.80$ μg/mL) was more active than α-tocopherol but not BHA. The extracts showed good activity on the radical cation ABTS•+ with IC_{50} values of

7.88 ± 0.20, 23.63 ± 0.75 and 7.12 ± 0.24 μg/mL for AN, AA and AS respectively as compared to the standards α-tocopherol and BHA with IC_{50} values of 35.50 ± 0.56 and 12.80 ± 0.08 μg/mL respectively. In the CUPRAC assay, AN ($A_{0.50}$ = 11.21 ± 0.35 μg/mL), AA ($A_{0.50}$ = 27.37 ± 0.55 μg/mL) and AS ($A_{0.50}$ = 10.54 ± 0.43 μg/mL) were more active than α-tocopherol ($A_{0.50}$ = 61.40 ± 0.75 μg/mL) but only AA and AS were more active than BHA ($A_{0.50}$ = 25.50 ± 0.43). In the metal chelation, EDTA (IC_{50} = 5.60 ± 0.45 μg/mL) was more active than AN (IC_{50} = 18.05 ± 0.29 μg/mL), AA (IC_{50} = 40.25 ± 0.77 μg/mL) and AS (IC_{50} = 19.57 ± 0.48 μg/mL) though their activities are moderate. Some compounds isolated from *A. ataxacantha* possessed antioxidant and antibacterial activities (Amoussa, Lagnika, et al., 2016; Maroyi et al., 2018). *Acacia nilotica* has been reported as a good source of bioactive phenolic compounds and these phenolics can contribute to high antioxidant activities (Foyzun et al., 2022; Kalaivani & Mathew, 2010; Sadiq, Tharaphan, et al., 2017; Zheleva-Dimitrova et al., 2021). The phytochemicals identified or isolated from *A. nilotica* can be grouped mainly as polyphenolic compounds, phenolic acids and their derivatives, alkaloids, flavonoids and their glycosides, saponins, volatile oils, phenols and phenolic glycosides, tannins, resins, steroids and terpenoids (Mitra & Sundaram, 2007; Banso, 2009; Kalaivani et al., 2010; Jigam et al., 2010; Singh, Singh, Sarma, & Singh, 2009; Singh et al., 2010). The polyphenolic extract of *Acacia nilotica* possess antioxidant activity measured as inhibition of lipid peroxidation, total antioxidant and scavenging ability (Foyzun et al., 2022). It can be concluded that the extracts of the three acacia species *A. sieberiana* **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA)** have all shown very good antioxidant activity measured by five complementary methods including β-Carotene-linoleic acid peroxidation assay, $DPPH^{\bullet}$ scavenging assay, $ABTS^{\bullet+}$ scavenging assay, CUPRAC reduction assay and metal chelating assay and this good antioxidant activity is attributable to their phenolic compounds.

3.3. Inhibition of violacein production in C. violaceum CV12472 and quorum sensing in C. violaceum CV026 by extracts

C. violaceum CV12472 is a Gram-negative bacterium that produces purple violacein pigment that acts as an antioxidant protecting the bacterial membrane against oxidative stress. The purple colour of violacein produced can be easily measured and it reflects quorum-sensing process in bacteria. Prior to violacein inhibition, the MIC values on *C. violaceum* CV12472 were determined and were found to be 0.5 mg/mL for AN and AA and 0.25 mg/mL for AS. The ability of the extracts to inhibit violacein production was measured at MIC and sub-MIC concentrations and reported on Table 3. All extracts exhibited, 100% inhibition at MIC. The most active extract AS showed further inhibition of 100% at MIC/2 and MIC/4 and was the only extract to inhibit violacein at MIC/16 and MIC/32. The mutant strain *C. violaceum* CV026 does not produce violacein except and external acylhomoserine lactone (AHL) is supplied to it. The quorum sensing inhibition zones corresponding to the cream or semitransparent zones were determined (diameters measured in millimeters) at concentrations of MIC and sub-MIC and reported on Table 4. The MIC values were 0.1562 mg/mL for AA and AS while AN had MIC value of 0.3125 mg/mL. All extracts showed anti-QS zones at MIC to MIC/4 concentrations while only AN and AS showed inhibition zones at MIC/8. Since anti-QS activity was concentration-dependent, the best quorum sensing was exhibited at MIC for AN (19.0 ± 1.5 mm), AA (18.0 ± 1.2 mm) and AS (18.5 ± 1.3 mm). The QS inhibition zones and violacein inhibition plates are found in Fig. 3. Acacia species have also been used as remedy for infectious diseases and their antimicrobial activities have been documented but with very few studies on their quorum sensing effects and antibiofilm potentials. Microbial resistance is promoted through quorum-sensing (QS) mediated processes. Quorum-sensing is a cell-to-cell means of communication within microbial colonies using small signal molecules and it is useful for the establishment of microbial biofilm, toxin production, resistance to antibiotics, motilities and spread of pathogenic microbes (Tamfu et al., 2020a, 2022a). Phenolic extracts of medicinal and food plants are known to exhibit anti-quorum sensing activity of food pathogens and other bacteria and can be useful to reduce food contamination and microbial resistance (Takó et al., 2020; Tamfu et al., 2020b, 2020c). Pigment production is one of the easily measurable QS traits and violacein pigment produced by *C. violaceum* bacteria acts a protective antioxidant molecule that can prevent the bacterial cell wall from oxidative stress and substance and violacein is produced by a QS-mediated process (Alfred et al., 2020; Beddiar et al., 2021; Boudiba et al., 2021; Popova et al., 2021). The acacia plant extracts displayed good violacein inhibition in *C. violaceum* CV12472, which is the strain that produces its own violacein naturally and equally the extracts showed good anti-QS effects on *C. violaceum* CV026, a mutant strain that does not produce violacein except with an externally supplied AHL. This is interesting as the extracts could block signal molecule production in CV12472 and also signal reception represented by the presence of the AHL added unto CV026. It can be said that the three acacia species *A. sieberiana* (AS), *A. nilotica* (AN) and *A. ataxacantha* (AA) can disrupt signal emission and signal reception in *C. violaceum* which is a suitable strategy to stop bacteria form controlling colony behavior, expressing virulence and developing resistance. Since growth was not inhibited in the *C. violaceum* 12472 and *E. coli*, the inhibition of pigment production as response to AHL could have been stopped by the acacia extracts through disruption of QS network and this is a desirable effect with selective pressure that avoids development of resistance (Omwenga et al., 2017; Singh, Singh, Singh, et al., 2009).

Table 3
Inhibition of violacein production in *C. violaceum* CV12472 by extracts.

Sample code	MIC (mg/mL)	Violacein inhibition (%)					
		MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC/32
AN	0.5	100 ± 0.00^{a}	45.1 ± 1.5^{b}	30.4 ± 1.0^{b}	15.3 ± 0.2^{d}	–	–
AA	0.5	100 ± 0.00^{a}	48.6 ± 0.9^{b}	25.1 ± 1.2^{c}	10.3 ± 0.5^{d}	–	–
AS	0.25	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	67.8 ± 1.5^{c}	44.1 ± 1.1^{b}	20.5 ± 0.9^{b}

Different subscripts in the same row indicate significant differences ($p < 0.05$).
-: No inhibition.

Table 4
Quorum sensing inhibition zones in *C. violaceum* CV026 by extracts.

Sample code	Anti-quorum sensing inhibition zones (mm)				
	MIC (mg/mL)	MIC	MIC/2	MIC/4	MIC/8
AN	0.3125	19.0 ± 1.5^{a}	15.5 ± 0.6^{b}	11.0 ± 0.5^{b}	09.5 ± 0.2^{c}
AA	0.1562	18.0 ± 1.2^{a}	14.5 ± 0.3^{c}	10.0 ± 0.8^{a}	–
AS	0.1562	18.5 ± 1.3^{a}	16.0 ± 0.5^{b}	13.5 ± 0.7^{c}	11.0 ± 0.1^{c}

Different subscripts in the same row indicate significant differences ($p < 0.05$).
-: No activity.

3.4. Swarming motility inhibition on P. aeruginosa PA01 by extracts

Swarming is one of the virulence factors in flagellated bacteria such as *P. aeruginosa* PA01 and bacteria use swarming motility to invade and colonize surfaces and this is an important step to prolonged surface contamination. Before evaluating swarming movement, the MIC values of the extracts on *P. aeruginosa* PA01 and the swarming inhibition determined at MIC, MIC/2 and MIC/4 concentrations and the results given on Table 5. The results varied in a concentration-dependent manner from 68.5 ± 2.1% at MIC to 20.0 ± 0.5% at MIC/4 for AN, from 35.9 ± 1.0% at MIC to 06.3 ± 0.1% at MIC/4 for AA and finally from 71.4 ± 1.6% at MIC to 29.0 ± 0.2% at MIC/4 for AS. AS was the

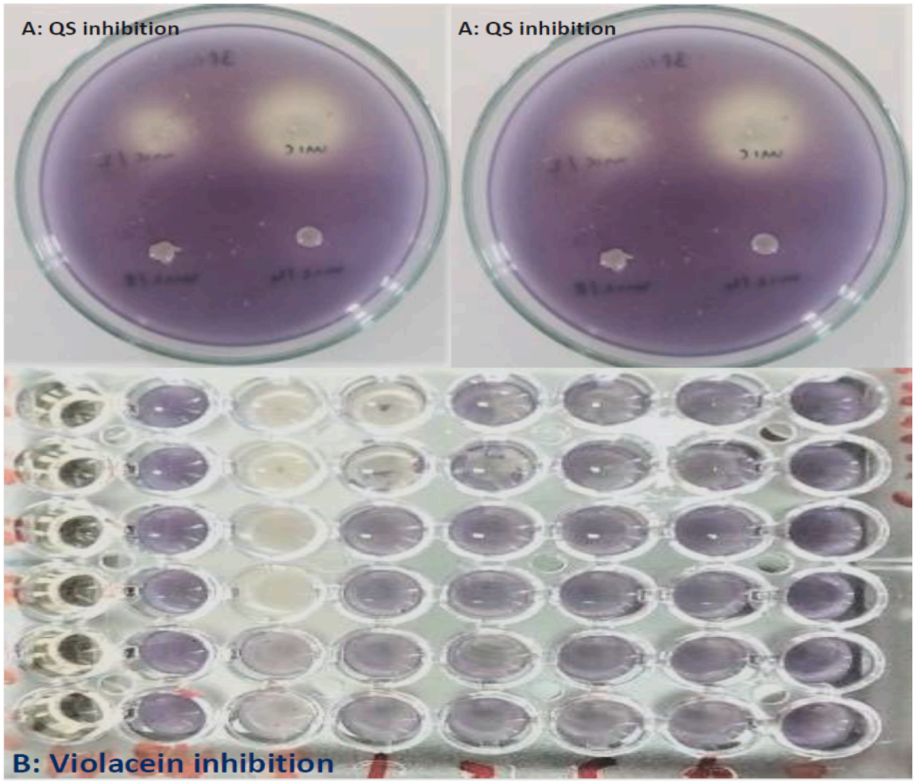

Fig. 3. A: Quorum-sensing inhibition plate; B: Violacein inhibition plate.

Table 5
Swarming motility inhibition on *P. aeruginosa* PA01 by extracts.

Sample code	Swarming inhibition (%)		
	MIC	MIC/2	MIC/4
AN	68.5 ± 2.1[a]	42.8 ± 1.1[b]	20.0 ± 0.5[c]
AS	71.4 ± 1.6[a]	47.9 ± 0.8[b]	29.0 ± 0.2[c]

Different subscripts in the same row indicate significant differences ($p < 0.05$). -: No inhibition.

most active extract. Swarming movement by flagellated bacteria *P. auroginosa* PA01 was inhibited by the extracts of the three plants indicating that they can prevent bacteria from moving and covering surfaces on which they can subsequently establish biofilms.

3.5. Antimicrobial and antibiofilm activities

Before evaluating the effects of the extracts on biofilm formation in bacteria, the antimicrobial activity of the extracts were measured and the antibiofilm was subsequently determined at MIC and sub-MIC concentrations and reported in Table 6. The antimicrobial activity was determined against *S. aureus, E. coli, C. albicans, E. faecalis,* S. Typhi and *P. aeruginosa*. MIC values varied from 0.1562 mg/mL to 2.5 mg/mL. The most susceptible bacteria to AN was *S. aureus* and *S. typhi* with MIC values of 0.3125 mg/mL while the least susceptible bacteria to this extract were *E. faecalis* and *P. aeruginosa* with MIC values of 1.25 mg/mL. The extract AA had its highest antimicrobial activity on *S. aureus* with MIC of 0.1562 mg/mL and its lowed activity on *E. coli*, *E. faecalis* and *P. aeruginosa*. The bacteria *S. aureus* and *S. typhi* were the most susceptible to AS with MIC of 0.1562 mg/mL while on *P. aeruginosa*, it had MIC of 2.5. The antimicrobial activity shows that all the plants are potential sources of good antimicrobial agents since they were able to inhibit all the tested strains. The antibiofilm activity of the extracts were evaluated at MIC and sub-MIC concentrations on against *S. aureus, E.*

Table 6
Antimicrobial and Anti-biofilm activity results of extracts.

Microorganism		AN	AA	AS
		MIC (mg/mL)		
S. aureus		0.3125	0.1562	0.15625
E. faecalis		1.25	1.25	0.625
P. aeruginosa		1.25	1.25	2.50
S. typhi		0.3125	0.625	0.156
E. coli		0.625	1.25	0.625
C. albicans		0.625	0.625	0.3125
Biofilm inhibition (%)				
S. aureus	MIC	23.44 ± 0.86[a]	28.11 ± 0.51[c]	30.20 ± 0.32[c]
	MIC/2	2.07 ± 0.05[b]	13.32 ± 0.27[d]	11.84 ± 0.05[e]
	MIC/4	–	–	–
	MIC/8	–	–	–
E. faecalis	MIC	42.39 ± 0.96[a]	52.34 ± 1.42[a]	14.62 ± 0.16[e]
	MIC/2	19.33 ± 0.21[c]	34.26 ± 0.82[a]	–
	MIC/4	–	12.01 ± 0.23[d]	–
	MIC/8		–	–
P. aeruginosa	MIC	23.58 ± 0.17[c]	31.50 ± 0.52[c]	19.15 ± 0.40[b]
	MIC/2	10.39 ± 0.06[b]	15.70 ± 0.21[e]	05.39 ± 0.02[e]
	MIC/4	–	6.81 ± 0.03[b]	–
	MIC/8	–	–	–
S. typhi	MIC	47.38 ± 1.06[a]	68.96 ± 1.86[a]	15.84 ± 0.32[d]
	MIC/2	21.15 ± 0.41[b]	46.12 ± 0.86[b]	2.16 ± 0.01[b]
	MIC/4	–	28.54 ± 0.22[d]	–
	MIC/8	–	8.35 ± 0.04[b]	–
E. coli	MIC	52.27 ± 1.79[a]	81.62 ± 2.34[a]	81.73 ± 2.48[a]
	MIC/2	30.54 ± 0.53[c]	65.34 ± 1.48[a]	62.16 ± 1.28[a]
	MIC/4	19.35 ± 0.14[c]	46.57 ± 1.10[c]	23.22 ± 0.69[b]
	MIC/8	–	26.64 ± 0.62[c]	8.84 ± 0.32[e]
C. albicans	MIC	28.75 ± 0.36[c]	56.87 ± 1.02[e]	20.65 ± 0.52[c]
	MIC/2	10.95 ± 0.06[b]	39.13 ± 0.49[d]	11.46 ± 0.24[d]
	MIC/4	–	21.27 ± 0.14[c]	–
	MIC/8	–	–	–

Different subscripts in the same row indicate significant differences ($p < 0.05$). -: No inhibition.

coli, C. albicans, E. faecalis, S. Typhi and *P. aeruginosa* using the crystal-violet staining method and the results given on Table 6. On the Gram positive bacteria, AA had the best biofilm inhibition percentage ranging from 28.11 ± 0.51% (MIC) to 13.32 ± 0.27 (MIC/2) against *S. aureus* and from The antibiofilm activity varied from 52.34 ± 1.42% (MIC) to 12.01 ± 0.23% (MIC/4) on *E. faecalis*. At MIC, AN and AS inhibited *S. aureus* biofilms with percentage inhibitions of 23.44 ± 0.86% and 30.20 ± 0.32% respectively and at MIC/2, 2.07 ± 0.05% and 11.84 ± 0.05% respectively. Against *E. faecalis*, AN inhibited biofilms at 42.39 ± 0.96% (MIC) and 19.33 ± 0.21 (MIC/2) while AS inhibited biofilms only at MIC (14.62 ± 0.16). *P. aeruginosa* biofilms were not very susceptible to the tested samples as the inhibition percentages varied from 47.38 ± 1.06% (MIC) to 21.15 ± 0.41% (MIC/2) for AN and from 15.84 ± 0.32% (MIC) to 2.16 ± 0.01% (MIC/2) for AS and finally from 31.50 ± 0.52% (MIC) to 06.81 ± 0.03% (MIC/4) for AA. The most active extract on S. Typhi was AA with percentage inhibition of biofilms of 81.62 ± 2.34% (MIC) and 26.64 ± 0.62% (MIC/8). Moderate biofilm inhibition was observed against S. Typhi by AN and varied from 47.38 ± 1.06% (MIC) to 21.15 ± 0.41 (MIC/2). AS had the lowest antibiofilm activity against S. Typhi. *E. coli* was the most suceptible Gram-negative bacterial biofilm to the tested samples and all samples had very good antibiofilm activity against *E. coli*. AA and AS had 81.62 ± 2.34% and 81.73 ± 2.48% inhibitions at MIC respectively, then 26.64 ± 0.62% and 8.84 ± 0.32% at MIC/8 for AA and AS respectively. AN antibiofilm against *E. coli* varied from 52.27 ± 1.79% (MIC) to 19.35 ± 0.14% (MIC/4) and no inhibition of biofilm at MIC/8. Against the yeast cells *C. Albicans*, AA was the most active sample with percentage inhibitions of biofilms ranging from 56.87 ± 1.02% (MIC) to 21.27 ± 0.14% (MIC/4) while AS and AN had low biofilm inhibitions at MIC and MIC/2 concentrations only. The antimicrobial effect of the extracts on different pathogenic microbes were very interesting as the MIC values ranged from 0.1562 mg/mL to 2.5 mg/mL. Various extracts of acacia species display antimicrobial activity and this is an indication that, the use of gum Arabica from acacia trees in food applications could help to deter food pathogens and reduce food contamination and decay. The good antimicrobial activity observed for *A. nilotica* against the test microorganisms containing Gram positive, Gram negative bacteria and yeasts, correlates with previous findings of other researchers (Ali et al., 2012; Banso, 2009; Kalaivani & Mathew, 2010; Kavitha et al., 2013; Okoro et al., 2014; Oladosu et al., 2013; Sadiq, Tarning, et al., 2017; Sharma et al., 2014; Yadav et al., 2015; Zheleva-Dimitrova et al., 2021). The ethanol extract of leaves, pods and barks of *A. nilotica* showed good antimicrobial activity even against drug resistant pathogens obtained from both food and clinical sources (Deshpande, 2013; Sadiq, Tharaphan, et al., 2017). *A. ataxacantha* (AA) ethanol extract also displayed good antimicrobial activity in this study and the results supports the findings reporting good antimicrobial activity of extracts and compounds from different parts of this plant against a range of pathogenic Gram positive, Gram negative fungi microbes (Aba et al., 2015; Amoussa et al., 2014, 2016a, 2016b; Zheleva-Dimitrova et al., 2021). The extracts of *A. sieberiana* (AS), equally had good antimicrobial activities. Various extracts of *A. sieberiana* have shown to be able to inhibit growth or kill fungi as well as Gram (+) and Gram (−) bacteria and the phytochemical compounds present in the extracts are considered to be responsible (Traoré et al., 2015a; Mshelia et al., 2017; Asase et al., 2008; Mary-Ann et al., 2019). *Cassia sieberiana* has been shown to contain saponins, tannins, polyphenols, flavonoids, alkaloids, steroids and triterpenes and these compounds were suggested to be responsible for the antibacterial activity of the plant extract acting as efflux pump substrates (Ambadiang et al., 2020). The microorganisms that can affect both human and nonhuman primates including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were shown to be highly susceptible to ethanol extracts of some plants including ethanol extracts of *A. sieberiana* (Kirabo et al., 2018). Furthermore, the antimicrobial activity of *A. nilotica* was said to higher than that of other acacia species (Saini et al., 2008). However in our study, the antimicrobial activity for *A. ataxacantha* (AA) and *A. sieberiana* (AS), was found to be higher than that of *A. nilotica* (AN) on some pathogens, such as *C. albicans, S. aureus* and *E. faecalis*. *A. sieberiana* (AS) was also more active than *Acacia nilotica* (AN) on S. Typhi. Despite the numerous studies of the antimicrobial activity of various acacia species, there are no clear evaluations of their antibiofilm activities. Biofilm are protective coatings made up of three dimensional extracellular matrix containing nucleic acids, polysaccharides and proteins within which are structured bacterial cells that adhere to each other as well as unto an attachment and the bacterial cells inside the biofilm show resistance to antibiotics and can escape from hosts immune system (Selvaraj et al., 2020). Antibiofilm assays are performed at MIC and sub-MIC concentrations so as to eliminate the hypothesis of bactericidal effect of extracts that occurs at high concentrations (Kocak et al., 2021; Tamfu, Ceylan, Kucukaydin, et al., 2020). Most of the existing antibiotics can do away with the effects of planktonic bacteria, but are unable to inhibit biofilm formation or destroy established biofilms and the infections will resurface, whenever conditions are favorable and sessile biofilm communities break out and continue to attack the host and spread further (Ceylan et al., 2020; Miquel et al., 2016; Ngenge et al., 2021; Popova et al., 2021; Tamfu, Munvera, et al., 2022). Because of the ease of formation of biofilms on various surfaces and their resistance to antibiotics, it is necessary to seek new antimicrobial materials which are capable of inhibiting biofilms and many phenolic extracts of medicinal and food plants are finding applications in this domain (Arab et al., 2022; Tamfu, Ceylan, et al., 2022). The ethanol extracts of the three acacia species, *A. sieberiana* **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA),** have shown moderate to good antibiofilm activity and this is a very potential information of interest, since the extracts can reduce the adherence of the pathogens on both living and nonliving surfaces and reduce their virulence factors instead of inhibiting growth, thereby giving very little chance for the development of resistance. Also, inhibiting motility which preceeds biofilm formation and inhibiting biofilms themselves indicate that the plant extracts can be used to increase the exposure of the bacteria cells to antibiotics by reducing the toughness or impermeability of the biofilms or by eliminating the biofilms.

3.6. Enzyme inhibitory activities

The breakdown of acetylcholine, which is an important neurotransmitter that interferes in central and peripheral nervous systems, could lead to serious complications such as Alzheimer's disease and substances which can inhibit cholinesterases are employed as remedy. In this study, the anticholinesterase potential of the test samples were evaluated against two cholinesterases namely acetylchlinesterase (AChE) and butyrylcholinesterase (BChE) and their percentage inhibitions at 100 μg/mL as well as the IC_{50} were reported on Table 7. AN had the highest inhibition on AChE with and IC_{50} of 71.33 ± 0.47 μg/mL while AS had IC_{50} of 82.54 ± 0.75 μg/mL as compared to the standard galantamine (5.50 ± 0.20 μg/mL) used. AA did not show an IC_{50} within test concentrations for both AChE and BChE. Only IC_{50} values for AN (55.81 ± 0.76 μg/mL) and AS (68.91 ± 0.80 μg/mL) were determined within tested conventrations on BChE and the values were good compared to that of galantamine (42.20 ± 0.44 μg/mL).

Two key enzymes, α-glycosidase and α-amylase are responsible for breakdown of carbohydrates into sugars especially glucose and inhibition of these two enzymes can slow the conversion of carbohydrates into glucose and lower blood glucose blood levels, hence, a suitable strategy to remedy type 2 diabetes. Inhibitions were by all samples on the α-glycosidase and α-amylase enzymes at appreciably levels within tested concentrations with highest sample concentration of 100 μg/mL, although IC_{50} values were observed only for AN (58.33 ± 0.27) and AS (40.57 ± 0.63), compared to acarbose (20.52 ± 0.84 μg/mL) on α-glycosidase and IC_{50} value determined only for AS (54.73 ± 0.47 μg/mL) compared to acarbose (32.57 ± 0.78 μg/mL) on α-amylase.

Certain pathogenic bacteria are ureolytic, that is, they produce

Table 7
Antidiabetic, anticholinesterase, anti-urease and anti-tyrosinase activities of the extracts.

Extracts/ Standards	Cholinesterase inhibitory activity				Anti-diabetic activity				Urease inhibitory		Tyrosinase inhibitory	
	AChE		BChE		α-glucosidase		α-amylase					
	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)	Inhibition (%) (at 100 μg/mL)	IC_{50} (μg/mL)
AN	59.75 ± 0.73[b]	71.33 ± 0.47	68.88 ± 0.45[c]	55.81 ± 0.76	65.78 ± 0.73[b]	58.33 ± 0.27	40.62 ± 0.93[e]	>100	73.64 ± 0.87[d]	44.36 ± 0.84	39.94 ± 0.57[b]	>100
AA	17.11 ± 0.61[d]	>100	22.43 ± 0.78[b]	>200	37.15 ± 0.85[c]	>100	33.43 ± 0.41[c]	>100	21.65 ± 0.36[e]	>100	27.54 ± 0.70[b]	>100
AS	55.61 ± 0.90[e]	82.54 ± 0.75	63.71 ± 0.52[b]	68.91 ± 0.80	75.91 ± 0.56[a]	40.57 ± 0.63	66.82 ± 0.35[b]	54.73 ± 0.47	77.82 ± 0.93[c]	35.96 ± 0.78	45.22 ± 0.68[c]	>100
Galantamine	85.48 ± 0.62[a]	5.50 ± 0.20	74.63 ± 0.25[a]	42.20 ± 0.44	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*
Acarbose	*NT*	*NT*	*NT*	*NT*	82.40 ± 0.70[b]	20.52 ± 0.84	77.59 ± 1.10[a]	32.57 ± 0.78	*NT*	*NT*	*NT*	*NT*
Thiourea	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	83.84 ± 0.58[c]	8.20 ± 0.32	*NT*	*NT*
Kojic acid	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	79.48 ± 0.32[b]	23.75 ± 0.24

Different subscripts in the same row indicate significant differences ($p < 0.05$).
NT: not tested.

urease which enables their survival and hence inhibiting urease could help to combat them. Percentage inhibitions of urease by test samples at 100 μg/mL indicates good activity with IC_{50} values of 44.36 ± 0.84 (AN) and 35.96 ± 0.78 (AS) compared to that of the standard which was 8.20 ± 0.32 (thiourea).

Tyrosinase intervene in some hyperpigmentation, browning of fruits, skin diseases and skin bleaching. It is beneficial to seek tyrosinase inhibitors which can slow down the processes. The extracts of the various acacia species under investigation exhibited moderate tyrosinase inhibitions at the tested concentrations and at 100 μg/mL, AN (39.94 ± 0.57%), AA (27.54 ± 0.70%) and AS (45.22 ± 0.68%) exhibited substantial inhibitions when compared to the standard kojic acid (79.48 ± 0.32%).

A. sieberiana **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA),** are amongst the various plants popurlarly used in Africa to treat neuropsychiatric health problems because they have psychoactive properties and therefore can have effects on the nervous system and the brain (Kinda et al., 2017). This suggests that they can act as cholinesterase inhibitors. Cholinesterase is responsible for the breakdown of acetylcholine and the reduction of acetylcholine which leads to adverse effects within the central nervous system and causing illnesses such as Alzheimer's disease (AD) associated with dementia (Tamfu, Kucukaydin, Yeskaliyeva, et al., 2021). Various anticholinesterases from natural and synthetic sources are employed as remedy to this situation (Maleu et al., 2022; Sharma, 2019; Tamfu, Kucukaydin, Yeskaliyeva, et al., 2021). *A. sieberiana* (AS) and *A. nilotica* (AN) demostrated very good anticholinesterase activity and were capable of significantly inhibiting acetylcholinestaerase and butyrylcholinesterase meanwhile the anticholinesterase activity of *A. ataxacantha* (AA) was low. The results here agree with those reported previously where *A. ataxacantha* had low AChE activity while *A. nilotica* had high AChE inhibition (Zheleva-Dimitrova et al., 2021). It is beleived and has been proven scientifically that acetylcholinesterase inhibition is a suitable treatment of Alzheimer's disease and *A. nilotica* is capable of inhibiting AChE and exhibiting an effect on the central nervous system (Crowch & Okello, 2009; Osama et al., 2015). Different solvent extracts including ethanol extracts of *A. sieberiana* and *A. nilotica* extracts amongst other plant extracts displayed good anticholinesterase activities and these plants are used traditionally to improve cognitive functions and remedy some symptoms usually associated with Alzheimer's disease (Eldeen et al., 2005). The various phenolic compounds present in the extracts under investigation are amongst the chemical compounds responsible for anticholinesterase activities. Cholinergic deficiency is responsible for brain deterioration, memory impairment and AD that affects over 25 million people in the world and therefore it is necessary to seek for long-term treatment with great efficiency and few side effects, which can inhibit cholinesterase and remove amyloid-beta(Aβ) deposits (Tamfu, Kucukaydin, Yeskaliyeva, et al., 2021).

While α-amylase breaks down long-chain carbohydrates, α-glucosidase converts carbohydrate to glucose directly in the small intestine and so both enzymes are responsible for the transformation of starchy foods and carbohydrates into sugars which will subsequently increase the blood sugar levels when absorbed (Khan et al., 2022; Tundis et al., 2015; Yao et al., 2010). Although all the extracts showed appreciable percentage inhibitions within the tested concentrations with maximum concentration of 100 μg/mL, *A. sieberiana* (AS) was the most active of all with IC_{50} value of 40.57 ± 0.63 μg/mL and 54.73 ± 0.47 μg/mL in the α-glucosidase and α-amylase inhibitory assays respectively. Some of the tested Acacia species extracts have demonstrated carbohydrate digestive enzyme inhibitions in several reports and the results vary from one study to another depending on extraction solvent and plant origin and activity was attributed to phenolic compounds (Ali et al., 2012; Majeed et al., 2021; Roozbeh et al., 2017; Saha et al., 2018; Salehi et al., 2019; Zheleva-Dimitrova et al., 2021). Our results show lower activity for ethanolic extract of the stem bark of *A. nilotica* compared those obtained elsewhere (Jaiswal et al., 2012). The results for moderate antidiabetic potential demonstrated through inhibition of both support the findings reported previously that *Acacia ataxacantha* (AA) extracts have antidiabetic potential (Arise et al., 2014). Inhibition of α-glucosidase and α-amylase can slow down the starch hydrolysis and glucose generation and is considered as a suitable approach for controlling blood glucose levels in the blood of Type 2 diabetes mellitus patients (Quan et al., 2019). Though some drugs such as acarbose and miglitol can be used to remedy for the situation, they are usually unavailable to some populations which could rely on effective plant medications and plant and their compounds have been exploited for this purpose (Feunaing et al., 2021; Salehi et al., 2019; Tamfu, Roland, et al., 2022; Tchuente Djoko et al., 2021). The findings here indicate the potential of the ethanol extracts of *A. sieberiana* **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA),** in inhibiting the carbohydrate metabolic enzymes and therefore being able to lower post-prandial glucose levels in the blood as a suitable remedy for hyperglycemia. The phenolic compounds in the plant extracts could be responsible for the antidiabetic potential of the plant extracts since phenolic compounds are amongst the potential inhibitors

of α-glucosidase and α-amylase (He et al., 2007; McDougall et al., 2005).

A. sieberiana **(AS)** and *A. nilotica* **(AN)** displayed very good antiurease activity while that of *A. ataxacantha* **(AA)** was low. The same trend was observed in the tyrosinase inhibition. In the presence of water, urease, a nickel-dependent enzyme, hydrolyzes urea compound to give ammonia and carbon dioxide. Ureolytic bacteria cause various infectious diseases most especially the pathogenic bacteria *Helicobacter pylori*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Svane et al., 2020). Urease activity increases the production of ammonia which promotes the activity of *Helicobacter pylori* creating damage of stomach mucosa, gastric and peptic ulcers as well as gastroduodenal infection (Yang et al., 2022). Therefore, the consumption of these acacia species as nutraceuticals with good urease activity could help to control certain infectious diseases caused by ureolytic bacteria in the digestive system and the urinary system. In agriculture, ureolytic bacteria can also cause great loss of nitrogen from fertilizers and manure.

Tyrosinase is a multifunctional glycosylated and copper-containing enzyme which plays a key role in melanogenesis but an overproduction of melanin is liable to create skin problems like disorders which can be treated with skin-whitening products and substances that reduce hyperpigmentation such as tyrosinase inhibitors (Obaid et al., 2021). Tyrosinase inhibition is a suitable approach to cure hyperpigmentation and phenolic compounds and their derivatives notably hydroxyl-substituted benzoic and cinnamic acid derivatives have been indicated as effective tyrosinase inhibitors (Nazir et al., 2022; Obaid et al., 2021). The extracts of *A. sieberiana* **(AS),** *A. nilotica* **(AN)** and *A. ataxacantha* **(AA)** under this study have shown to possess high amounts of phenolic compounds and coupled with their good antioxidant effects, suggests that the phenolic compounds are responsible for the tyrosinase inhibition. This is possible because the production of melanin in living systems can be initiated by free radicals and the inhibition of free radicals in an antioxidant process can reduce the production of melanin (Obaid et al., 2021). Various nutraceuticals and functional foods with excellent anti-tyrosinase activity are currently being developed since they are and one of their important mechanisms of action includes the inhibition of oxidation (Song et al., 2022). Tyrosinase inhibition can also help to slow down the ripening of fruits and skin problems arising from hyperpigmentation.

It is worthy to recall that phenolic compounds possess antioxidant and antimicrobial activities, and for this reason, phenolic rich extracts can find applications in cosmetic and food industries as additives which can be used as preservatives to increase the commercial shelf life of products and as biocontrol substances for food safety to reduce the growth of foodborne pathogens (Caballero-Guerrero et al., 2022).

4. Conclusion

Many Acacia species represent a source of gum Arabica and are used in many African communities as a local nutraceutical. Nutraceuticals or functional foods include nutritious foods and their excipients which possess important beneficial and protects the body from disease and nutrient deficiencies while promoting healthy growth and development. Ethanol was used as a suitable solvent for extraction of phenolic rich extracts from *Acacia sieberiana* (AS), *Acacia nilotica* (AN) and *Acacia ataxacantha* (AA). HPLC-DAD enabled the detection of a number of phenolic compounds and quantified them in the extracts and this showed good amounts of target phenolics quantified in mg/g of extract. All the extracts displayed good antioxidant activities measure through five complementary assays. The extracts were able to disrupt quorum sensing processes in bacteria as they inhibited violacein production possibly through signal production and reception in *C. violaceum* CV12472 and *C. violaceum* CV026. Good antimicrobial activities and inhibition of bacterial biofilms were exhibited on Gram (−), Gram (+) and candida species. These extracts showed potential to remedy Alzheimer's disease and diabetes type 2 as they respectively inhibited cholinesterases (AChE and BChE) and carbohydrate hydrolysis enzymes (α-amylase and α-glucosidase). The results here showed the relevance of the ethanol extracts of the acacia species in food applications as antimicrobial and nutraceuticals.

CRediT authorship contribution statement

Koudoro Yaya Alain: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Alfred Ngenge Tamfu:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Selcuk Kucukaydin:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Ozgur Ceylan:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Project administration. **Agbangnan Dossa Cokou Pascal:** Conceptualization, Formal analysis, Methodology, Investigation, Data curation, Supervision. **Avlessi Félicien:** Conceptualization, Formal analysis, Investigation, Methodology, Data curation, Supervision. **Sohounhloue Codjo Koko Dominique:** Conceptualization, Formal analysis, Investigation, Data curation, Supervision. **Mehmet Emin Duru:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Project administration, Supervision. **Rodica Mihaela Dinica:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Project administration, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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