Chemical constituents from fruits of *Cnestis ferruginea* Vahl ex. DC (Connaraceae) and evaluation of their anticholinesterase and antiradical activities

Thérèse Christelle Maleua, Rostan Mangoua Tallaa, Michael Hermann Kengne Kamdem, Tamfu Alfred Ngenge, Selcuk Kucukaydin, Edwin Mpho Mmutlane, Derek Tantoh Ndinteh, Celine Djama Mbazoaa and Jean Wandji

ABSTRACT

The phytochemical investigation of the DCM/MeOH (1:1) extract of the fruits of *Cnestis ferruginea* led to the isolation and characterization of one new quinic acid derivative, ferruginoic acid (1), together with six known compounds 2–7. Compounds 3–7 were reported for the first time from this species. The structures of compounds 1–7 were elucidated on the basis of 1D and 2D NMR spectroscopic data, mass spectrometry and by comparison of spectroscopic data with those from the literature. The anticholinesterase (AChE and BChE) activity and DPPH free radical scavenging assay of compounds 1, 3, 4 and 7 were evaluated. Ferruginoic acid (1) exhibited moderate anticholinesterase activity with IC50 value of 36.18 ± 1.78 μg/mL against AChE. Compounds 3, 4 and 7 showed high activity against free radical (DPPH) scavenging assay (DPPH) with IC50 values 40.09 ± 0.96 μg/mL, 61.70 ± 0.78 μg/mL and 41.87 ± 0.62 μg/mL respectively. These results indicate that *C. ferruginea* and its constituents could be employed in the management of Alzheimer’s disease.
1. Introduction

Alzheimer’s disease (AD) is the most common form of dementia mostly in old people, characterized by low acetylcholine levels and oxidative stress, involving progressive neurodegeneration with formation of amyloid-β deposits in the brain (Tamfu et al. 2020c; Birsan et al. 2021). Worldwide, more than 115 million people will be affected by this disease by 2050 with a majority of them aged above 65 years (Rahman and Choudhary 2015). A proper strategy to overcome AD is through the inhibition of the cholinesterase enzymes, both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which helps to increase acetylcholine levels in the brain and this is necessary for neurotransmission, memory, reasoning, and other cognitive activities (Uddin et al. 2021). Though, cholinesterase inhibitors, such as: rivastigmine, donepezil and galantamine are usually employed as remedy to AD, there is a growing interest in the search of new cholinesterase inhibitors from natural sources due to the draw backs of synthetic ones (Tamfu et al. 2019). Cnestis is a genus, belonging to Connaraceae family and includes about 13 species occurring mostly in the tropics of Africa and Asia (Wiart 2006). Plants of this genus commonly occur as lianes or shrubs and are widely distributed in the tropical regions of South America, Africa and Asia. Scientific studies of these plants reveal interesting biological and chemical potentials, thereby providing new drug leads (Nunes et al. 2020). Some species of this genus were reported to show antioxidant, antimicrobial, antifungal and hypoglycemic activities (Parvez and Rahman 1992; Nijveldt et al. 2001; Adisa et al. 2004, 2011). Various parts of C. ferruginea including roots, fruits, barks, branches and leaves are used in traditional medicine and have been reported as remedy for diabetes, wounds, diarrhea, periodontitis, headache, snakebites, gum pain, inflammatory conditions, syphilis, dysentery, gonorrhea, scabies, bronchitis, toothache, dysmenorrhea, pains, sinusitis, conjunctivitis and ear problems (Funsho et al. 2013; Catarino et al. 2016; Frazão-Moreira 2016; Ahmed 2017; Lautenschläger et al. 2018; Nunes et al. 2020). These properties of C. ferruginea have attracted much scientific studies and some biological assays have revealed antimicrobial, antioxidant, antiepileptic, anti-inflammatory, analgesic, laxative, anti-convulsant, aphrodisiac, hypoglycemic, hepatoprotective, antidepressant properties of the plants (Yakubu and Nurudeen 2012; Owope et al. 2016; Ahmed 2017; Akwasi et al. 2018; Ojo et al. 2019). Phytochemical investigation of some Cnestis species from Africa and Asia report the isolation and characterization of several classes of natural products such as phytosteroids, pentacyclic triterpenoids, flavonoids, coumarin, cinnamoyle (Adisa et al. 2011), and phenolic compounds (Soro et al. 2012).

In this study, C. ferruginea was subjected to phytochemical investigation, in search for bioactive secondary metabolites which were in turn evaluated for their anticholinesterase and DPPH radical scavenging activities. The methylene chloride/methanol (1:1) extract of the fruits of C. ferruginea was subjected to column chromatography, leading to the isolation and characterization of one new compound together with six known secondary metabolites. In addition, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities as well as DPPH radical scavenging activity of compounds 1, 3, 4 and 7 are reported.
2. Results and discussion

The DCM/MeOH (1:1) extract of the fruits of C. ferruginea was subjected to repeated silica gel column chromatography (CC) which afforded one new quinic acid derivative, together with six known secondary metabolites 1–7 (Figure 1).

Compound 1 was isolated as a brownish powder. The HRESIMS (Figure S1) shows the pseudo-molecular ion peak at \( m/z \) 175.0603 \([M+H]^+\) (calcd for \( C_7H_{11}O_5^+ \), 175.0606) from which the molecular formula \( C_7H_{10}O_5 \) was deduced. It contains three double bond equivalents. The \(^1\)H NMR spectrum of compound 1 (Figure S3) exhibits signals of three oxygen-methine protons at \( \delta_H \) 3.50 (1H, dd, \( J = 3.3, 9.5 \) Hz, H-4); \( \delta_H \) 3.99 (1H, td; \( J = 4.2, 9.9 \) Hz, H-3) and \( \delta_H \) 4.11 (1H, br t, \( J = 3.6 \) Hz, H-5); one diastereotopic methylene group at \( \delta_{Ha} \) 1.87 and \( \delta_{Hb} \) 2.10 (2H, dd, \( J = 11.0, 13.7 \) Hz H-2a, H-2b).

Figure 1. Chemical structure of compounds 1–7.
and one methylene group at \( \delta_H 2.01 \) (2H, m, H-7). The \(^{13}\)C NMR (Figure S2) spectrum of compound 1 shows signals of seven carbon atoms, and in combination with signals of DEPT 135 experiment (Figure S4) and HSQC spectrum (Figure S5), the carbons were distinguished and classified as one carbonyl carbon at \( \delta_C 177.7 \), one oxygenated quaternary carbon at \( \delta_C 75.6 \) (C-1), three oxygenated methine carbons at \( \delta_C 74.8 \) (C-3), 69.9 (C-4), 66.4 (C-5) and two methylene carbons at \( \delta_C 40.2 \) (C-2), 36.8 (C-7). Since the carbonyl carbon occupies one degree of unsaturation, it is suggested that compound 1 possesses a bicyclic ring system to attain the three double bond equivalents. In \(^1\)H-\(^1\)H COSY spectrum (Figure S6) cross correlations from the following pairs of adjacent protons H-2/H-3, H-3/H-4, H-4/H-5 and H-5/H-7 allowed the establishment of the long chain C-2/C-3/C-4/C-5/C-7. Moreover, the HMBC correlations of H-2 to C-1, C-3 and C-7; H-3 to C-2, C-4 and C-5; H-4 to C-2 and C-3; H-5 to C-1, C-3, C-4 and C-7; H-7 to C-1, C-4 and C-5 were used to unambiguously establish the membered rings of compound 1 (Figures S7 and S9). The relative stereochemistry of compound 1 was established based on the NOESY of H-2/H-4, H-2\( \alpha \)/H-3, H-4/H-7 and H-5/H-4 (Figures S8 and S9). Based on the \(^1\)H and \(^{13}\)C NMR data (Table S1), compound 1 was assigned the structure of 3,4-dihydroxy-6-oxabicyclo[3.1.1]heptane-1-carboxylic acid, an unknown quinic acid derivative, to which the trivial name ferruginoic acid is given. The biosynthesis pathway of compound 1 is proposed (Figure 2).

The known compounds \( \beta \)-sitosterol 3-O-\( \beta \)-D-glucopyranoside (2) (Oliveira et al. 2010), scopeletin (3) (El-Demerdash et al. 2009), vitexin (4), 3’-hydroxyvitexin (5) (Thenmozhi and Subasini 2016), bergenin (6) (Olugbade et al. 1982) and 2-(1,3,4,5-tetrahydroxycyclohexyl)acetic acid (7) (Figure 1) were identified by comparison of their NMR and MS data with those reported in the literature. The known compounds 3–7 were reported for the first time from \( C. ferruginea \).

Compounds 1, 3, 4 and 7 were evaluated for their anticholinesterase activity measured by spectrophotometric method on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) as well as their radical scavenging potential on DPPH* (2, 2-diphenyl-1-picrylhydrazylhydrazyl) free radical. It was shown previously that the antidepressant activity of \( C. ferruginea \) involves enzymatic action notable cholinergic, monoaminergic and L-arginine-nitric oxide pathways (Owope et al. 2016). The antidepressant-like activity of \( C. ferruginea \) equally involves antioxidant defense and neuroendocrine systems (Ishola et al. 2016). This information suggests that, this plant can possess enzyme inhibitory activity such as anticholinesterase activity. The results obtained (Table S2) show that, ferruginoic acid (1) exhibited moderate activity against anticholinesterase
by inhibiting acetylcholinesterase enzyme with \( IC_{50} \) values of \( 36.18 \pm 1.78 \, \mu g/mL \) compared to the standard Galantamine whose \( IC_{50} \) value was found to be \( 5.30 \pm 0.16 \, \mu g/mL \). Compound 1 exhibited moderate anticholinesterase by inhibiting butyrylcholinesterase enzyme with a percentage inhibition (\%) of the enzyme of \( 34.41 \pm 0.90\% \) at \( 50 \, \mu g/mL \). These type of low molecular weight terpenoids are major components of essential oils and are known to possess anticholinesterase activities because they are able to interact with AChE and BChE and act as either competitive or noncompetitive inhibitors of cholinesterase due to their lipophilicity and small molecular sizes, which makes them more likely to cross the blood-brain barrier and exert their effect (Alfred et al. 2021; Tamfu et al. 2021). This compound 1 also exhibited moderate activity in the free radical (DPPH\(^*\)) scavenging assay with a percentage inhibition (\%) of \( 19.39 \pm 0.30\% \) at \( 50 \, \mu g/mL \). Moreover, compounds 3, 4 and 7 showed good radical (DPPH\(^*\)) scavenging activity with \( IC_{50} \) values of \( 40.09 \pm 0.96 \, \mu g/mL \), \( 61.70 \pm 0.78 \, \mu g/mL \) and \( 41.87 \pm 0.62 \, \mu g/mL \) respectively compared to \( \alpha \)-tocopherol with an \( IC_{50} \) value of \( 45.31 \pm 0.17 \, \mu g/mL \). The same compounds (3, 4 and 7) exhibited weak activity against anticholinesterase activity determined on AChE and BChE at \( 50 \, \mu g/mL \) with percentage inhibition values of \( 10.25 \pm 0.55\% \), \( 14.37 \pm 0.21\% \) and \( 7.38 \pm 0.35\% \) respectively for AChE and \( 28.03 \pm 0.67\% \), \( 17.45 \pm 0.39\% \) and \( 20.96 \pm 0.83\% \) for BChE respectively. Antiradical and anticholinesterase potential of the compounds is an indication of their possible application as remedy for oxidative stress and Alzheimer’s disease (AD). Various medicinal plant extracts and natural compounds have gained special attention due to their specific modes of action and their advantages of low toxicity and high efficiency in the treatment of oxidative stress related ailments and neuroprotective diseases such as AD (Beddiah et al. 2021; Tamfu et al. 2021).

3. Experimental

3.1. General experimental procedures

High resolution mass spectrum was obtained with a TOF spectrometer (Bruker, South Africa) equipped with an ESI source. The spectrometer was operated in positive and negative modes (mass range: 50–1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 1 ppm deviation using Na Formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (4 L/min). 1 D NMR spectra (\(^1\)H NMR, 400 MHz; \(^{13}\)C NMR and DEPT 135; 100 MHz) and 2 D NMR spectra (HSQC, HMBC, COSY and NOESY) were measured on a Bruker Top Spin 3.0 spectrometer equipped with cryoprobe, with TMS as an internal reference. Chemical shifts (\( \delta \)) are expressed in ppm with reference to TMS and coupling constants (\( J \)) are given in Hz. Column chromatography (CC) were carried out on silica gel (0.040-0.063 mm). Thin layer chromatography (TLC) were performed on Merck precoated silica gel 60F\(_{254}\) aluminum. Spots were visualized under UV light (254 and 366 nm) and by spraying the plates with 10% \( H_2SO_4 \) in EtOH followed by heating at about 110°C.
3.2. Plant material

The fruits of *C. ferruginea* were collected in Kribi locality in the South Region of Cameroon, during the month of December 2013 and was identified by Victor Nana, a botanist of National Herbarium of Cameroon, where a voucher specimen was deposited under the reference 2003/SRF K.

3.3. Extraction and isolation

The fruits were chopped, air-dried and pulverized to yield 578.0 g of powder. The resulting powder was macerated three consecutive times in 7.0 L of a mixture of methylene chloride/methanol (1:1) for 72 h at room temperature. The solvent was evaporated under vacuum to afford 83.0 g of methylene chloride/methanol crude extract. A portion of the methylene chloride/methanol extract (80.0 g) was subjected to column chromatography (CC, size: 90 cm, diameter: 5.50 cm) over silica gel (350.0 g) and eluted with a mixture of *n*-hexane-ethyl acetate and ethyl acetate-methanol gradients polarity. A total 100 fractions of 125 mL each were collected and combined according to TLC profile monitoring to four sub-fractions (F1 to F5). Sub-fraction F2 (3.6 g) was purified employing a step gradient of *n*-hexane-ethyl acetate and ethyl acetate-methanol to yield (3) (3.1 mg) and (2) (19.2 mg). Sub-fraction F3 eluted with a gradient ethyl acetate-methanol yielded (4) (4.2 mg), (5) (2.8 mg), (6) (2.7 mg). Sub-fraction F5 (28.0 g) was subjected to column chromatography on silica gel employing a step gradient of methylene chloride-methanol as eluent, to yield (7) (5.1 mg) and (1) (8.5 mg).

3.4. Ferruginoic acid (1)

Brownish powder. $^1$H NMR (400 MHz, D$_2$O). $\delta_{H}$ (ppm): 3.50 (1H, dd, $J = 3.3$, 9.5 Hz, H-4); $\delta_{H}$ 3.99, (1H, td; $J = 4.2$, 9.9 Hz, H-3) and $\delta_{H}$ 4.11 (1H, br t, $J = 3.6$ Hz, H-5); $\delta_{H}$ 1.87 and 2.10 (2H, dd, $J = 11.0$, 13.7 Hz H-2a, H-2b), $\delta_{H}$ 2.01 (2H, m, H-7). $^{13}$C NMR (100 MHz, D$_2$O) $\delta_{C}$ (ppm): 177.7, $\delta_{C}$: 75.6 (C-1), $\delta_{C}$: 74.8 (C-3), $\delta_{C}$: 69.9 (C-4), $\delta_{C}$: 66.4 (C-5), $\delta_{C}$: 40.2 (C-2), $\delta_{C}$: 36.8 (C-7). (+) HRESIMS m/z [M + H]$^+$ 175.0603 (C$_7$H$_{11}$O$_5$$^+$ calcd. 175.0606).

3.5. Anticholinesterase activity

The anticholinesterase activity was measured spectrophotometrically by determining acetylcholinesterase and butyrylcholinesterase enzyme inhibition according to the method described by Ellman with minor modifications (Ellman et al. 1961; Tamfu et al. 2020a). Briefly, 130 μL of 100 mM sodium phosphate buffer (pH 8.0), 10 μL of sample solution dissolved in ethanol at various concentrations, and 20 μL of enzyme (AChE or BChE) solution in buffer were mixed and incubated for 15 min at 25°C, followed by 20 μL of 0.5 mM DTNB (5,5'-Dithio-bis (2-nitrobenzoic) acid) was added. The reaction was then initiated by addition of 0.71 mM, 20 μL of acetylthiocholine iodide, or 0.2 mM, 20 μL of butyrylthiocholine chloride. The formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, was monitored spectrophotometrically using a 96-well microplate reader at a
wavelength of 412 nm. The results were expressed as a percentage inhibition (%) of the enzyme at 50 μg/mL concentration of the compounds.

3.6. DPPH free radical scavenging assay

The free radical scavenging activity of the compounds were determined by the DPPH• (2,2-diphenyl-1-picrylhydrazylhydrazyl) assay as described previously (Tamfu et al. 2020b). In its radical form, DPPH• absorbs at 517 nm, but upon reduction by an antioxidant or antiradical species its absorption decreases. Briefly, a 0.1 mmol/L solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of samples solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

\[
\text{DPPH radical scavenging} \% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

4. Conclusion

Oxidative stress occurs as a result of imbalance between radical species production and antioxidants defense and causes several chronic diseases such as AD. Inhibition of radicals can be a possible solution to these diseases and many plants and their compounds have found applications as natural antioxidants used in the management of oxidative stress related illnesses. In this study, C. ferruginea fruits crude extract and compounds were evaluated for antiradical and anticholinesterase activities. Ferruginoic acid (1) exhibited moderate anticholinesterase activity while compounds 3, 4 and 7 showed good radical (DPPH•) scavenging activity. The biological activities of some isolated compounds as cholinesterase (AChE and BChE) inhibitors, key enzymes involved in hydrolysis of choline leading to AD, partially justify the use of the plant in the treatment of oxidative stress diseases.

Acknowledgements

The authors are grateful to Assoc. Prof. Derek Tantoh Ndinteh, Centre for Natural Product Research (CNPR), Chemical Sciences Department, University of Johannesburg, South Africa for performing NMR analyses and mass of compounds.

Disclosure statement

The authors declare no conflict of interest.

Funding

The author(s) reported there is no funding associated with the work featured in this article.
References

Adisa R, Abass Khan A, Oladosu I, Ajaz A, Choudhary MI, Olorunsogo O, Rahman AU. 2011. Purification et caractérisation des composés phénoliques des feuilles de *Cnestis ferruginea* (De Candolle): Enquête de la propriété antioxydante. Res J Phytochem. 5(4):177–189.

Adisa R, Choudhary E, Adewoye O. 2004. Propriétés hypoglycémiques et biochimiques de *Cnestis ferruginea*. Afr J Tradit Altern Med. 7:185–194.

Ahmed HA. 2017. Therapeutic potentials of *Cnestis ferruginea*: a review. J Pharmacogn Phytochem. 6(6):1397–1401.

Akwasi A, Samuel O, Clement OA, Kennedy AB. 2018. Antioxidant, antimicrobial and FTIR analysis of methanol root extract of *Cnestis ferruginea* and ethanol root extract of *Citrus limon*. J Pharmacogn Phytochem. 7(4):2938–2946.

Alfred NT, Kucukaydin S, Ceylan O, Duru ME. 2021. Evaluation of enzyme inhibition and anti-quorum sensing potentials of *Melaleuca alternifolia* and *Citrus sinensis* essential oils. Nat Prod Commun. 16(9):1–8.

Beddiah H, Boudiba S, Benahmed M, Tamfu AN, Ceylan O, Hanini K, Kucukaydin S, Elomri A, Bensouici C, Laouer H, et al. 2021. Chemical composition, anti-quorum sensing, enzyme inhibitory, and antioxidant properties of phenolic extracts of *Clinopodium nepeta* L. Kuntze. Plants. 10(9):1955..

Birsan RI, Wilde P, Waldron KW, Rai DK. 2021. Anticholinesterase activities of different solvent extracts of brewer’s spent grain. Foods. 10(5):930..

Catarino L, Havik PJ, Romeiras MM. 2016. Medicinal plants of Guinea-Bissau: therapeutic applications, ethnic diversity and knowledge transfer. J Ethnopharmacol. 183:71–94..

El-Demerdash A, Dawidar AM, Keshk EM, Abdel-Mogib M. 2009. Coumarins from *Cynanchum acutum*. Latin Am J Chem. 37(1):65–69.

Ellman GL, Courtney KD, Andres V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88–95..

Frazão-Moreira A. 2016. The symbolic efficacy of medicinal plants: practices, knowledge, and religious beliefs amongst the Nalu healers of Guinea-Bissau. J Ethnobiol Ethnomed. 12(1):24..

Funsho OO, Yinusa R, Olajire AA, Mathew OO. 2013. Haematological and some biochemical profiles in male rats treated with *Cnestis ferruginea* (de Candolle) root extract and its pure fractions. African J Pharm Pharmacol. 7(20):1231–1235.

Ishola IO, Akinleye MO, Oduola MD, Adeyemi OO. 2016. Roles of monoaminergic, antioxidant defense and neuroendocrine systems in antidepressant-like effect of *Cnestis ferruginea* Vahl ex DC (Connaraceae) in rats. Biomed Pharmacother. 83:340–348.

Lautenschläger T, Monizzi M, Pedro M, Mandombe JL, Brânquima MF, Heinze C, Neinhuis C. 2018. First large-scale ethnobotanical survey in the province of Uige, Northern Angola. J Ethnobot Ethnomed. 14(1):51..

Nijveldt RJ, Nood E, Hoorn P, Boelens G, Pam L. 2001. La mesure de la protéine avec du phénol de Folin réactif. J Biol Chem. 193:265–275.

Nunes APLF, Patrocínio TCA, Lima da Paz JR, Picolotto A, Ballardin G, Souza VC, Salvador M, Moura S. 2020. Connaraceae: an updated overview of research and the pharmacological potential of 39 species. J Ethnopharmacol. 261:112980..

Ojo ES, Ishola IO, Ben-Azu B, Afolayan OO, James AB, Ajayi AM, Umukoro S, Adeyemi OO. 2019. Ameliorative influence of *Cnestis ferruginea* vahl ex DC (Connaraceae) root extract on kainic acid-induced temporal lobe epilepsy in mice: Role of oxidative stress and neuroinflammation. J Ethnopharmacol. 243:112117.
Oliveria PV, Jesus CJF, Fabyanne SM, Gerson SL, Fernando MO, Patricia ESO, Lucia MC, Ana MG, Rosangela PLL. 2010. Larvicidal activity of 94 extracts from ten plant species of northeastern Brazil against Aedes aegypti L. (Diptera: Culicidae). Parasitol Res. 107(2):403–407.

Olugbade TA, Oluwadiya JO, Yisak WA. 1982. Constituants chimiques de Cnestis ferruginea DC. Fraction de l’ether de petrole I. J Ethnopharmacol. 6(3):365–370.

Owope TE, Ishola IO, Akinleye MO, Oyebade R, Adeyemi OO. 2016. Antidepressant effect of Cnestis ferruginea Vahl ex DC (Connaraceae): involvement of cholinergic, monoaminergic and l-arginine-nitric oxide pathways. Drug Res (Stuttg). 66(05):235–245.

Parvez M, Rahman A. 1992. A novel antimicrobial isoflavone galactoside from Cnestis ferruginea (Connaraceae). J Chem Soc Pak. 14:221–223.

Rahman AU, Choudhary MI. 2015. (Eds.) Drug design and discovery in Alzheimer’s disease; Elsevier: Amsterdam, The Netherlands; p. 784.

Soro Y, Kassi ABB, Bamba F, Siaka S, Touré SA, Coustard JM. 2012. Flavonoids and gallic acid from leaves of Santaloides afzelii (Connaraceae). Rasayan J Chem. 5(3):332–337.

Tamfu AN, Ceylan O, Fru GC, Ozturk M, Duru ME, Shaheen F. 2020a. Antibiofilm, anti-quorum sensing and antioxidant activity of secondary metabolites from seeds of Annona senegalensis, Persoon. Microb Pathog. 144:104191.

Tamfu AN, Ceylan O, Kucukaydin S, Duru ME. 2020. HPLC-DAD phenolic profiles, antibiotic, anti-quorum sensing and enzyme inhibitory potentials of Camellia sinensis (L.) O. Kuntze and Curcuma longa L. LWT-Food Sci Technol. 133:110150.

Tamfu AN, Ceylan O, Kucukaydin S, Ozturk M, Duru ME, Dinica RM. 2020c. Antibiofilm and enzyme inhibitory potentials of two Annonaceous food spices, African pepper (Xylopia aethiopica) and African nutmeg (Monodora myristica). Foods. 9(12):1768.

Uddin MJ, Russo D, Rahman MM, Uddin SB, Halim MA, Zidorn C, Milella L. 2021. Anticholinesterase activity of eight medicinal plant species: in vitro and in silico studies in the search for therapeutic agents against Alzheimer’s Disease. Evid Based Complement Alternat Med. 2021:9995614.

Wiart C. 2006. Medicinal plants of Asia and Pacific. London: CRC Press.

Yakubu MT, Nurudeen QO. 2012. Effects of aqueous extract of Cnestis ferruginea (Vahl ex De Cantolle) root on paroxetine-induced sexual dysfunction in male rats. Asian Pac J Reprod. 1(2):111–116.