A new abietane-type diterpenoid from roots of *Burkea africana* Hook (Fabaceae) with \(\alpha\)-amylase inhibitory potential

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**ABSTRACT**

A new abietane-type diterpenoid, rubesanolidic acid (1), alongside six known compounds including \(\beta\)-sitosterol (2), lupeol (3), betulinic acid (4) ursolic acid (5), \(\beta\)-sitosterol 3-\(\beta\)-D-glucopyranoside (6) and stigmasterol 3-\(\beta\)-D-glucopyranoside (7) were isolated from the roots of *Burkea africana* through column chromatography. Their structures were elucidated from spectroscopic analyses (UV, IR, MS, 1D and 2D NMR) data and by comparison with data from previous studies. The extract and compounds were tested for their \(\alpha\)-amylase inhibition. The extract was more active than the isolated compounds with a percentage inhibition of 51.0 ± 2.5% at 400 \(\mu\)g/mL and was the only sample showing above 50% inhibition at this dose. Amongst the isolated compounds and at the dose of 400 \(\mu\)g/mL, the new diterpenoid Rubesanolidic acid exhibited the highest percentage inhibition of \(\alpha\)-amylase of 38.2 ± 2.0% while \(\beta\)-sitosterol showed the lowest inhibition of 9.6 ± 0.5%. The results indicate that *B. africana* is a potential source of antidiabetic compounds.
1. Introduction

The Fabaceae is a family of flowering leguminous plants commonly referred to as bean or pea family of plants comprising of about 18,000 species classified into over 650 genera (Abouelela et al. 2019). Fabaceae plants are mostly herbs but include also shrubs and trees found in both temperate and tropical areas. *Burkea africana* is an example of Fabaceae plant of average height that can attain 10–12 meters and at times up to 20 meters. It is known in North Cameroun under traditional names such as ‘Gourong’ in Moundang language and ‘Jorokijigahi’ in ‘fulfude’ language. The parts of this plant are used in Africa for the treatment of various ailments. The barks, roots and leaves are used in treating cough, migrain, epilepsy, vertigo, inflammation, headache and gonorrhea. The stems are used as an antidote for stings and venomous bites, parasitic skin infections, convulsions and pulmonary problems (Elin et al. 2002; Yaro et al. 2016). In Australia, various parts of *Burkea africana* are used for tooth ache, stomach problems, antidote, cough and catarrh, gonorrhoea and syphillis. The stem barks have been shown to have antidiarrhea, antioxidant and antibacterial activities (Mair et al. 2018). The extracts and compounds from *B. africana* have been shown to possess anticancer, analgesic, antibacterial, antiproliferative, cytotoxic and antioxidant activities (Eboji et al. 2017).

From *Burkea africana*, mostly flavonoids and saponins have been isolated (Mair et al. 2018; Eboji et al. 2020). Plants of the Fabaceae family in general and the genus Burkea in particular are known to be rich in diverse secondary metabolites principally flavonoids (Werner 2012), saponins (Mair et al. 2018) and tannins (Elin et al. 2002) which are classes of compounds known for biological activities including antidiabetic property. Although diabetes mellitus can be managed using oral hypoglycemic agents. These medications can have some undesirable side effects, and so scientists are looking for alternative therapies from plants and other natural sources which are considered to have less severe or no side effects (Singh et al. 2015; Bindu and Narendhirakannan 2019). The phytochemicals contained in medicinal plants used for management of diabetic disorders are responsible for this activity. For this reason, this study involves the isolation and characterisation of secondary metabolites from *B. africana* and evaluation of their inhibitory potential on α-amylase.
2. Results and discussion

The structures of the compounds isolated from roots of *B. africana* were determined and are presented in Figure 1. A total of seven compounds were isolated including a new abietane-type diterpene acid, rubesanolidic acid (1) together with six known compounds: \( \beta \)-sitosterol \( 2 \) (Habib et al. 2007), lupeol \( 3 \) (Talla et al. 2017), betulinic acid \( 4 \) (Tamfu et al. 2020), ursolic acid \( 5 \) (Tamfu et al. 2020); \( \beta \)-sitosterol 3-\( \beta \)-D-glucopyranoside \( 6 \) and stigmasterol 3-\( \beta \)-D-glucopyranoside \( 7 \) (Ngenge et al. 2019; Eve et al. 2020).

Compound 1 was obtained in the eluent system hexane/ethyl acetate (90/10) in the form of white powder and was soluble in chloroform. Its HREI-MS presents a pseudo-molecular ion peak \([M + H]^+\) at \( m/z = 333.2240 \) (Calcd for \( C_{20}H_{28}O_4 + H \), 333.2234) and another diagnostic peak at \([M + Na]^+\) at \( m/z = 355.2066 \) compatible with the molecular formula of the adduct \( C_{20}H_{28}O_4 + Na \) with seven double bond equivalents. The IR spectrum shows 2 absorption maxima between 1650 and 1800 cm\(^{-1}\) indicating two carbonyl groups (C=O) for the acid and the ester functional groups.

The NMR spectra of compound 1 showed characteristic features of an abietane-type diterpenoid closely related to Rubesanolide E previously described from *Isodon*...
rubescens (Zou et al. 2012). The presence of the lactone system in compound 1 was confirmed by the presence of the ester carbonyl at δC 179.4 ppm (C-20) together with the carbon signals at 83.8 ppm (C-8) and 48.9 ppm (C-10) and their positions were confirmed via HMBC correlations with H-9 (δH 1.77 ppm, m, 2H). Compound 1 differed from Rubesanolide E by a rearrangement effected on the side chain whereby the methyl group at position 17 migrated to the olefinic carbon C-14, leaving the methyl protons of H-16 to appear as a triplet at δH 0.99 ppm confirmed by HMBC and COSY cross-peaks with H-15 (δH 2.00 ppm, m, 2H). The signals of the now olefinic methyl proton H-17, appeared at δH 1.72 ppm as a singlet of three protons and exhibited HMBC correlation signals with the olefinic carbons at δC 140.87 ppm (C-14) and δC 123.82 ppm (C-13). Another difference with Rubesanolide E was the absence of signals of the methyl group protons at position 18 and the appearance of carboxyl group at δC 182.9 ppm attributable to a carboxylic acid function thereby suggesting the presence of acid group at position 18 which was confirmed by HMBC correlations of H-3, H-5 and H-19 with this carboxylic acid function. In Rubesanolides C and E, the methyl 18 is in the beta position, and methyl 19 in the alpha position, therefore the absence in this compound, of the NOESY correlation between H-18 and the proton H-5 that exists in Rubesanolide E, reinforces the orientation of the acid function at position 18 behind the plane and the orientation of the methyl group at position 19 in front of the plane. There is no observed NOESY correlation between H-5 and the methyl 19, therefore methyl 19 must be in the beta position. This could be supported by the observable NOESY correlation peaks between H-19 (1.29 ppm) with H-1β (1.95 ppm) and H-2β (2.18 ppm). The relative configuration was further supported by the ROESY experiment spectrum, which showed correlation peaks between the following pairs of protons: H-5α (2.27 ppm) and H-1α (1.18 ppm) and H-6α (1.72 ppm). In addition, correlation peak was observed between H-1β (1.95 ppm) and H-19 α/β (1.29 ppm) which substantiates the α-orientation of this methyl group. H-6β (1.78 ppm) and H-7β (2.38 ppm) showed correlation peaks on the ROESY spectrum.

Furthermore, the 1H NMR spectrum of compound 1 indicated signals corresponding to methyl groups at δH 0.99 ppm (H-16; t), 1.30 ppm (H-19; s) and 1.71 ppm (H-17; s). Two double of doublets signals appeared at δH 2.27 (1H, dd, J = 2.28 Hz) and 2.39 (1H, dd, J = 2.38 Hz) corresponding to the protons H-5 and H-7 respectively. The analysis of the 13C NMR spectrum broadband showed signals of 20 carbon atoms, characteristic of diterpenes. The 13C NMR together with DEPT135 experiments enabled the attribution of characteristic carbon atoms including: three methyl groups at δC 11.9 ppm (C-16), 12.9 ppm (C-17) and 14.9 ppm (C-19). Eight methylene carbon signals appeared at δC 27.5 ppm (C-1), 17.5 ppm (C-2), 37.2 ppm (C-3), 23.0 ppm (C-6), 31.8 ppm (C-7), 19.9 ppm (C-11), 27.9 ppm (C-12) and 26.8 ppm (C-15). Other signals corresponding to two sp3 methine carbon atoms δC 44.8 ppm (C-5) and 55.8 ppm (C-9). Signals of seven quaternary carbon signals were deduced and grouped as follows: four sp2 carbon atoms at δC 123.8 ppm (C-13), 140.9 ppm (C-14), 182.9 ppm (C-18) and 179.4 ppm (C-20), three others were sp3 carbon atoms at δC 46.3 ppm (C-4), 83.8 ppm (C-8) and 48.9 ppm (C-10).

Signals of the carbon atoms of a substituted double bond were observed δC 123.8 (C-13) and 140. 9 ppm (C-14). On its HMBC spectrum, correlations were observed
between: H-5 (2.27) and C-19/C-6/C-4/C-20; H-7 (2.38) and C-6 (23.04)/C-5(44.75)/C-9(55.84)/C-8(83.80); H-9 (1.77) and C-11 (19.89)/C-7(31.75)/C-5(44.75)/C-10(48.97)/C-8(83.80)/C-20(179.43); H-15 (2.11 and 2.00) and C-16 (11.89)/C-12(27.47)/C-13(123.82)/C-14(140.87); H-16 (0.99) and C-15(26.76)/C-14(140.87); H-17 (1.72) and C-8 (83.80)/C-14(140.87)/C-13(123.82); H-19 (1.29) and C-3 (37.21)/C-4(46.26)/C-18(183.97).

The isolated compounds were evaluated for their antidiabetic potential by measuring their inhibition potential of α-amylase enzyme. Diabetes mellitus is a metabolic disease which results from high blood sugar levels. Usually, the system of the patient is unable to effectively manage the metabolism of glucose resulting from carbohydrate digestion. This implies that the retardation of starch or carbohydrate breakdown and digestion will contribute to reducing blood glucose levels and control of diabetes. This can be achieved through the inhibition of enzymes which are responsible for carbohydrate breakdown such as α-amylase. The antidiabetic potential extract and isolated compounds from B. africana were evaluated and reported as percentage inhibitions of α-amylase at 400 μg/mL and presented on Table S1 (Supplementary material). The extract was more active than the isolated compounds with a percentage inhibition of 51.0 ± 2.5% at 400 μg/mL and was the only sample showing above 50% inhibition at this dose. Amongst the isolated compounds and at the dose of 400 μg/mL, the new diterpenoid Rubesanolidic acid exhibited the highest percentage inhibition of α-amylase of 38.2 ± 2.0% while β-sitosterol showed the lowest inhibition of 9.6 ± 0.5%. The fact that the extract is more active than the isolated compounds, suggests that the compounds might be acting in synergy to inhibit the α-amylase enzyme. The compounds isolated are terpenes and sterols and can be responsible for the α-amylase inhibitory activity of the extract of B. africana. It has been shown that the amount of terpenes and sterols in medicinal plants is directly proportional to its α-amylase inhibition and thus its antidiabetic potential (Snezana et al. 2020). These results are moderate compared to acarbose which is one of the inhibitors currently in clinical use. Some of these synthetic hypoglycemic agents are non-specific and have side effects which may limit their use. Many mechanisms are usual involved in the antidiabetic activity of medicinal plants and their compounds such as stimulation of beta cells of islets of Langerhans for insulin secretion, renal glucose reabsorption, insulin degradative processes inhibition, decreasing the resistance of insulin, and regenerating or repairing the pancreatic beta cells by increasing the size and number of the cells in islets of Langerhans (Singh et al. 2015; Subramani et al. 2019).

3. Experimental

3.1. General experimental procedure

Column chromatography was performed on glass column using silica gel (Merck 230–400 mesh). Thin layer chromatography was realized on TLC cards with silica gel 60 F254 of 0.5mm thickness and revealed with the aid of UV lamp at 254 and 365 and also by spraying with dilute sulphuric acid. NMR spectra were recorded on Bruker 500 at 500 MHz for proton and 1H et 125 MHz 13C with TMS as reference. HREIMS was carried out on a Compact Bruker MS instrument. Optical densities for enzyme
inhibition were recorded on a Multiplate Reader (TECAN Infinite M 200 Pro, Männedorf, Switzerland).

3.2. Plant material

The roots of *Burkea africana* were collected in Wack, Ngan-ha subdivision in the Adamawa region during the month of July 2019. The plant material was identified by Dr. Victor NANA of the National herbarium as a voucher specimen number 14878/SFR.Cam. exists.

3.3. Extraction and isolation

The roots of *B. africana* were dried and ground into powder. 1 kg of the powder was extracted with CH$_2$Cl$_2$-MeOH (1:1) at room temperature. 70 g of the crude extract were subjected to column chromatography using silica gel and gradient system of hexane/ethyl acetate (0–100%) and ethyl acetate/methanol (0–100%) to afford four fractions (A-D). Fraction A (1.3 g), obtained in the eluent system Hex/AcOEt was rechromatographed on silica gel column with eluent Hex/AcOEt (97.5: 2.5) to give compound 2 (12 mg). Fraction B (1.44 g) was purified on silica gel column in Hex/AcOEt (80:20) to obtained compound 1 (14.1 mg) and compound 2 (20.4 mg). Fraction C (2.4 g) was subjected to silica gel column chromatography using gradient system hexane-ethyl acetate and ethyl acetate-methanol to yield compound 3, compound 4 (18.9 mg) and compound 5 (2.9 mg). Finally, fraction D (1.2 g) was purified on gradient system hexane-ethyl acetate and ethyl acetate-methanol and this yielded compound 6 (20.9 mg) and compound 7 (14.6 mg).

3.4. Key data of compound 1

**Compound 1** (Rubesanolidic acid): White Powder; Mp: 178.5–180; HR-EI-MS$^+$ m/z = 333.2240 [M + H]$^+$; IR: $\lambda_{\text{max}}$ 2995, 1750, 1690, 915 cm$^{-1}$; $^1$H NMR ($\delta$, CDCl$_3$, 500 MHz): $\delta_H$ 2.39 (H-7, dd, $J = 13.6$, 4.5 Hz), 2.27 (H-5, dd, $J = 12.7$, 5.0 Hz), 1.71 (H-17; s), 1.29 (H-19; s), 0.99 (H-16; t), 1.18 (H-1a; m), 1.95 (H-1b; m), 1.63 (H-2a; m), 2.18 (H-2b; m), 1.70 (H-3a; m), 1.82 (H-3b; m), 1.72 (H-6a; m), 1.79 (H-6b; m), 1.77 (H-9; m), 1.97 (H-11a; m), 2.08 (H-11b; m), 1.28 (H-12a; m), 1.69 (H-12b; m), 2.07 (H-15; q) and 2.12 (H-15b, m). $^{13}$C NMR (125 MHz): $\delta_C$ 182.9 (C-18), 179.4 (C-20), 140.9 (C-14), 123.8 (C-13), 83.8 (C-8), 55.8 (C-9), 48.9 (C-10), 46.3 (C-4), 44.8 (C-5), 37.2 (C-3), 27.9 (C-1), 31.8 (C-7), 27.5 (C-12), 26.8 (C-15), 23.0 (C-11), 19.9 (C-6), 17.5 (C-2), 14.9 (C-19), 12.9 (C-16) and 11.8 (C-17).

3.5. In vitro $\alpha$-amylase inhibition assay

The $\alpha$-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNS) method (Wickramaratne et al. 2016) with slight modifications. Each sample was dissolved in minimum amount of 10% DMSO and was further dissolved in phosphate buffer, pH = 6.8 ((Na$_2$HPO$_4$/NaH$_2$PO$_4$ (0.01 M), NaCl (6 mM) at pH 6.8) to give
concentrations of 400 μg/mL. A volume of 40 μL of α-amylase solution (4 mg/mL) was mixed with 80 μL of sample and was incubated for 20 min at 37 °C. Thereafter 140 μL of the starch solution (1% in water (w/v)) was added to each tube and incubated for 30 min. at 37 °C. The reaction was terminated by the addition of 400 μL of 1% DNS reagent and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 800 μL buffer, and the absorbance was measured at 540 nm using a microplate reader (iTeCan Microplate). The blank with 100% enzyme activity was prepared by replacing the samples with buffer. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

\[
\% \text{α-amylase inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100
\]

4. Conclusion

Some medicinal plants have been proven to be highly effective antidiabetic therapies and could thus be used as alternatives to conventional antidiabetic drug agents which are usually associated with undesirable side effects. Plants of the fabaceae family fall in this category. Phytochemical study of crude extract of the roots of *Burkea africana* led to the isolation and characterisation of seven compounds out of which one was a new compound. The inhibitory effect of the extract and compounds on α–amylase at a dose of 400 μg/mL was appreciable as the extracts and some compounds showed significant antidiabetic potential and this indicates that, *B. africana* extract and compounds can be use to alleviate and treat type 2 diabetes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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