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Evaluation of Radical Scavenging and Metal Chelating Potential of Cameroonian Propolis and Isolation of Some Chemical Constituents

Mathieu Sawalda [*,1], Alfred Ngenge Tamfu [2,3*], Fadimatou [1], Cadet François Essongue [1], Emmanuel Talla [1,2], Henoumont Céline [4], Laurent Sophie [4], Farzana Shaheen [3] and Joseph Tanyi Mbafor [5]

[1]*Department of Chemistry, Faculty of Science, University of Ngaoundéré, P.O. BOX 454, Ngaoundere, Cameroon*

[2]*Department of Chemical Engineering, School of Chemical Engineering and Mineral Industries, University of Ngaoundéré, P.O. BOX 454, Ngaoundere, Cameroon*

[3]*Third World Center for Science and Technology, H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*

[4]*Department of General, Organic and Biomedical Chemistry, Faculty of Science, University of Mons-Hainaut, NMR and Molecular Imaging Laboratory, B-7000 Mons, Belgium*

[5]*Department of Organic Chemistry, Faculty of Sciences, University of Yaoundé 1, P.O. BOX 812, Yaounde, Cameroon*

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Abstract: Five extracts were prepared from different propolis samples and denoted PR1-PR5. They were subjected to two complementary antioxidant assays, DPPH radical scavenging and metal chelation. The propolis extracts PR4 and PR5 showed good radical scavenging power with IC_{50} 20.45 ± 1.65 µg/mL and 17.23 ± 1.40 µg/mL respectively as well as appreciable chelating power of IC_{50} 7.52± 0.61 µg/mL and 3.47± 0.61 µg/mL for PR4 and PR5 respectively. The PR1 extract showed a moderate radical scavenging activity with an IC_{50} = 230.08 ± 18.60 µg/mL and exhibited a high chelating power with an IC_{50} of 17.40 ± 1.41 µg /mL. This potent antioxidant effects of the propolis indicates its possible application in food science. Some compounds were isolated and characterized by 1D-, 2D-NMR studies as 7-O-ß–glucopyranosylapigenine, fridelin, lupeol, β-sitosterol, 3'-hydroxypentyltetratretracontanoate, 3'-hydroxybutyltetracontanoate, propylhexatetracontanoate, methyl-tetratretraconta-noate, 1′-O-eicosanylglycerol, and 2',3'-dihydroxypropyltetraeicosanoate which was further acetylated through hemi-synthesis to yield 2',3'-diacetylpropyltetraeicosanoate.

Keywords: Propolis; radical scavenging; metal chelation; acetylation; 2',3'-diacetylpropyltetraeicosanoate.

1. Introduction

Five extracts were prepared from different propolis samples and denoted PR1-PR5. They were subjected to two complementary antioxidant assays, DPPH radical scavenging and metal chelation. The propolis extracts PR4 and PR5 showed good radical scavenging power with IC_{50} 20.45 ± 1.65 µg/mL and 17.23 ± 1.40 µg/mL respectively as well as appreciable chelating power of IC_{50} 7.52± 0.61 µg/mL and 3.47± 0.61 µg/mL for PR4 and PR5 respectively. The PR1 extract showed a moderate radical scavenging activity with an IC_{50} = 230.08 ± 18.60 µg/mL and exhibited a high chelating power with an IC_{50} of 17.40 ± 1.41 µg /mL. This potent antioxidant effects of the propolis indicates its possible application in food science. Some compounds were isolated and

* Corresponding authors: E-mail: macntamfu@yahoo.co.uk (A.N. Tamfu); sawaldamathieu@yahoo.fr (M.Sawalda)

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characterized by 1D-, 2D-NMR studies as 7-O-ß–glucopyranosylapigenine, fridelin, lupeol, β-sitosterol, 3'-hydroxypentyltetratretracontanoate, 3'-hydroxybutyltetracontanoate, propylhexatetracontanoate, methyl-tetratretraconta-noate, 1′-O-eicosanylglycerol, and 2',3'-dihydroxypropyltetraeicosanoate which was further acetylated through hemi-synthesis to yield 2',3'-diacetylpropyltetraeicosanoate.

Free radicals are chemical species having, one or more singlet electrons on their outer most shells, which conferes unto them a certain instability and a high reactivity capable of attacking biomolecules in living systems. At low physiological concentrations, free radicals play very important roles in cells signalling, migration and differentiation, but at high concentrations, they can induce cellular damage and cell death or apoptosis [1]. This situation creates an instability in the equilibrium between antioxidants and pro-oxidants including free radicals in favor of the later. This disequilibrium leads to oxidative stress, causing lipid peroxidation and attacks on nitrogen and amino acid sources. Thus, the appearance or acceleration of pathogenic diseases such as cancer, diabetis, cardiovascular diseases and digeranative diseases like Alzheimer's disease and Parkinson's diseases [2]. Antioxidants are defined as chemical species, complex or not, which diminishes or reduces oxidative stress in living organism. They can prevent free radicals synthesis by inhibiting their chain reaction initiation stage or directly disactivating the oxygen reactive species. According to their mode of action, they can be classified as enzymatic antioxidant systems, oxidant enzyme inhibitors, metal chelators and free radical scavengers. Antioxidants are mostly known for their free radical scavenging effects by neutralizing radicals via reduction chemical reactions. They are a heterogeneous group composed of endogenous systems, enzymatic, vitamins, oligo-elements or even polyphenols [2]. Many antioxidants are obtained from natural sources and they have shown interesting potentials to act as natural antioxidants and this motivates researchers to focus on new nontoxic alternatives, to substitute the unstable and toxic synthetic antioxidants [3]. Antioxidants are exogenous or endogenous compounds from natural or synthetic origin, capable of removing free radicals, scavenging and inhibiting formation of reactive oxygen species (ROS) and reactive nitrogen species and their precursors and chelating metals which intervene in the catalysis of ROS generation which can prevent oxidative stress that leads to several pathological conditions [4]. Bee products notably honey and propolis are sources of natural non-toxic antioxidants.

Bees are social insects that have existed for more than one hundred million years ago and they are easily identified with honey and other interesting products that have fascinated man who gradually learned to breed them. *Apis Mellifera* is the most interesting species in apiculture [5, 6]. Bees produce substances that ensure their sustainability, and these substances have always serve man as food and medicine. Amongst the products made by bees there is honey, wax, venom, pollen, royal jelly and propolis. Propolis is a resinous beehive product of plant resin and exudates origin, manufactured by the worker bees of the *Apis mellifera* species, from selected plants to which they may add their own secretions in order to be able to use them as protection in the hive and it has been used by humans therapeutically to treat many ailment [7-8]. Propolis is considered one of the oldest cures for humanity, known and used since the highest antiquity, its beneficial effects on the organization's defense system.

Propolis has been traditionally used to treat wounds, burns, sore throat, ulcers, tooth ache, asthma, skin rashes, boils, diabetes as well as for ornamental works and mending of calabashes [9-10]. Propolis possesses many biological activities like antioxidant [11-12], antibacterial [13-14], anti-inflammatory, immunomodulatory [15], antifungal [16], antioxydant, analgesic-anesthesic [12, 17-18], antiviral [19], anticancer [20], pest management [21], antiallergic [22] and regenerative [23] properties which have been shown to correlate with its complex and variable chemical composition. The chemical composition of propolis varies from one region to another and according to the vegetation [24]. Therefore, the biological properties of propolis as well will necessarily depend on the phytochemicals it contains, local vegetation, geographical zone and seasons and more than 300 compounds have characterized in propolis belonging to terpenoids, phenolic compounds aromatic and fatty acids [25].

This study focuses on the isolation and characterization of secondary metabolites, as well as the assessment of antioxidant potential of the propolis extracts. Also, to produce a new derivative through acetylation of 2',3'-dihydroxypropyltetraeicosanoate isolated from the propolis extract.

2. Materials and Methods

2.1. Collection of the Raw Material, Extraction and Isolation of Compounds

Propolis samples for this study were collected from two different regions of Cameroon (Adamawa and West regions) in March 2018. They were collected from the Bini locality, Vina Division of Adamawa region (ECh-1), from Dschang locality of Menoua Division (ECh-2) and from Mbouda, Bamboutos Division (ECh-3) of West region.

Propolis from Bini, Adamawa Region labelled ECh-1 (205 g) was extracted by maceration for 48 hrs. in 03 L of dichloromethane: methanol (50:50) solvent mixture followed by a filtration and evaporation on a rotatory evaporator. The operation was repeated three times to obtain 95 g of crude extract (PR1). 40 g of the crude extract was subjected to column chromatography separation on silica gel and eluted with mixture of solvents as eluent on a gradient as follows: hexane: ethyl acetate (0-100) and ethyl acetate: methanol (0-100). A total of 420 fractions of 200 mL each collected from the column and the solvent removed with the aid of a rotatory evaporator. They were regrouped on the basis of TLC (Thin Layer Chromatography) profiles to 20 pooled fractions A to T and allowed to stand for 24 hrs. In some fractions, powder matter settled at the bottom and they were then filtered and washed with pure hexane: acetone (80:20) mixture to afforded pure compounds as indicated: fractions C, D and Q afforded compounds **5** (20.8 mg) **10** (40 mg) et **2** (10.3 mg). The powder from fraction B was purified on column chromatography using the isocratic system Hexane: ethyl acetate (20 :75) to yield compound **4** (25.1 mg).

Propolis from Mbouda, West Region labelled ECh-2 (575 g) of was extracted by maceration in 05 L of dichloromethane: methanol (50:50) solvent mixture for 48 hours, followed by filtration and evaporation under reduced pressure with a rotatory evaporator to obtain 200 g of crude extract (PR2). 50 g of the crude extract underwent silica gel column chromatography and eluted with a gradient system of with solvents as follows: hexane-ethyl acetate (0-100) and ethyl acetate-methanol (0-100). This lead to 480 fractions each of 200 mL each collected at the base of the column, which were combined into 5 major fractions (F1 to F5) based on their TLC profiles. Fraction F1 was filtered and washed with ethyl acetate to afford compound **8** (5.7 mg). Fraction F4 was purified on silica gel column eluted with hexane-ethyl acetate (20-80%) and afforded compounds **6** (75.4 mg), **7** (103 mg) and **9** (19 mg). Recrystallization of fraction F3 in dichloromethane afforded compound **3** (15.9 mg).

Propolis from Dschang West Region labelled ECh-3 (957g) was extracted by maceration in 10 L of dichloromethane: methanol (50:50) solvent mixture for 48 hours. The mixture obtain was filtered and the solvent evaporated under reduced pressure with a rotatory evaporator. The crude extract obtained was dissolved in water and the resultant aqueous solution was re-extracted using liquid-liquid partitioning with hexane and then ethyl acetate in order to obtain the crude extracts PR3 and PR4 respectively. The residual aqueous extract on the other hand was lyophilized and the crude extract PR5 obtained.

2.2. Structural Elucidation of Pure Compounds

The melting points were measured on a Buchi M-560 capillary device. The mass spectra recorded on Jeol Msroute-600H and Q-T of Ultima spectrometers. The ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded in $CDCl_3$, CD_3OD and DMSO-D_6 solvents. TLC was performed on the TLC plates covered with silica gel (Merck, PF254, 20x20 cm, 0.25 mm). The plates were revealed using UVITEC 07-22243 at 254 Nm and 365 Nm and 10% H_2SO_4 acid.

2.3. Radical Scavenging Activity (DPPH Assay) of Crude Extracts

The scavenging power of our extracts were carried out by the method of the free synthetic radical $DPPH^{\bullet}$ according to the protocol used elsewhere [17].

2.4. Metal Chelating Activity

The chelating power, determined as ferric iron chelation (FIC) of our extracts were evaluated according to the method used previously [12].

*2.5. Chemical Transformation by Acetylation of Compound **11***

Compound **11** formerly obtained from propolis extract of Nohmedjoh [24] underwent an acetylation according to the method describe elsewhere [26]. In an assay tube containing 2 mL of acetic anhydride, 10 mg of compound **11** was dissolve and 2 mL of pyridine were added. The mixture was allowing to rest in the dark for 12 hrs. Some drops of hydrochloric acid were then added to the mixture and it was suspended several times in methylene chloride. The organic phase collected was passed under the rotatory evaporator so as to remove solvent found inside. A comparative TLC was performing between the concentrated organic phase and the starting product in order to obtain the acetylated product. The reaction that took place is as follows:

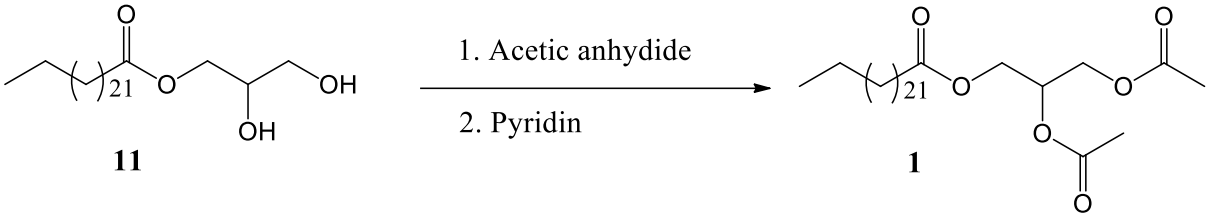

Figure 1. Reaction scheme for the acetylation process

3. Results and Discussion

3.1. Characterization of Isolated Compounds and the Semi-Synthetic Product

The isolated compounds were characterized and it is worthy to note that most of them were triterpenes and fatty compounds, mostly fatty acid esters. Their structures are given in Figure 2. Only one of them is a phenolic compound. However, this is in agreement with some previous works done on Cameroonian propolis in which triterpenoids and fatty compounds were described. The compounds isolated from propolis are usually subjected directly to bioassays or used as starting material for synthesis in view of obtaining their derivatives or synthetic analogues with enhanced biological activities [24]. In this study, the fatty ester of glycerol obtained in high yields was subjected to an acetylation reaction and the success of the reaction was evidence by the comparison of the spectra of the substrate compound and the semi-synthetic product obtained as shown in the supporting information sheet as Figures S1 and S2 for ^{1}H NMR and Figure S3 and S4 for ^{13}C NMR. Semi-syntheses and chemical changes are the basis of research and development of innovative drugs obtained from chemically modified natural compounds with various functional groups that greatly improve their biological properties and this has been evidenced in some recent studies [27-28].

3.2. NMR Data of Isolated Compounds

*2',3'-Diacetylpropyltetraeicosanoate (**1**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 173.3 (C-1), 34.0 (C-2), 31.9 (C-3), 29.1 – 29.7 (C-4 to C-21), 24.9 (C-22), 22.7 (C-23), 14.1 (C-24), 62.3 (C-1'), 69.1 (C-2'), 62.0 (C-3'), 170.1 (C-1''), 20.7 (C-2''), 170.5 (C-1'''), 20.9 (C-2'''). ^{1}H NMR ($CDCl_3$, 500 MHz): 2.32 (H-2, *t*, *J*=14 Hz), 1.62 (H-3, *m*), 1.23 – 1.31 (H-4 to H-21, *m*), 1.31 (H-22, *m*), 1.26 (H-23, *m*), 0.86 (H-24, *t*, *J*=14 Hz), 4.30 (H-1', *dd*, *J*=10 Hz *J*=3.5 Hz), 5.25 (H-2', *m*), 4.15 (H-3', *m*), 2.07 (H-2''), 2.05 (H-2''').

*7-O-ß–glucopyranosylapigenine (**2**)*: ^{13}C NMR (DMSO, 125 MHz) : 161.0 (C-2), 104.5 (C-3), 181.2 (C-4), 162.4 (C-5), 98.1 (C-6), 163.9 (C-7), 81.7 (C-8), 160.2 (C-9), 103.9 (C-10), 121.5 (C-1'), 128.9 (C-2' and C-6'), 115.8 (C-3' and C-5'), 155.9 (C-4'), 102.4 (C-1''), 73.3 (C-2''), 70.8 (C-3''), 70.5 (C-4''), 78.6 (C-5''), 61.3 (C-6''). ^{1}H NMR (DMSO, 500 MHz) : 6.78 (H-6), 6.26 (H-8), 8.03 (H-2' and H-6'), 6.89 (H-3' and H-5'), 4.69 (H-1''), 3.81 (H-2''), 4.61 (H-3''), 3.34 (H-4''), 3.24 (H-5''), 3.73, 3.52 (H-6'').

*Fridelin (**3**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 22.2 (C-1), 41.4 (C-2), 213.0 (C-3), 57.9 (C-4), 42.1 (C-5), 41.2 (C-6), 18.4 (C-7), 52.9 (C-8), 37.4 (C-9), 59.7 (C-10), 35.2 (C-11), 30.5 (C-12), 39.7 (C-13), 38.0 (C-14), 32.2 (C-15), 36.1 (C-16), 30.1 (C-17), 42.7 (C-18), 35.4 (C-19), 28.1 (C-20), 32.7 (C-21), 39.6 (C-22), 7.84 (C-23), 14.7 (C-24), 17.6 (C-25), 20.2 (C-26), 18.8 (C-27), 32.0 (C-28), 35.0 (C-29), 31.7 (C-30). ^{1}H NMR ($CDCl_3$, 500 MHz):1.89 (H-1), 2.27, 2.34 (H-2), 2.40 (H-4), 1.60 (H-6), 1.54 (H-7), 1.43 (H-8), 1.42 (H-10), 1.67 (H-11), 1.77 (H-12), 1.61 (H-15), 1.59 (H-16), 1.44 (H-18), 1.50 (H-19), 1.66 (H-21), 1.63 (H-22), 0.88 (H-23), 0.73 (H-24), 0.87 (H-25), 1.01 (H-26), 1.05 (H-27), 1.18 (H-28), 0.95 (H-29), 1.00 (H-30),

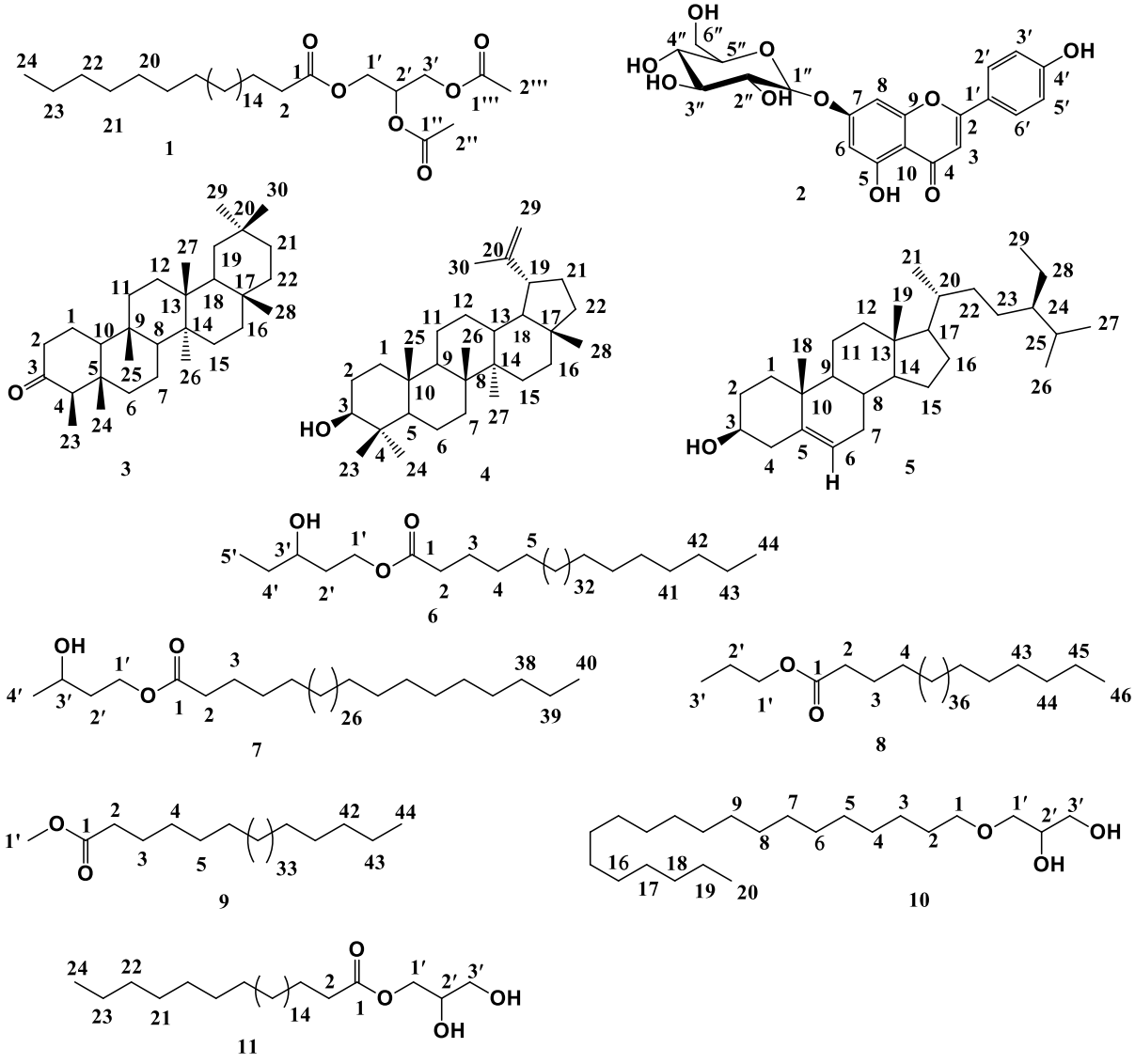

Figure 2. Structures of isolated compounds and semi-synthetic products

*Lupeol (**4**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 39.3 (C-1), 27.5 (C-2), 79.6 (C-3), 39.9 (C-4), 56.7 (C-5), 18.6 (C-6), 35.4 (C-7), 40.0 (C-8), 51.7 (C-9), 37.2 (C-10), 22.0 (C-11), 25.3 (C-12), 38.5 (C-13), 44.0 (C-14), 28.7 (C-15), 36.6 (C-16), 43.0 (C-17), 49.5 (C-18), 47.8 (C-19), 151.5 (C-20), 30.8 (C-21), 40.3 (C-22), 28.6 (C-23), 16.2 (C-24), 16.8 (C-25), 15.6 (C-26), 15.0 (C-27), 18.1 (C-28), 110.2 (C-29), 19.4 (C-30). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.98 (H-l), 2.20 (H-2), 3.19 (H-3), 0.69 (H-5), 1.45 (H-6), 1.40 (H-7), 1.17 (H-9), 1.40 (H-11), 1.88 (H-12), 1.67 (H-13), 1.75 (H-15), 1.38 (H-16), 1.35 (H-18), 2.38

(H-19), 1.83 (H-21), 1.42 (H-22), 1.04 (H-23), 0.97 (H-24), 1.40 (H-25), 0.84 (H-26), 0.79 (H-27), 1.26 (H-28), 4.56, 4.65 (H-29), 1.69 (H-30).

*β-sitosterol (**5**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 37.5 (C-1), 31.9 (C-2), 72.0 (C-3), 42.5 (C-4), 140.1 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.1 (C-10), 21.1 (C-11), 39.9 (C-12), 42.6 (C-13), 56.9 (C-14), 26.3 (C-15), 28.5 (C-16), 56.3 (C-17), 111.8 (C-18), 19.2 (C-19), 43.2 (C-20), 26.3 (C-21), 46.1 (C-22), 26.3 (C-23), 46.1 (C-24), 29.4 (C-25), 20.1 (C-26), 19.6 (C-27), 19.0 (C-28), 12.0 (C-29). ^{1}H NMR ($CDCl_3$, 500 MHz): 1.83 (H-1), 1.80 (H-2), 3.50 (H-3), 2.29 (H-4), 5.33 (H-6), 1.97 (H-7), 1.45 (H-8), 1.00 (H-9), 1.51 (H-11), 1.02 (H-12), 1.04 (H-14), 1.56 (H-15), 1.24 (H-6), 1.17 (H-17), 0.92 (H-18), 0.98 (H-19), 2.29 (H-20), 1.01 (H-21), 1.56 (H-22), 1.66 (H-23), 0.84 (H-24), 0.80 (H-25), 0.82 (H-26), 0.89 (H-27), 0.78 (H-28), 0.78 (H-29).

*3'-hydroxypentyltetratretracontanoate (**6**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 172.7 (C-1), 34,2 (C-2), 26.3 (C-4), 29.7 – 23.2 (C-4 to C-26), 14.1 (C-27), 62.1 (C-1'), 39.5 (C-2'), 67.2 (C-3'), 25.8 (C-4'). ^{1}H NMR ($CDCl_3$, 500 MHz): 2.28 (H-2, *t*, *J*=7.5 Hz), 1.62 (H-3, *m*), 1.57 – 1.26 (H-4 to H-43), 0.87 (H-44, *t*, *J*=14 Hz), 4.05 (H-1', *t*, *J*=6.5Hz), 1.83 (H-2'), 3.52 (H-3'), 1.47 (H-4'), 0.82 (H-4', *t*, *J*=14 Hz).

*3'-hydroxybutyltetracontanoate (**7**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 172.9 (C-1), 34,2 (C-2), 26.3 (C-4), 29.7 – 23.2 (C-4 to C-26), 14.1 (C-27), 62.1 (C-1'), 39.5 (C-2'), 67.2 (C-3'), 25.8 (C-4'). ^{1}H NMR ($CDCl_3$, 500 MHz): 2.29 (H-2, *t*, *J*=7.5 Hz), 1.44 (H-3, *m*), 1.39 – 1.23 (H-4 to H-26), 0.87 (H-27, *t*, *J*=7.5 Hz), 4.05 (H-1', *t*, *J*=6.5Hz), 1.67 (H-2', *m*), 3.80 (H-3', *m*), 1.17 (H-4').

*Propylhexatetracontanoate (**8**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 171.8 (C-1), 34,6 (C-2), 25.3 (C-4), 29.6 – 32.2 (C-4 to C-33), 14.1 (C-34), 65.6 (C-1'), 22.5 (C-2'), 11.2 (C-3'). ^{1}H NMR (CD_3OD, 500 MHz): 2.30 (H-2, *t*, *J* = 7.5 Hz), 1.62 (H-3), 1,37-1.23 (H-4 to H-33), 0.87 (H-34, *t*, *J* = 7.5 Hz), 4.05 (H-1', *t*, *J* = 6.5 Hz), 1.50 (H-2'), 0.85 (H-3').

*Methyltetratretracontanoate (**9**)*: ^{13}C NMR ($CDCl_3$, 125 MHz): 172.4 (C-1), 35,6 (C-2), 26.3 (C-4), 29.7 – 32.1 (C-4 to C-29), 14.1 (C-30), 54.6 (C-1'). ^{1}H NMR (CD_3OD, 500 MHz): 2.29 (H-2, *t*, *J*=7.5 Hz), 1.19 (H-3, *m*), 1.23 – 1.11 (H-4 to H-28), 1.53 (H-29), 0.87 (H-30, *t*, *J*=7.5 Hz), 3.64 (H-1', *t*, *J*=6.5Hz).

*1'-O-eicosanylglycerol (**10**)*: ^{13}C NMR (CD_3OD, 125 MHz): 64.9 (C-1), 33.1 (C-2), 27.0 (C-3), 31.0 - 31.2 (C-4 to C-18), 14.1 (C-20), 73.1 (C-1'), 72.7 (C-2'), 72.2 (C-3'). ^{1}H NMR (CD_3OD, 500 MHz): 3.49 (H-1), 1.61 (H-2), 1.37 (H-3), 1.32 – 1.20 (H-4 to H-18), 0.93 (H-20), 3.61 (H-1'), 3.52 (H-2'), 3.77 (H-3').

*2',3'-Dihydroxypropyltetraeicosanoate (**11**)*: m.p. 69.5 - 70.9°C. ^{13}C NMR ($CDCl_3$, 125 MHz): 174.3 (C-1), 34.2 (C-2), 31.9 (C-3), 29.1-29.7 (C-4 to C-21), 24.9 (C-22), 22.7 (C-23), 14.1 (C-24), 65.5 (C-1'), 70.3 (C-2'), 63.34 (C-3'). ^{1}H NMR ($CDCl_3$, 500 MHz): 2.32; 2.35 (H-2, *t*, *J=14* Hz), 1.62 (H-3, *m*), 1.23 – 1.31 (H-4 to H-21, *m*), 1.31 (H-22, *m*), 1.26 (H-23, *m*), 0.86 (H-24, *t*, J=14 Hz), 4.21; 4.18 (H-1', *dd*, *J*=10 Hz, *J*=3.5 Hz), 3.92 (H-2', *m*), 3.58; 3.68 (H-3', *m*), 2.43 (2'-OH), 1.97 (3'-OH).

*3.3. Characterization of Compound **1** Obtained by Acetylation of Compound **11***

The NMR ^{1}H spectrum (Figure S1) of compound **11** reveals signals of hydroxyl group at δ_H 2.43 (*2′*-OH) and at δ_H 1.97 (*3′*-OH). On this same spectrum we can notice the existence of signals at δ_H 4.21, 4.18 (H-1', dd, J=10 Hz, J=3.5 Hz), 3.92 (H-2', m), 3.58; 3.68 (H-3', m). We can observe the absence the hydroxyl groups signals on Figure S2 as compound **11** was transformed into compound **1** and the appearance of a signal at δ_H 2.07 (*s*) integrating for six protons corresponding to the two-methyl linked to the carbonyl's esters. This enabled us to say that the two OH groups have been acetylated during the reaction since their signals disappeared in Figure S2.

The ^{13}C NMR spectrum (Figure S3) of compound **11** compared with that of compound **1** (Figure S4) shows a wide similarity with the exception of the signals at 170.1 (C-1"), 20.7 (C-2"), 170.5 (C-1'"), 20.9 (C-2'") corresponding to the two methyl carbons of the acetyl groups and the two additional carbonyl esters.

3.4. Radical Scavenging and Metal Chelating Activities

The extracts PR4 and PR5 showed good radical scavenging capacity with IC_{50} values of 20.45±1.65 µg/mL and 17.23±1.40 µg/mL, as well as appreciable chelating power with IC_{50} values of 7.52±0.61 µg/mL and 3.47±0.61 µg/mL, respectively. The PR1 extract showed a moderate radical scavenging activity with an IC_{50} = 230.08 ± 18.60 µg/mL and exhibited a high chelating power with an IC_{50} of 17.40 ± 1.41 µg /mL as shown on Table 1. These results are higher compared to those obtained by some authors [24] and [17]. DPPH radical scavenging and metal chelation were used to evaluate antioxidant effects of Polish propolis and in both models the propolis extracts exhibited scavenging activity comparable to the standard antioxidant butylated hydroxytoluene (BHT) and a chelating power almost half of that of EDTA standard chelator [29].

This difference in the results obtained is partly due to the composition of secondary metabolites of the extracts and as well as the extraction method used. The hexane extract PR3 was inactive in the DPPH radical scavenging and chelation assays. This may be due to the unavailability in this extract of compounds responsible for the scavenging activity and metal chelation such as phenolic compounds.

Table 1. Radical scavenging activity (DPPH assay), and chelating activity (FIC assay)

Compounds	**DPPH (IC_{50} µg/mL)**	**FIC (IC_{50} µg/mL)**
PR1	230.08 ± 18.60	17.40 ± 1.41
PR3	>1000	>1000
PR4	20.45 ± 1. 65	7.52± 0.61
PR5	17.23± 1.40	3.47± 0.61
Vitamin C	3.34 ± 0.27	-
BHT	-	1.24±0.10

The pharmacological action of natural products has significant acceptance and their use is becoming more popular in the form of food supplements, complementary and alternative medicine [30-31]. Propolis, from both sting and stingless bees, is one of the numerous natural products that has gained populace since it possesses beneficial pharmacological properties such as anti-inflammatory, antibacterial, immune-stimulating, antiviral, cytotoxic, hepatoprotective and antioxidant, and its preparations find applications in food and cosmetics as pure extracts or in combined form [32-34]. Therefore, the propolis extracts and the compounds obtained from there can find applications in cosmetics, foods and nutraceuticals. The compounds found in propolis extracts are usually consumed by people from many countries as food complements, nutraceuticals, or alternative medicine. Oftenly the propolis can be used alone or in combination with other natural products, usually as a nutraceutical supplement and also as a natural antioxidant in food and related products. Evidently, propolis possesses numerous health benefits but has vary variable and inconsistent chemical composition which makes it difficult for propolis to be universally accepted into health care systems and this requires continuous chemical characterization, quality control and standardization, bringing out possible phytochemical markers of propolis from each region [35-36]. The antioxidant capacity of Cameroonian propolis samples could be attributed to the triterpene and phenolic compounds that they contain [36, 37]. Equally, honey samples from Cameroon have displayed antioxidant activity, though with poor phenolic content [38]. Propolis just as some other edible natural products have shown antioxidant activity and are able to inhibit reactive oxygen species (ROS) and this is an indication of their possibility in preventing metabolic illnesses and finding applications as food additives or food supplements [39-42].

4. Conclusion

Summarily, it could be observed that propolis is a natural substance used in traditional medicine in Cameroon to treat several diseases. This is because propolis has numerous therapeutic effects as demonstrated from its biological activities reported in scientific papers and these activities are conferred by the wide variety of phytochemicals and secondary metabolites found in propolis. Propolis is safe for consumption because it shows low toxicity even at high doses and therefore is a valuable source of

bioactive molecules for development of drugs [43]. The different chromatographic separations carried out on the Cameroonian propolis extracts led to the isolation of ten compounds including pentacyclic triterpenes, fatty acid esters, glycerol, flavonoid glycoside and steroid. The extracts also showed important antioxidant, potential through two assays: radical scavenging and metal chelation power. The results obtained show that propolis is a potential alternative source of antioxidants that could find applications in the prevention and treatment of oxidative stress. 2 ',3'-dihydroxypropylterecosanoate (**11**) was successfully acetylated to obtain a new triester derivative of glycerol, 2',3'-diacetylpropyl-erterecosanoate (**1**).

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Supporting Information

Supporting information accompanies this paper on https://acgpubs.org/journal/records-of-agricultural-and-food-chemistry

ORCID
Mathieu Sawalda: 0000-0001-6885-2603
Alfred Ngenge Tamfu: 0000-0001-6683-3337
Fadimatou: 0000-0002-8642-1388
Cadet François Essongue: 0000-0002-4703-7017
Emmanuel Talla: 0000-0003-4145-4095
Henoumont Céline: 0000-0002-3280-2441
Laurent Sophie: 0000-0002-2589-3250
Farzana Shaheen: 0000-0003-0057-3960
Joseph Tanyi Mbafor: 0000-0002-8326-716X

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