Research Article

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A New fatty acid and some triterpenoids from propolis of Nkambe (North-West Region, Cameroon) and evaluation of the antiradical scavenging activity of their extracts

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Abstract: The aim of this work was to evaluate *in vitro* antiradical scavenging activity of propolis from Nkambe (North-West, Cameroon). The polyphenol content of the acetone extract was evaluated using the Folin-Ciocalteu reagent as 0.166±0.008 gGAE/100 gRM. Antiradical scavenging activity of hexane and acetone extracts was carried out on DPPH using ascorbic acid as standard. The results showed that the extracts possess antiradical activity with IC_{50} of 141 µg/mL and 267 µg/mL for acetone and hexane extracts, respectively. The column chromatography separation on silica gel of the hexane fractionyielded compounds **1** to **3**. The structures of these compounds were elucidated by NMR and mass spectrometry data as Lupenone (**1**), a mixture of *α* and *ß*-Amyrin (**2**) and lastly Hexatriacontanoic acid (**3**) which was described for the first time from propolis.

Keywords: Propolis; antiradical activity on DPPH; polyphenol content; hexatriacontanoic acid.

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1 Introduction

Propolis is a resinous, sticky and balsamic substance of viscous consistency, produced by bees principally collected from resin of buds and exudates of plants and their secretions [1]. This substance has important pharmacological activities such as antiplasmodial [2], analgesic [3], antimicrobial [4,5], vasodilatory [1], anti-inflammatory [6], antifugal [7], antioxidant [8], anti-ulcer [9], ostrogenic [10], antiviral, antiprotozoal, antiparastic, antitumor, hepatoprotective and cardioprotective properties [11]. In Cameroun, propolis is used locally to treat illnesses such as dysentery, stomachache, asthma, infertility, ulcers, tooth aches, fever, burns and different forms of inflammation [3,9]. Many studies on different samples show that propolis chemical composition is difficult to standardize because it is highly dependent on a number of factors such as local flora and environmental conditions of the site of collection of resin from plants for production of propolis. This is why propolis from areas not yet studied seems to be a promising source of new bioactive molecules [12]. From different botanical and geographical origins of the world, more than 300 compounds including volatile organic compounds, flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpenes, quinones, coumarins, steroids, amino acids were reported to have been isolated from propolis [8, 13]. Amongst these compounds, 241 of them were reported for the first time from the year 2000 to 2012 [14]. Despite the chemical variability of propolis, it always possesses promising and considerable biological activities [15].

This present study consisted of evaluating the *in vitro* antiradical activity of the hexane and acetone extracts of propolis from Nkambe and to purify the hexane extract in order to obtain pure compounds which will be identified based on their spectroscopic data (^{1}H NMR, ^{13}C NMR and MS).

2 Materials and Methods

The propolis sample under investigation was harvested during the month of April 2014 by a bee farmer in Njap village, Nkambe central subdivision, Donga-Mantung division, the North-West Region of Cameroon.

2.1 Extraction and isolation

To extract secondary metabolites, 1 kg of propolis was extracted with 5 L of acetone by maceration with intermittent stirring at intervals of 3 hours during 72 hours after which it was filtered on a N°1 Whatman filter paper and evaporated using a rotary evaporator to near dryness to obtain a crude acetone extract. This process was repeated three times in order to optimize the extraction process. The acetone extract obtained was partitioned using liquid-liquid extraction with hexane to obtain the hexane extract. 60 g of this hexane extract was subjected to column chromatography on 360 g of silica gel with the gradient eluting system hexane (Hex)-dichloromethane (CH_2Cl_2) (100:0 → 0:100) followed by CH_2Cl_2-methanol (MeOH) (100:0 → 70:30). Fractions of 100 mL where collected regularly and concentrated on a rotavapor. This process yielded three compounds: Lupenone (**1**, 200 mg) [16], a mixture of *α*-amyrin (**2a**) and *β*-amyrin (**2b**) (85 mg) [17] and hexatriacontanoic acid (**3**, 25 mg).

Lupenone ($C_{30}H_{48}O$) (**1**)**:** White crystals; ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 38.7 (C-1); 27.4 (C-2); 218.2 (C-3); 38.8 (C-4); 54.9 (C-5); 19.7 (C-6); 34.2 (C-7); 40.8 (C-8); 49.7 (C-9); 37.1 (C-10); 20.9 (C-11); 25.1 (C-12); 30.1 (C-13); 42.8 (C-14); 27.4 (C-15); 35.5(C-16); 43.1 (C-17); 48.2 (C-18); 47.9 (C-19); 150.9 (C-20); 29.8 (C-21); 40.0 (C-22); 26.6 (C-23); 21.1 (C-24); 15.9 (C-25); 15.8 (C-26); 14.4 (C-27); 18.1 (C-28); 109.4 (C-29) and 19.3 (C-30). ^1H NMR ($CDCl_3$, 500 MHz) *δ* (ppm): 4.58 (1H, d, H-29a); 4.72 (1H, d, H-29b); 2.55 (1H, m, H-2a); 2.40 (1H, m, H-2b); 1.10 (3H, s, H-23); 1.08 (3H, s, H-24); 1.06 (3H, s, H-26); 0.96 (3H, s, H-25); 0.98 (3H, s, H-27); 0.80 (3H, s, H-28) and 1.72 (3H, s, H-30) [16].

***α*-amyrin** ($C_{30}H_{50}O$) (**2a**): White powder; ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 38.7 (C-1); 27.4 (C-2); 79.1 (C-3); 38.8 (C-4); 55.3 (C-5); 18.4 (C-6); 32.8 (C-7); 40.8 (C-8); 49.7 (C-9); 36.9 (C-10); 23.3 (C-11); 121.7 (C-12); 145.2 (C-13); 42.1 (C-14); 28.4 (C-15); 26.6 (C-16); 33.8 (C-17); 59.1 (C-18); 39.8 (C-19); 40.1 (C-20); 31.3 (C-21); 41.6 (C-22); 28.6 (C-23); 15.4 (C-24); 15.6 (C-25); 16.8 (C-26); 23.1 (C-27); 28.4 (C-28); 17.4 (C-29) and 21.3 (C-30). ^1H NMR ($CDCl_3$, 500 MHz) *δ* (ppm): 3.15 (1H, dd, H-3); 5.15 (1H, t, H-12); 0.84 (3H, d, *J*=5.6 Hz, H-29); 0.90 (3H, d, *J*=7.6 Hz, H-30); 0.72 (3H, s); 0.78 (3H, s); 0.93 (3H, s); 0.95 (3H, s); 0.99 (3H, s) and 1.07 (3H, s) [17].

***β*-amyrin** ($C_{30}H_{50}O$) (**2b**): White powder; ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 38.7 (C-1); 27.4 (C-2); 79.1 (C-3); 38.8 (C-4); 55.3 (C-5); 18.4 (C-6); 32.8 (C-7); 40.8 (C-8); 49.7 (C-9); 36.9 (C-10); 23.3 (C-11); 121.7 (C-12); 145.2 (C-13); 42.1 (C-14); 28.4 (C-15); 26.6 (C-16); 33.8 (C-17); 59.1 (C-18); 39.8 (C-19); 40.1 (C-20); 31.3 (C-21); 37.2 (C-22); 28.6 (C-23); 15.4 (C-24); 15.6 (C-25); 16.8 (C-26); 26.0 (C-27); 28.4 (C-28); 33.6 (C-29) and 23.7 (C-30). ^1H NMR ($CDCl_3$, 500 MHz) *δ* (ppm): 3.15 (1H, dd, H-3); 5.15 (1H, t, H-12); 0.72 (3H, s); 0.78 (3H, s); 0.84 (3H, s); 0.90 (3H, s); 0.93 (3H, s); 0.95 (3H, s); 0.99 (3H, s) and 1.07 (3H, s) [17].

Hexatriacontanoic acid ($C_{36}H_{72}O_2$) (**3**): White powder: ESI TOF-MS: $[M+H]^+$ *m/z* 537.3, Key fragment ions *m/z* = 185.0; 227.1; 409.2; 445.3 and 532.3 (see figure 2). ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 178.8 (C-1); 33.8 (C-2); 24.7 (C-3); 31.9 (C-34); 22.7 (C-35); 14.1 (C-36) and 29.4 (C-4 → C-33); ^1H NMR ($CDCl_3$, 500 MHz) *δ* (ppm): 0.80 (3H, t, 3H-36); 1.50 (2H, m, 2H-3); 2.30 (2H, t, 2H-2); 1.20-1.32 [$(CH_2)_{32}$, *brs*] and 10.70 (1H, s, OH).

2.2 Total polyphenol content

This was done according to the method described elsewhere [18]. A 100 µL of extract (200 µg/mL) were added to 200 µL of Folin-Ciocalteu followed by addition of 2000 µL of distilled water. The mixture was agitated for 3 minutes. After this, 1000 µL Na_2CO_3 (20%) was added to the mixture and incubated in a dark cupboard at room temperature for 1 hour. The absorbance of the resulting solution was then measured at 760 nm on a spectrophotometer, with a methanol solution used as negative control. The preparation of the positive control, gallic acid (200 µg/mL) was subjected to the same treatment as the test sample. The results were expressed in term of gram equivalent of gallic acid per 100 g of raw matter (gGAE/100 gRM). The quantification of polyphenolic compounds was done with respect to a linear standardization curve obtained at different concentrations (20 to 120 µg/mL) of gallic acid in the form *y*=2.428x+0.033 whose correlation coefficient R was 0.994.

2.3 Evaluation of DPPH antiradical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) antiradical scavenging activity of the extracts was done according to the method described elsewhere [12] with slight modifications. To 2 mL of the solution of the test sample prepared at different concentrations, 1 mL of a methanol

solution of DPPH (100 µg/mL) was added. The mixture obtained was then stored at room temperature for 1 hour after which the absorbance was then measured at 517 nm against a negative control (6 mL of MeOH and 1 mL of DPPH solution) on a spectrophotometer. The positive control was ascorbic acid. A mother solution of sample at the concentration of 120 µg/mL was prepared by dissolving 5000 µg of extract 41.6 mL of MeOH. From this, five other solution concentrations were obtained from the mother solution making a total of six solutions at six concentrations: 120, 100, 80, 60, 40 and 20 µg/mL. The test at each concentration was done in triplicates, the absorbance was mesured and the percentage inhibition calculated according to the following equation [12].

$$\% \text{ inhibition} = \frac{A_{t0} - A_{t1}}{A_{t0}} \times 100$$

Where A_{t0} = absorbance of negative control (without any anti-oxidant substance); A_{t1} = absorbance of tested samples.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Isolation and structural elucidation of compounds

The Hexane fraction of propolis of Nkambe was separated by column chromatography of silica gel yielded four compounds (**1**-**3**) among which one new compound (**3**). The structures of known compounds Lupenone (**1**), *α*-Amyrin (**2a**), and *β*-Amyrin (**2b**) were elucidated by comparison of their spectral data with those described in the literature [16, 17].

Compound **3** was obtained has a white powder from the Hex/CH_2Cl_2 9:1 fraction. Its molecular formula was established as $C_{36}H_{72}O_2$ by TOF-MS-ESI+ analysis which showed a quasi-molecular ion peak at *m/z* 537.3 $[M+H]^+$. In its ^1H NMR spectrum a signal of three protons triplet at δ_H 0.80 (3H, t, J = 6.0 Hz) indicates the presence of a terminal methyl group. A signal at δ_H 1.20-1.32 (6H, brs, $-(CH_2)_{32}-$) indicated the presence of a straight chain of 32 carbon atoms. The spectrum displayed a signal at δ_H 2.30 (2H, t, $-CH_2-COOH$) for methylene protons attached to a carboxylic group. On the basis of the NMR spectra it was inferred that compound **3** is an aliphatic acid. The ^{13}C NMR spectrum of compound **3** confirmed this suggestion by exhibiting important signals for carboxylic carbon at δ_C 180.1 and methyl carbon at δ_C 14.3 (C-36). The remaining methylene carbon resonated between δ_C 34.1-29.0. Mass spectral studies offered further support to the above assignment. The molecular ion peak at *m/z* 537.3 gave the molecular formula $C_{36}H_{72}O_2$. The peak at *m/z* 532.3 was due to the loss of two H_2 from molecular ion. The loss of an C_9H_{19} radical from the parent ion gave an ion which appeared at *m/z* 409.2 and this ion underwent successive loss of $C_{13}H_{27}^{\bullet}$ and $C_3H_7^{\bullet}$ units to give ions appearing at *m/z* 227.1 and 185.0 successively (Figure 2). The isolated aliphatic acid was therefore identified as Hexatriacontanoic acid.

3.2 Total polyphenol content and antiradical activity

The total polyphenol content was performed only on the acetone extract since phytochemical screening indicated that no phenolic compound was present in hexane extract. These results indicated the presence of polyphenolic compounds in the ethyl acetate extract and the total phenolic content of the said acetone extract was found to be 0.166±0.008 gGAE/100gRM. This value is less that that obtained by Talla and co-workers for propolis of Ngaoundal 2.32 ± 0.37-8.64 ± 0.47 gGAE/100gRM [18] and also those of Njintang and co-workers 10.99 ± 2.56-12.12 ± 2.24 g/100g [19] for some Cameroonian samples. This difference could be explained the difference in environmental conditions and local flora of site of collection of the propolis samples.

According to Melo and co-workers [20], anti-oxidant activity can be classified based on the performance of crude extracts as follows: good activity (IC_{50} < 69 µg/mL); moderate activity (69 µg/mL < IC_{50} < 161 µg/mL); low activity (IC_{50} > 161 µg/mL) [20]. The percentage inhibition was dose-dependent or concentration-dependent. The IC_{50} were deduced by graphical means and presented in table 1 and higher values imply low activity and vice versa. The acetone extract had a higher activity with IC_{50} of 141 µg/mL compared to hexane extract with IC_{50} 267 µg/mL. This could be explained by the absence of polyphenols in the hexane extract because the higher activity of acetone extract corroborates with its polyphenol content 0.166 ± 0.008 gGAE/100gRM. Ascorbic acid (vitamin C) possesses higher anti-radical activity (IC_{50} = 9 µg/mL) than the tested samples.

4 Conclusion

Evaluation of antiradical activity carried out on the acetone extract showed a moderate one with percentage

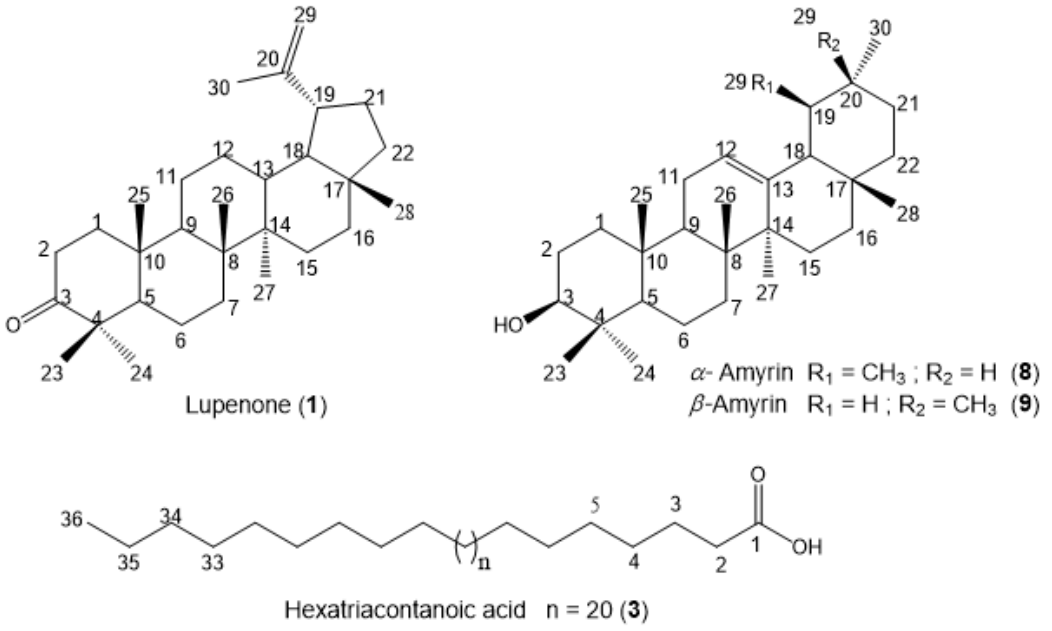

Figure 1: Structures of the compounds isolated.

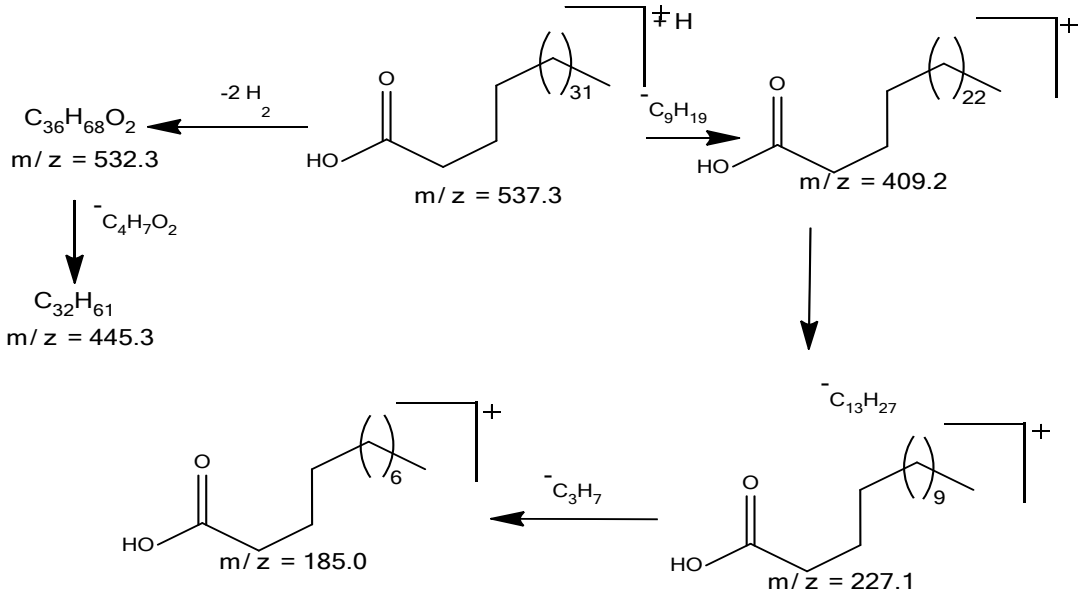

Figure 2: Proposed fragmentation pattern of compound **3.**

Table 1: IC_{50} values of tested samples.

Sample	IC_{50} (µg/mL)	Percentage inhibition (%)
AE	141	50.37
HE	267	38.75
Vitamin C	9	85.63

AE: Acetone extract. HE: Hexane extract.

inhibition of 50.37% with an IC_{50} of 140 µg/mL while that of the hexane hexane extract showed low antiradical activity with percentage inhibition of 38.75% and IC_{50} of 267 µg/mL. The acetone extract has low polyphenols content of 0.166 ± 0.008 gGAE/100gRM. Column chromatographic separation on silica gel of the hexane extract yielded compounds **1** to **3.** The structures of these compounds were elucidated based on NMR and mass spectroscopic data

lup-20(29)-en-3-one (**1**), a mixture of α and β-amyrin (**2**) and lastly hexatriacontanoic acid (**3**) which is described for the first time from propolis.

Conflict of interest: Authors declare no conflict of interest.

References

[1] Ntchapda F, Talla E, Sakava P, Tanzi F, Tchuenguem FF, Mbafor TJ, et al. Nitric oxide-dependent vasodilation and Ca2+ signalling induced by erythrodiol in rat aorta. APJTD. 2015;05:1–10.

[2] Kaewmuangmoon J, Pawornrat N, Atsalek R, Winayanuwattikun P, Chanchao C. Preliminary screening for various bioactivities in honey and propolis extracts from Thai bees. EJMP. 2012;02(2):74–92.

[3] Talla E, Dabole B, Taïwe GS, Ngo-Bum E, Mbafor JT, Atchade AD, et al. Antinociceptive pentacyclic triterpenoids from the cameroonian brown propolis. Pharmacologia. 2013;04(3):218–27.

[4] Sakava P, Talla E, Matchawe C, Tchinda TA, Zeuko'o ME, Laurent S, et al. Pentacyclic triterpenes and crude extracts with antimicrobial activity from cameroonian brown propolis samples. J Appl Pharm Sci. 2014;04(7):1–9.

[5] Segueni N, Kadour B, Bousseboua H, Fairouz M, Amar Z, Mesbah L, et al. Antibacterial activity of two algerians propolis. IJPSRR. 2014;25:106–10.

[6] Valenzuela-Barra G, Castro C, Figueroa C, Barriga A, Silva X, de Las Heras B, et al. Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago, Chile. J Ethnopharmacol. 2015 Jun;168:37–44.

[7] Irlan AF, Severino MA, Pedro LR. A pharmacological perspective on the use of Brazilian red propolis and its isolated compounds against human diseases. Eur J Med Chem. 2016;01:1–25.

[8] Zina M, Salim O, Abderezak T. Antioxydant activity of some algerian honey and propolis. Ind Crops Prod. 2016;01:1–6.

[9] Tamfu AN, Domgnim ME, Talla E, Tan PV, Mbafor TJ, Popova M, et al. Chemical Constituents and Anti-ulcer Activity of Propolis from the North-West Region of Cameroon. Res J Phytochem. 2016;10(2):45–57.

[10] Zingue S, Nde CB, Michel T, Ndinteh DT, Tchatchou J, Adamou M, et al. Ethanol-extracted Cameroonian propolis exerts estrogenic effects and alleviates hot flushes in ovariectomized Wistar rats. BMC Complement Altern Med. 2017 Jan;17(1):65.

[11] Khalil ML. Biological activity of bee propolis in health and disease. Asian Pac J Cancer Prev. 2006 Jan-Mar;7(1):22–31.

[12] Talla E, Tamfu NA, Gade SI, Yanda L, Mbafor TJ, Laurent S, et al. New mono-ether of glycerol and triterpenes with DPPH radical scavenging activity from Cameroonian propolis. Nat Prod Res. 2016;01:1–12.

[13] Gülçin I, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from Stephania rotunda: cepharanthine and fangchinoline. J Enzyme Inhib Med Chem. 2010 Feb;25(1):44–53.

[14] Huang S, Zhang CP, Wang K, Li GQ, Hu FL. Recent advances in the chemical composition of propolis. Molecules. 2014;19(12):19610–32.

[15] Bankova V, Popova M, Trusheva B. New emerging fields of application of propolis. Maced J Chem Chem Eng. 2016;35(1):1–11.

[16] Venkata VS, Indra P. Isolation and structural characterization of lupane triterpenes from Polypodium vulgare. Res. J. Pharmaceutical. Sci. 2012;01:23–7.

[17] Vázquez LH, Palazon J, Navarro-Ocaña A. The Pentacyclic Triterpenes α,β-amyrins: A Review of Sources and Biological Activities, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.), ISBN: 978-953-51-0296-0, InTech; 2012. http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/the-pentacyclic-triterpenes-amyrins-a-review-of-sources-and-biological-activities

[18] Talla E, Ngenge TA, Biyanzi P, Sakava P, Asobo FP, Mbafor TJ, et al. Phytochemical screening, antioxidant activity, total polyphenols and flavonoids content of different extracts of propolis from Tekel (Ngaoundal, Adamawa region, Cameroon). J. Phytopharmacol. 2014;03:321–9.

[19] Njintang YN, Tatsadjieu NL, Ngakou A, Danra D, Tchuenguem-Fohouo FN. Antiradical activity and polyphenol content of ethanolic extracts of Propolis. Int J Biosci. 2012;2(4):56–63.

[20] Gomes de Melo J, de Sousa Araújo TA, Thijan Nobre de Almeida e Castro V, Lyra de Vasconcelos Cabral D, do Desterro Rodrigues M, Carneiro do Nascimento S, et al. Antiproliferative activity, antioxidant capacity and tannin content in plants of semi-arid northeastern Brazil. Molecules. 2010 Nov;15(12):8534–42.