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Antioxidant Activity and Chemical Constituents from Stem Bark of *Ficus abutilifolia.* Miq (Moraceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TE, DTM and AAT designed the study, wrote the protocol and managed the literature searches. Authors DTM, HA and NJN performed the chromatographic isolation and structural determination. Authors SL and CH performed the spectroscopic analysis. Authors DTCL and DTM performed the antioxidant and statistical analysis. Authors DTM and NJN wrote the first draft of the manuscript. Authors TE and JMT advised and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was conducted to determine the antioxidant activity and chemical components of *Ficus abutilifolia*. Miq. (Moraceae).
Place and Duration of the Study: The study were performed at Department of chemistry, University of Ngaoundere, Cameroon, between July 2017 and September 2018.
Methodology: Phytochemical study was carried out on all extracts of stem barks. Besides, the total phenols contents (TPC) using the Folin-Ciocalteu and the antioxidant activities using DPPH

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and FRAP methods were also evaluated. The isolation of compounds from the EtOAc extract was done using column and thin layer chromatographic techniques on silica gel. The structures of isolated constituents were elucidated using mass spectrometry, 1D and 2D-NMR techniques.
Results: Phytochemical screening revealed that all the compounds tested were found to be present in the acetone extract; the hexane extract was the poorest in compounds. Acetone extract, DCM/MeOH (1:1) and MeOH extracts were found to be rich in phenols with TPC respectively (239. 849 ± 0.969), (232.676 ± 0.404) and (109.654 ± 0.724) mg EAG/100g EX. Except the n-hexane extract, all the others exhibit antioxidant activities, acetone extract being the most effective with an inhibitory concentration 50 (IC_{50}) of (0.038 ± 0.002) mg/mL for the DPPH antiradical scavenging activity and (0,021 ± 0,002) mg/mL for FRAP. A new fatty acid named Pentacosyl henicosanoate (**2**) along with six known compounds Octatriacontane (**1**), *β*–sitosterol (**3**), a mixture of Lupeol acetate (**4a**), *α*-amyrin acetate (**4b**), *β*-amyrin acetate (**4c**) and Daucosterol (**5**) were isolated from the column chromatography of the ethyl acetate extract. Daucosterol (**5**) exhibited antiradical activity with an IC_{50} of (13.005 ± 0.005) mg/mL. Only Daucosterol (**5**) has exhibited antiradical activity with an IC_{50} of (13.005 ± 0.005) mg/mL among the isolated compounds.
Conclusion: This study provides scientific evidence and support for the traditional uses of *F. abutilifolia* stem barks in the treatment of diseases associated with oxidative stress.

Keywords: Ficus abutilifolia; stem bark; antioxidant activity; DPPH; FRAP; isolated compound; Pentacosyl henicosanoate.

1. INTRODUCTION

Free radicals are naturally produced under aerobic conditions and usually promote the good functioning of the body and the health of mammals, but their excess can be harmful [1, 2]. In recent years there has been an overflow of information about the role of the oxidative stress in triggering a number of serious diseases, studies have indicated that free radicals are implicated in the pathogenesis of diabetes, liver damage, atherosclerosis, inflammation, cardiovascular disorders, neurological disorders and in the process of aging acids [1,3]. Antioxidants are known as substances able to suppress, delay or prevent oxidation process [4], they neutralize the harmful free radicals in human bodies. Antioxidants are free radical scavengers that prevent or slow damage done by these free radicals. Due to the overexposure to environmental factors (smoking, change of environment, increasing stress etc.), the excess of radicals can become difficult to be controlled by enzymes [5]. It is therefore urgent to find another source of antioxidants because of the harmfulness to human health, unavailability and high cost of the synthetic ones [6,7]. It has been shown that antioxidants are present in fruits and vegetables and in most of the plants [8]. Because of their bioactive substances, various plants extracts are used in the treatment of several diseases associated with oxidative stress [9,10]. These bioactive substances play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals [11].

Ficus abutilifolia is one of the rich floristic resources that Cameroon has reference to medicinal plants. It is commonly called "*dundeehi hooseere*" in Fufulde in the North Region of Cameroon and "large-leaved rock fig" or "rock wild fig" in English. The plant is a small to medium sized, deciduous to semi-deciduous tree that may grow up to 15 m high and belongs to the Moraceae family. It is traditionally used to treat various ailments such as typhoid fever, chronic dysentery, sexually transmitted infections, malaria, infertility and epilepsy [12,13,14]. Additionally, in the North region of Cameroon, *F. abutilifolia* is used against jaundice, hypertension, coughs, rheumatism and also to protect food stored. Antibacterial, anticonvulsant as well as qualitative phytochemical profile of leaf and root of *F. abutilifolia* have already been investigated [15,16,17]. To the best of our knowledge, there is a paucity of information regarding the antioxidant activity and isolated compounds from *F. abutilifolia*. This study was therefore performed to investigate the antioxidant activities of stem bark extracts of the plant and to isolate bioactive constituents.

2. MATERIALS AND METHODS

2.1 General Experimental Procedure

^{1}H-NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with a Bruker AV-500 Spectrometer, in $CDCl_3$ and DMSO-d_6 with TMS as internal reference. Chemical shifts were given in ppm. ESI-MS spectra were registered on a Q-

TOF Ultima spectrometer (Waters). Column chromatography (CC) was performed on silica gel normal phase 60 (Merck, 63-200 µm) with a gradient of n-hexane, n-hexane-EtOAc, EtOAc and EtOAc-MeOH as eluents. Analytical Thin layer chromatography (TLC) was carried out on silica gel precoated plates F-254 Merck (20 x 20 cm). Detection of the spots was achieved under UV light (254 and 365 nm) and by spraying with 10% sulfuric acid followed by heating at 105°C for few minutes. The absorbance in the experiments was read on a GENESYS 10S UV-VIS. DPPH (Aldrich, 95%) and Folin-Ciocalteu reagent (Sigma, 2N) were purchased from Sigma-Aldrich. Ascorbic acid (Riedel-De Haen, 99.7%), BHT (Sigma-Aldrich, 99,5%), and Gallic acid (Sigma-Aldrich, 97.5-102%), were used as standards. All organic solvents used for the tests were upgrade and water was distilled.

2.2 Plant Material

The stem bark of *F. abutilifolia* were collected in July 2017 in the locality of Poli in the North Region of Cameroon and identified by Pr. Mapongmetsem Jean Marie, botanist and agro-forester in the Department of Biological Science, University of Ngaoundere, Cameroon. The stem barks were cut in small pieces and dried at room temperature for 3 weeks, after that, the dried barks were ground into uniform powder to increase the surface area of the sample for extraction.

2.3 Extraction and Phytochemical Screening

The obtained stem barks powder (1.5 Kg) of *F. abutilifolia* were extracted by mechanical stirring successively with increasing polarity of Hexane, EtOAc, acetone, CH_2CL_2/MeOH (1:1) and MeOH (6 L each) for 48 h at room temperature. For each solvent the operation was repeated 3 times to exhaust the powder as much as possible. 20.8 g, 15.1 g, 63.5 g, 49.11 g, and 21.2 g of extracts were respectively obtained. These extracts were then subjected to preliminary phytochemical screening according to standard procedures [18] to investigate the various classes of natural compounds. They were tested for the presence of alkaloids, steroids, triterpenoids, phenolic compounds, flavonoids, saponins, tannins, carbohydrates and anthraquinons inspired by the works of Taiwo *et al*.,[15].

2.4 Compounds Isolation

Ethyl acetate extract (14 g) was chromatographed over silica gel column and eluted with a gradient of increasing polarity solvent. This fractionation lead to isolation of 7 compounds 1-5 (Fig. 3): **1**, (17 mg); **2**, (19 mg); **3** (15 mg); (20 mg) of a mixture of compounds **4a**, **4b** and **4c**) and **5** (31 mg) using respectively Hex/EtOAc (10:0; 9.5:0.5; 8.5:1.5; 7.0:3.0), EtOAc/MeOH (9.5: 0.5).

Octatriacontane 1: white amorphous solid, TOF-MS-ESI+ *m/z* 557.7 $[M+Na]^+$ for $C_{38}H_{76}$. ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 1.39 (4H, *m*, 2H-2, 2H-37), 1.05-1.17 [(CH_2)n] and 0.7 (6H, *t*, 3H-1, 3H-38).^{13}C-NMR ($CDCl_3$, 500MHz): *δ* 14.1 (CH_3, C-1, C-38), 22.71 (CH_2, C-2, C-37), 29.3 (CH_2, C-4, C-35), 29.7 (CH_2, C-5 to C-34), 32.08 (CH_2, C-3, C-36) [19].

Pentacosyl henicosanoate 2: TOF-MS-ESI+ *m/z* 699.4 $[M+Na]^+$ for $C_{46}H_{92}O_2$. Its ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.81 (6H, *t*, J=7.1 Hz, 3H-21, 3H-25'), 1.14 -1.57 [(CH_2)n], 2.25(2H, *t*, J=7.5 Hz, 2H-2) and 4.00 (2H, *t*, J=6.7 Hz, 2H-1').^{13}C-NMR ($CDCl_3$ 125 MHz): δ_C 14.12 (CH_3, C-21 and C-25'), 22.7-34.4 (C-4 to C-18 and C-3' to C-23'), 64.41 (CH_2, C-1'), 174.05 (C, C-1).

***β* - sitosterol 3:** colorless needles ($C_{29}H_{50}O$), ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 5.36 (1H, *t*, H-6), 5.16 (1H, *m*, H-22), 5.28 (1H, *m*, H-23), 3.54 (1H, *m*, H-3), 1.28 (3H, *s*, H-18), 0.93 (3H, *d*, H-21), 0.92(3H, *d*, H-29), 0.86 (3H, *d*, H-27), 0.83 (3H, *d*, H-26) and 0.72 (3H, *s,* H-19).^{13}C-NMR ($CDCl_3$,125 MHz): δ_C 36.1 (CH_2, C-1), 29.1 (CH_2, C-2), 71.8 (CH, C-3), 42.2 (CH_2, C-4), 140.7 (C-5), 121.7 (CH, C-6), 31.6 (CH_2, C-7), 31.9 (CH, C-8), 50.1 (CH, C-9), 36.5 (C, C-10), 24.3 (CH_2, C-11), 39.7 (CH_2, C-12), 42.3 (CH, C-13), 56.7 (CH, C-14), 24.3 (CH_2, C-15), 28.2 (CH_2, C-16), 56.0 (CH, C-17), 11.9 (CH_3, C-18), 19.0 (CH_3, C-19), 36.5 (CH_2, C-20), 18.7 (CH_3, C-21), 33.9 (CH_2, C-22), 26.1 (CH_2, C-23), 45.8 (CH, C-24), 33.9 (CH, C-25), 21.09 (CH_3, C-26), 19.8 (CH_3, C-27), 23.09 (CH_2, C-28), 11.8 (CH_3, C-29) [20].

Lupeol acetate 4a : white powder , TOF-MS-ESI+ m/z 537.3 $[M+3Na]^{3+}$ for $C_{32}H_{52}O_2$ ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 4.68 (d, J = 2.4 Hz, H-29b), 4.52 (br s, H-29a), 0.98 (s,H_3-23), 0.84 (s, H_3-24), 0.88 (s, H_3-25), 0.97 (s, H_3-26), 1.01 (s, H_3-27), 0.92 (s, H_3-28), 1.13 (s, H_3-30). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 38.4 (C-1), 27.4 (CH_2, C-2), 80.9 (CH, C-3), 39.6 (CH_2, C-4), 55.4 (C-5), 18.2 (CH, C-6), 34.2 (CH_2, C-7), 40.8 (CH, C-8), 50.3 (CH, C-9), 37.8 (C, C-10), 21.4 (CH_2, C-11), 25.9 (CH_2, C-12), 42.8 (CH, C-13), 42.8 (CH, C-14), 26.9 (CH_2, C-15), 35.5 (CH_2, C-16), 43.01 (CH, C-17), 48.3 (CH_3, C-18), 48.02 (CH_3, C-19), 150.9 (CH_2, C-20), 29.8 (CH3, C-

21), 40.02 (CH2, C-22), 28.08 (CH_2, C-23), 15.9 (CH, C-24), 16.7 (CH, C-25), 16.5 (CH_3, C-26), 14.5 (CH_3, C-27), 18.02 (CH_2, C-28), 109.3 (CH_3, C-29), 20.9 (CH_3, C-30), 171.02 (C- 1'), 21.33 (C- 2') [21].

α - amyrin acetate 4b : white powder, TOF-MS-ESI+ m/z 537.3 $[M+3Na]^{3+}$ for $C_{32}H_{52}O_2$, ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 5.12 (t, J = 3.6 Hz, H-12), 0.83 (s, H-23), 0.84 (s, H-24), 0.80 (s, H-25), 0.92(s, H_3-26), 1.01 (s, H_3-27), 0.96 (s, H_3-28), 0.85 (d, H_3-29), 0.83 (d, H_3-30). ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ_C 38.4 (CH_2, C-1), 28.7 (CH_2, C-2), 80.9 (CH, C-3), 38.4 (CH_2, C-4), 55.2 (C-5), 18.2 (CH, C-6), 32.7 (CH_2, C-7), 40.02 (CH, C-8), 46.8 (CH, C-9), 37.1 (C, C-10), 23.2 (CH_2, C-11), 124.3 (CH_2, C-12), 139.6 (CH, C-13), 42.09 (CH, C-14), 27.4 (CH_2, C-15), 26.6 (CH_2, C-16), 33.7 (CH, C-17), 59.08 (CH_3, C-18), 39.63 (CH_3, C-19), 39.67 (CH_2, C-20), 31.2 (CH_3, C-21), 41.5 (CH_2, C-22), 28.7 (CH_2, C-23), 16.1 (CH, C-24), 15.7 (CH, C-25), 16.88 (CH_3, C-26), 23.2 (CH_3, C-27), 28.1 (CH_2, C-28), 17.5 (CH_3, C-29), 21.4 (CH_3, C-30), 171.02 ,(C- 1'), 21.33 (C- 2') [21].

β - amyrin acetate 4c : White powder, TOF-MS-ESI+ m/z 537.3 $[M+3Na]^{3+}$ for $C_{32}H_{52}O_2$, ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 5.18 (t, J = 3.6 Hz, H-12), 0.97 (s, H_3-23), 0.92 (s, H_3-24), 0.80 (s, H_3-25), 0.96 (s, H_3-26), 1.07 (s, H_3-27), 1.03 (s, H_3-28), 0.87 (s, H_3-29), 0.85 (s, H_3-30). ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ_C 38.06 (CH_2, C-1), 27.9 (CH_2, C-2), 80.9 (CH, C-3), 39.6 (CH_2, C-4), 55.2 (C-5), 18.2 (CH, C-6), 32.8 (CH_2, C-7), 40.05 (CH, C-8), 47.6 (CH, C-9), 37.7 (C, C-10), 23.3 (CH_2, C-11), 121.6 (CH_2, C-12), 145.2 (CH, C-13), 41.5 (CH, C-14), 27.9 (CH_2, C-15), 25.9 (CH_2, C-16), 32.8 (CH, C-17), 47.6 (CH_3, C-18), 46.8 (CH_3, C-19), 31.1 (CH_2, C-20), 34.2 (CH_3, C-21), 37.1 (CH_2, C-22), 28.1 (CH2, C-23), 15.7 (CH, C-24), 15.9 (CH, C-25), 16.82 (CH_3, C-26), 25.9 (CH_3, C-27), 28.08 (CH_2, C-28), 33.7 (CH_3, C-29), 23.7 (CH_3, C-30), 171.02 ,(C- 1'), 21.33 (C- 2') [21].

Daucosterol 5 : white powder ($C_{35}H_{60}O_6$) ^{1}H-NMR (DMSO-*d6*, 500 MHz): δ_H 5.34 (H-6), 4.88 (1H, d, H-1'), 3.14 (1H, m, H-5'), 4.24 (1H, t, H-4'), 2.91 (1H, m, H-2'),0.97, 3.67 (1H, m, H-3'), 4.42 (1H, m, H-6'), 0.93 (3H, d, H-21), 1.00 (3H, s, H-19), 0.82 (3H, d, H-26), 0.85 (3H, d, H-29), 0.68 (3H, s, H-18). ^{13}C-NMR (DMSO-*d6*, 125 MHz) : δ_C 36.8 (CH_2, C-1), 31.3 (CH_2, C-2), 76.9 (CH, C-3), 38.3 (CH_2, C-4), 140.4 (C, C-5), 121.2 (CH, C-6), 31.4 (CH_2, C-7), 29.2 (CH, C-8), 49.6 (CH, C-9), 35.4 (C, C-10), 20.6 (CH_2, C-11), 39.3 (CH_2, C-12), 41.8 (C, C-13), 56.1 (CH, C-14), 23.9 (CH_2, C-15), 27.7 (CH_2, C-16), 55.4 (CH, C-17), 11.7 (CH_3, C-18), 18.9 (CH_3, C-19), 36.2 (d, C-20), 18.6 (CH_3, C-21), 33.3 (CH_2, C-22), 25.4 (CH_2, C-23), 45.1 (CH_2, C-24), 28.7 (CH, C-25), 19.1 (CH_3, C-26), 19.7 (CH_3, C-27), 22.6 (CH_2, C-28), 11.6 (CH_3, C-29), 100.8 (CH, C-1'), 73.5 (CH, C-2'), 76.9 (CH, C-3'), 70.1 (CH, C-4'), 76.9(CH, C-5'), 61.1 (CH_2, C-6') [22].

2.5 Determination of Total Phenolic Content (TPC)

The analyses were based on reduction produced by phenolics in the presence of the phenol Folin-Ciocalteu reagent by using the method previously describe by López-Mejía *et al.,* [23] with slight modifications. To 100 µL of each extract, 200 µL of Folin-Ciocalteu reagent, and 2000 µL of distilled water were added. The mixture was incubated for 3 min, after what, 1000 µL of Na_2CO_3 20% was added. The final solution, 3300 µL, was incubated for 60 min in darkness at room temperature and the absorbance determined at 765 nm. Gallic acid was used as standard and prepared in same conditions as above at different concentrations (0.2; 0.4; 0.6; 0.8 and 1) mg/mL. The blank was prepared with 2100 µL of water and 200 µL of Folin-Ciocalteu reagent. Absorbance was measured at 765 nm in a GENESYS 10S UV-VIS spectrophotometer. The determination of the total phenolic compounds was carried out using a straight-line equation obtained from the standard Gallic acid calibration graph. The total phenolic content was measured as milligrams of Gallic acid equivalent per 100 g of dry extract.

2.6 *In vitro* Antioxidant Activities

2.6.1 DPPH radical-scavenging activity assay

The effect of different extracts of *F. abutilifolia* on DPPH radical (1,1-diphenyl-2 picrylhydrazyl) was investigated using the method described by Olorunnisola *et al*., [24] with slight modifications. Each extract were prepared in MeOH at different concentrations (0.02; 0.04; 0.06; 0.08 and 0.1 mg/mL) by successive dilutions of sample stock solution 0,1 mg/mL in MeOH. For each concentration, 1000 µL of $DPPH^{\bullet}$ (0.3 mM in MeOH) was added to 2500 µL of sample or extract. The mixtures were shaken vigorously and incubated for 30 min at 25°C in the dark. After that, the absorbance of the fractions were measured at 517 nm in a UV–VIS spectrophotometer against a blank (2500 µL of MeOH in 1 000 µL of DPPH). Acid ascorbic were used as standard and were also prepared at similar concentrations and his absorbance were

determined, in comparison with those of extracts. The ability to scavenge DPPH radical was calculated according to the following equation:

DPPH radical scavenging activity (%) = [(Abs control – Abs test) / (Abs control)] × 100

Where,

Abs control is the absorbance of DPPH radical + methanol; Abs test is the absorbance of DPPH radical + sample extract.

2.6.2 Evaluation of the Ferric Reducing antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power in the extracts were determined according to the method described by Oyaizu [25] which is based on the reduction of iron (Fe^{3+}) present in the $K_3Fe(CN)_6$ complex into Fe^{2+}, through and electron transfer mechanism. 1 mL of the extract at different concentrations (0.02 ; 0.04; 0.06; 0.08 and 0.1 mg/mL) were mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a solution of potassium ferricyanide $K_3Fe(CN)_6$ 1%. The whole solution was incubated in a water bath at 50 °C for 20 minutes then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous $FeCl_3$ solution. The absorbance of the reaction medium was read at 700 nm against a similarly prepared blank, by replacing the extract with distilled water. The positive control was a solution of a standard antioxidant; ascorbic acid whose absorbance has been measured under the same conditions that the samples. Lower absorbance indicated a higher iron chelating capacity. Butylhydroxytoluen (BHT) were used as standard and were also prepared at similar concentrations as those of extracts. The Ferric Reducing Antioxidant Power was calculated accordingly by comparing the absorbance of the test samples with that of the negative control by the following equation.

Ferric Reducing Antioxidant Power = [(A control – A extract) / A control] x 100

2.7 Statistical Analysis

All the experiments were carried out in triplicate, and the results were presented as mean ± SD (standard deviation). Curves and graphs were obtained by using Microsoft Excel. The comparisons between the dependent variables were determined using the analysis of variance (ANOVA) by STATGRAPHICS Centurion.16.1.11. The Duncan statistical test (LSD: least significant difference) were used in the comparison of means. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of the qualitative phytochemical analysis of the stem barks extracts of *F. abutilifolia* are giving in Table 1.

The results showed that each extract contained at least one class of secondary metabolites, the acetone, the CH_2Cl_2/MeOH (1:1) and the MeOH extracts are the richest extracts containing the greatest number of organic compounds which include flavonoids, phenols, sterols and triterpenoids. Whereas hexane and EtOAc extracts contained respectively one and three classes of secondary metabolites. The phytochemical constitution of the methanol extract corroborates with the results of previous work carried out on the leaves and the root bark of *F abutilifolia* [14,15].The presence of those compounds in the studied extracts could therefore explain the observed activities and justified the traditional uses.

Table 1. Results of the phytochemical screening

Phytochemicals constituents	Extracts				
	Hexane	EtOAc	Acetone	CH_2Cl_2/MeOH (1:1)	MeOH
Terpenes and/or steroids	+	+	+	+	+
Phenols	-	-	+	+	+
Flavonoids	-	-	+	+	+
Carbohydrates	-	+	+	+	+
Saponins	-	+	+	-	-
Tannins	-	-	+	+	+
Anthraquinones	-	-	+	+	+

3.2 Total Phenolic Contents Results

Phenolic compounds are known to exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals [26]. They are also known to inhibit various types of oxidizing enzymes. These potential mechanisms make the diverse group of phenolic compounds an interesting target in the search for health beneficial phytochemicals [27]. The results obtained for the determination of total phenolic contents (Table 2) revealed a significant level of phenolic compounds in both extracts of *F. abutilifolia* except hexane and ethyl acetate extracts which were phenol-negative during phytochemical screening (Table 1). The acetone extract had the higher concentration of total phenolic compounds (239.849 ± 0.969) followed by the CH_2Cl_2/MeOH (1:1) extract (232, 676 ± 0, 404) which is in its turn two times higher than the MeOH extract (109.654 ± 0.724) mg EAG/100 gEX. These results may be justified by the presence in our extracts of metabolites such as flavonoids, anthraquinones and tannins which are part of phenolic compounds. The difference observed may be due to the respective polarities of solvent [26].

3.3 Antioxidant Activities

3.3.1 DPPH activity results test

The DPPH radical test as described above was implemented to evaluate the ability of extracts to trap DPPH radicals. The evaluation of the antioxidant power was made on all the extracts and all the isolated compounds by the trap capacity of radical DPPH, to know the active samples, this transformation leads to a color change from purple to yellow. This change in color was observed for all extracts except the hexane one. among the isolated compounds, only compound 5 daucosterol has exhibited an antiradical activity. The results obtained show a variation of the percentage inhibition as a function of the concentration of extracts and ascorbic acid used as reference (Fig. 1). The IC_{50} of each sample was determined by calculation using the logarithmic regression line. The IC_{50} of the tested samples are recorded in the (Table 2).

Table 2. DPPH radical scavenging activity, FRAP activity and TPC

Sample	Antioxidant activity		TPC (mgEAG/100 gEX)
	DPPH IC_{50} (mg/mL)	FRAP IC_{50} (mg/mL)	
EtOAc	4.532 ± 0.002^{b}	0.931 ± 0.006^{d}	/
Acetone	0.038 ± 0.002^{a}	0.021 ± 0.002^{b}	239.849 ± 0.969
CH_2Cl_2/MeOH (1:1)	0.053 ± 0.001^{a}	0.039 ± 0.004^{b}	232.676 ± 0.404
MeOH	0.060 ± 0.002^{a}	0.059 ± 0.002^{c}	109.654 ± 0.724
Daucosterol	13.005 ± 0.005^{c}	/	/
Ascorbic acid	0.010 ± 0,001^{a}	/	/
BHT	/	0.004 ± 0.001^{a}	/

Means followed by the same letter in a column do not differ significantly (n= 3; p < 0,05)

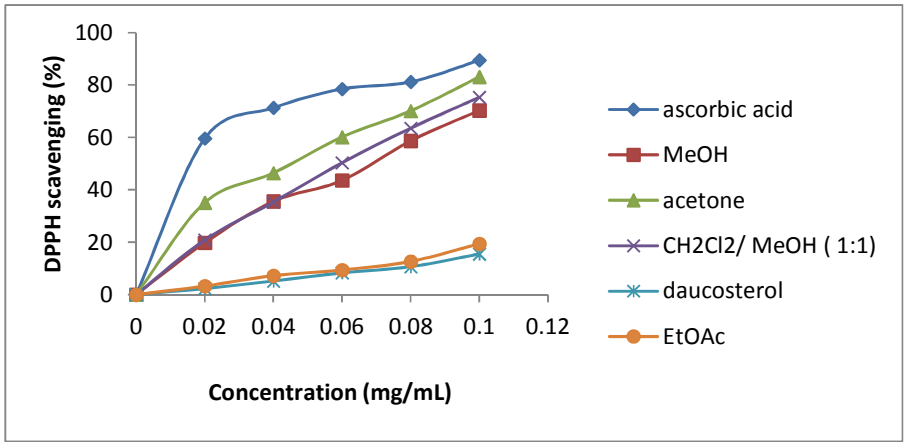

Fig. 1. DPPH inhibition assay of Daucosterol (4) and *F. abutilifolia* extracts

All the tested extracts are endowed with antioxidant activity except the hexane one. Scavenging of DPPH radical was found to increase with the sample concentrations (Fig. 1). The ascorbic acid uses as reference had the highest scavenging activity with an IC_{50} value of 0.010 mg/mL, followed by acetone extract with 0.038 mg/mL, then comes the CH_2Cl_2/MeOH (1:1) extract with 0.053 mg/mL followed by the methanol. This can be explained by their phytochemical composition which is related to their total phenolic contents. In fact, some studies showed that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes [28]. The hexane extract is not endowed with any antioxidant activity. Among all the actives extracts, EtOAc extract had the lowest scavenging activity with an IC_{50} value of 4.53 mg/mL. Indeed, this extract seems to not contain phenolic compounds according to the results of phytochemical screening, its activity could therefore certainly be due to the synergy of the other metabolites it contains. Daucosterol had an IC_{50} value of 13.01 mg/mL. These results are different from those obtained by Isaac *et al.,* [29] which evaluated the antioxidant activity of Daucosterol isolated from the $CHCl_3$ extract of the stem barks of *Ficus exasperata* with an IC_{50} value of 0.22 mg/mL. This difference could be explained by several experimental parameters as sample concentrations among others [30].

3.3.2 FRAP assay results test

Previous studies have shown that the reducing power of a sample can serve as a significant indicator of its potential antioxidant activity [31,32]. The presence of reducing agents in plant extracts causes the reduction of the Fe^{3+}/ ferricyanide complex into the ferrous form Fe^{2+}. The results of the test show that the FRAP activity of extracts increased proportionally with the concentration of extracts (Fig. 2). This can be explained by the fact that increasing the concentration of the extract lead to the formation of greater amount of Fe^{2+} complexes by increasing the reducing agent concentration [33]. At the concentration of 0.1 mg/mL, the reducing power of the acetone extract of *F. abutilifolia* is much higher than the one of the EtOAc, MeOH, DCM/MeOH (1:1) extracts, but lower than that of BHT used as positive control. The reducing power of *F. abutilifolia* extracts is probably due to the presence of hydroxyl groups in phenolic compound that can serve as electron donors. Therefore, antioxidants are considered as reducing and inactivating oxidants. This result follows the same order of reactivity of the extracts as in the DPPH test.

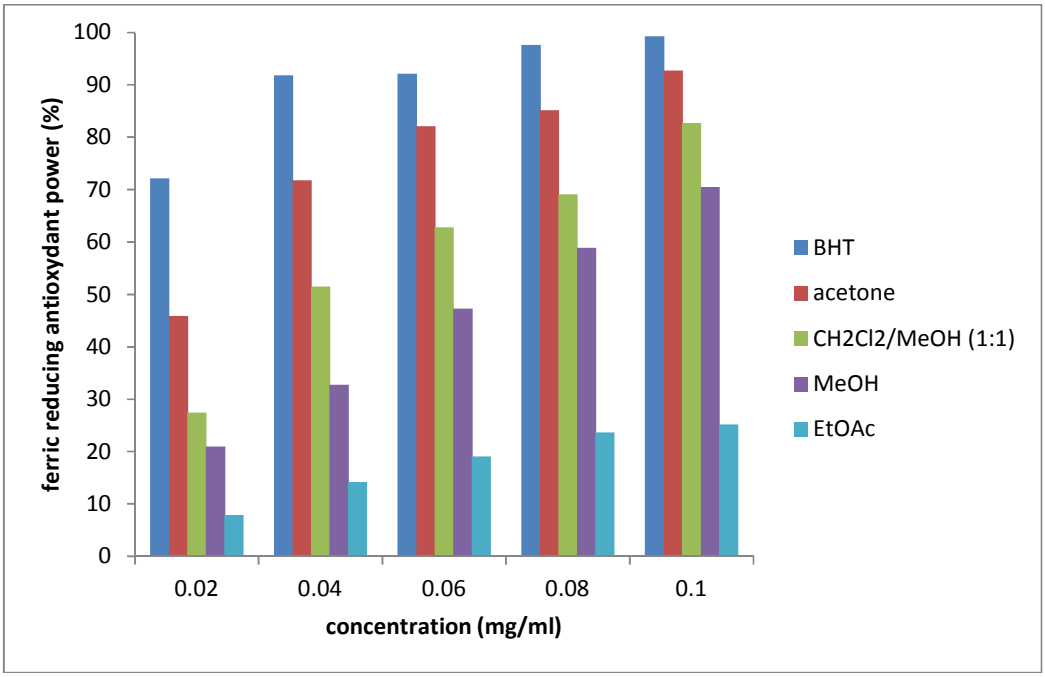

Fig. 2. Ferric Reducing antioxidant power of *F. abutilifolia* extracts

3.4 Compounds Isolation

The ethyl acetate extract of *F. abutilifolia* stem bark that was separated by column chromatography on silica gel yielded five compounds which to the best of our knowledge, are reported for the first time from this species The structures of these compounds were elucidated using ESI-TOF MS, NMR spectroscopy and by comparison with previous reported data of similar compounds. The known compounds were identified as: Octatriacontane 1; *β* - sitosterol 3; Daucosterol (*β*-sitosterol-3-*O*-*β*-D-glucoside) 5; lupeol acetate 4a; *α* –amyrin acetate 4b; and *β* –amyrin acetate; The last three compounds were obtained as a mixture, this mixture crystallizes as a white powder in Hex/EtOAc (7.0:3.0). The ratio of the mixture is about 1:5:2, deduced from the intensities of the ^{1}H NMR resonances for the olefinic protons of 4a at δ 4.68 (d, *J* = 2.4 Hz H-29b) and 4.52 (br s, H-29a) [21], 4b at δ 5.12 (t, *J* = 3.6 Hz, H-12) [34] and 4c at δ 5.18 (t, *J* = 3.6 Hz, H-12) [34], and the intensities of carbons at *δ* 150.9 (C-20), 109.3 (C-29); *δ* 124.3 (C-12),139.6 (C-13); *δ* 121.6 (C-12), 145.2 (C-13) [21], characteristics of pentacyclic triterpene skeletons respectively of lupan, ursan and oleanan type.

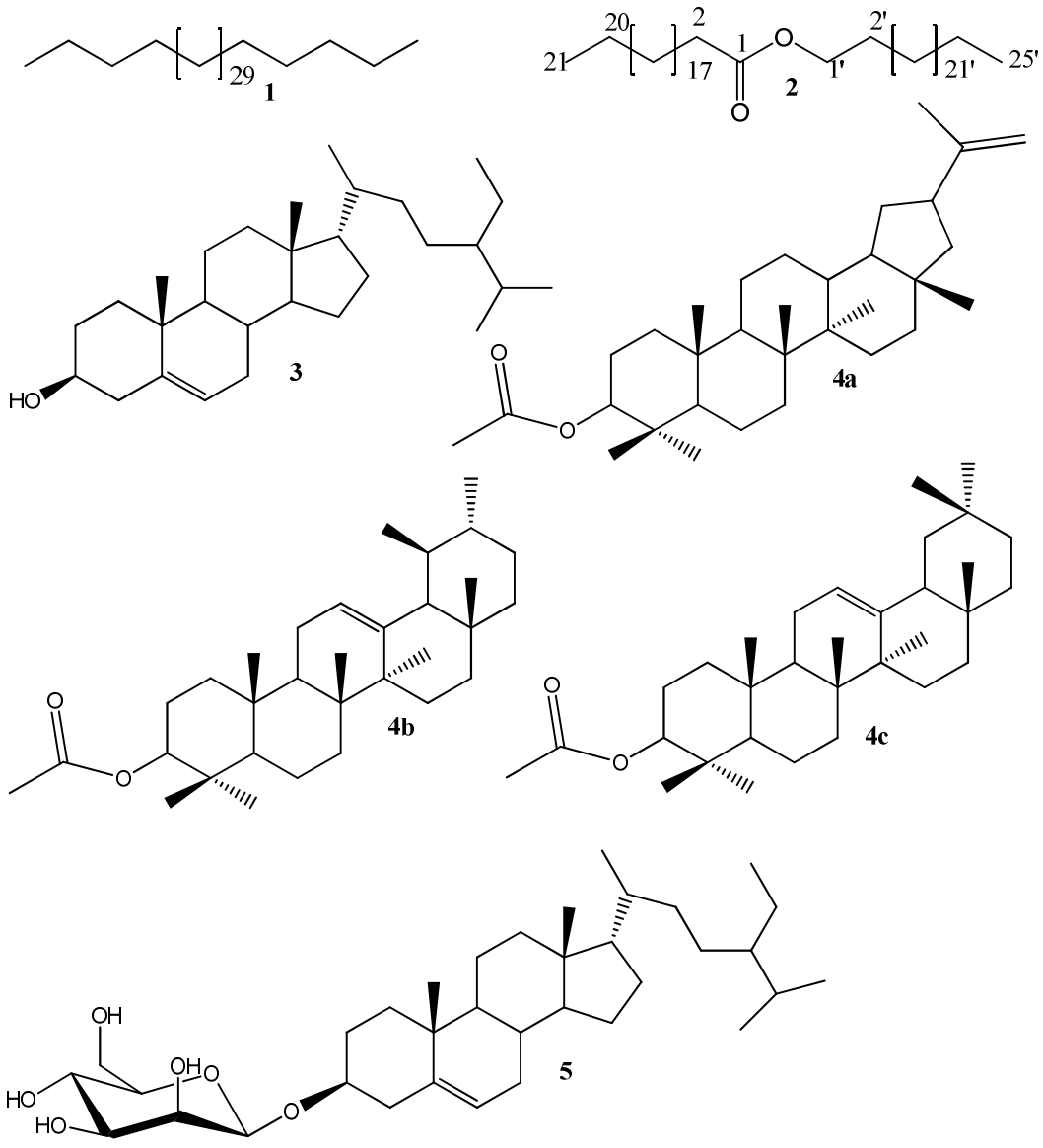

Fig. 3. Isolated compounds from *F. abutilifolia* stem bark

Compound 2 was obtained as a white amorphous solid. Its molecular formula found to be $C_{46}H_{92}O_2$ deduced by the TOF-MS-ESI$^+$ spectra that showed pseudo-molecular ion peak $[M+Na]^+$ at *m/z* 699.4. Its ^{1}H NMR ($CDCl_3$, 500 MHz) revealed the presence of a triplet of six protons at δ_H 0.81 (6H, *t*, H-21 and H-25') corresponding to two terminal methyl groups. The spectrum also revealed at δ_H 4.00 two protons triplet (2H, *t*, H-1') probably deshielded by the closeness of the ester function. At δ_H 1.14 and δ_H 1.57 the spectrum showed a broad signal corresponding to a linear hydrocarbon sequence which revealed an integration of 76 protons. At δ_H 2.25, the spectrum also displayed a signal of two protons triplet (2H, *t*, H-2) reference to the methylene protons linked to the carbon located in *α* of the ester group. Its ^{13}C NMR spectrum recorded in $CDCl_3$ at 125 MHz showed characteristic signals of a fatty ester among which the signal of the carbonyl ester function (C-1) at δ_C 174.05; the signal of a methylene carbon (C-1') deshielded by the nearness of the ester function at δ_C 64.41; a signal corresponding to the terminal methyl groups (C-21 and C-25') at δ_C 14.1; between δ_C 22.7 and δ_C 34.4 appeared the signals corresponding to a long carbon chain of methylene. From the HSQC spectra of compound 2, direct correlations were detected between the protons 2H-1' at δ_H 4.00 and carbon C-1' at δ_C 64.4; 2H-2 at δ_H 2.25 and C-2 at δ_C 34.4; (3H-21, 3H-25') at δ_H 0.81 and (C-21, C-25') at δ_C 14.13; correlations were also observed between protons from δ_H 1.14 to δ_H 1.57 and carbon from δ_C 22.7 to δ_C 31.9.

The COSY spectra of compound 2 showed the correlations between the methyl protons at δ_H 0.81(6H, *t*, H-21 and H-25') and protons at δ_H 1.26 which also correlated with the methylene protons at δ_H 1.57. The correlations of the methylene at *δH* 1.54 with the ones at δ_H 2.25 (2H, *t*, H-2) and the ones at δ_H 4.00 (2H, *t*, H-1') respectively were also visible (Fig. 5). From the HMBC spectra, we observed the correlations between the protons at δ_H 0.81 (6H, H-21 and H-25') and carbon C-20, C-24' (δ_C 22.7) and C-19, C-23' (δ_C 31.9). Another correlation is observed between the protons at δ_H 1.26 (2H, H-20 and H-24') with carbon (C-17, C-21') at δ_C 29.7. A set of correlation is also observed between the methylene at δ_H 2.25 (2H- 2) and carbon (C-1) at δ_C 174.05 (Fig. 5).

All the above information allowed us to identify compound 2 as Pentacosyl henicosanoate. The study of its TOF-Electro Spray mass spectrum in positive mode made possible the identification of some characteristic fragments indicated in Fig. 4.

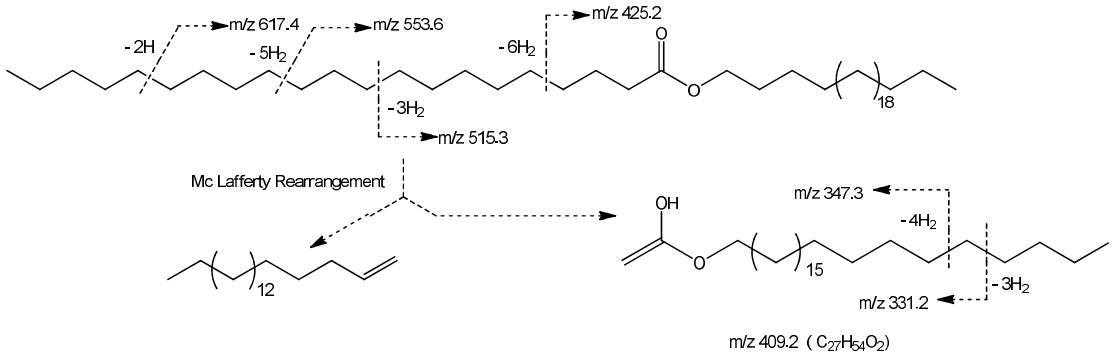

Fig. 4. Proposed fragmentation mechanism of compound 2 from TOF MS ESI$^+$ analysis

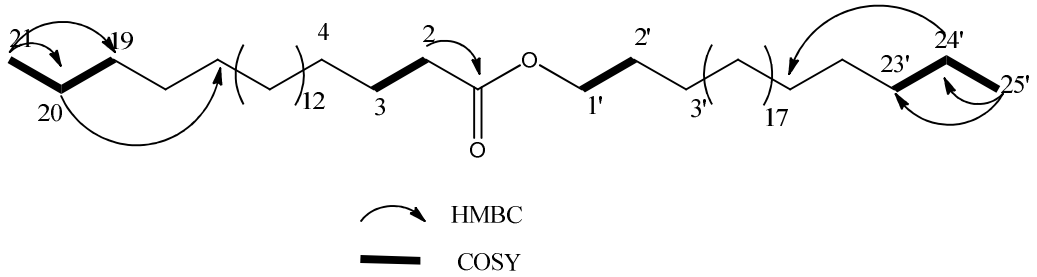

Fig. 5. Important HMBC and COSY correlations of compound 2

4. CONCLUSION

This work has demonstrated that extracts of the stem bark of *F. abutilifolia* exhibits considerable antioxidant activities. The study of ethyl acetate extract from the stem barks of *F. abutilifolia* resulted in the isolation and structural elucidation of a new fatty acid, with four known compounds. As far as we are aware, this is the first report regarding the isolation of compounds and antioxidant activities of *F. abutilifolia.* The study provides scientific evidence for the use of *F. abutilifolia* stem barks for the treatment of diseases associated with oxidative stress. So the plant could be a good source of natural free radical scavengers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pelli K, Marika L. Les antioxydants dans l'alimentation. Biotechnology Finlande. 2003;28.
2. Hangum-balkir Y, Mckenney ML. Determination of Antioxidant Activities of Berries and Resveratrol.Green Chemistry Letters and Reviews. 2012;5:147-153. DOI:10.1080/17518253.2011.603756
3. Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. Antioxidant and Antimicrobial Activities of Laetiporus sulphurus (Bull.) Murvill. Food Chemistry. 2007;101:267 273. DOI:10.1016/j.foodchem.2006.01.025
4. Klein BP, Kurilich AC. Processing effects on dietary antioxidants from plant foods. Hort Science. 2000;35:580-584.
5. Perry EK, Pickering AT, Wang WW, Houghton PJ, Perru NS. Medicinal plants and alzheimer's disease: From ethnobotany to phytotherapy. J. Pharm. Pharmacol. 1999;51: 527-534.
6. Chen C, Pearson AM, Gray JI. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds; Food Chem. 1992; 43:177-183. DOI:10.1016/0308-8146(92)90170-7
7. Ito N, Fukushima S, Hagiwara A, Shibata MT. Ogiso; Carcinogenicity of butylated hydroxyanisole in F344 rats; J. Natl. Cancer Inst. 1983;70:343-352.
8. Food and Nutrition Board of the National Academy of Science. Dietary reference intakes: Proposed definition and plan for review of dietary antioxidants and related compounds. Natl. Acad. Press. 1998;64-24.
9. Grassmann J. Terpenoids as plant antioxidants. Vitam Horm. 2005;72:505-535. DOI:10.1016/S0083-6729(05)72015-X
10. Dimitrios B. Sources of natural phenolics and antioxidants. Trends Food Sci Technol. 2006;17:506-512. DOI:10.1016/j.tifs.2006.04.004
11. Mihai CM, Liviu AM, Daniel SD, Lavinia B. Correlation between polyphe-nolic profile and antioxidant activity of propolis from Transylvania. Animal Sci. and Biotechnol. 2011;44(2):100-103.
12. Ukwulibe CA. Phytochemical screening and physical constant evaluation of *Ficus abutilifolia* Miq. (Moraceae) leaves, stem barks and roots for quality control. Scientific Journal of Crop Science. 2014;3(9):98-108.
13. Burring J; 2006. Available:http:/www.plantzafrica.com/plantefg/ficusabuti.htm.
14. Danmalam UH, Agunu A, Abdurahman EM, Ilyas1 N, Magaji MG.et Yaro AH. Anticonvulsant studies on a traditional antiepileptic mixture used by theHausa people of north-western Nigeria. Research Journal of Pharmacognosy (RJP). 2017;4(3):113-116.
15. Taiwo FO, Fidelis AA, et Oyedeji O. Antibacterial Activity and Phytochemical Profile of Leaf Extracts of *Ficus abutilifolia*. British Journal of Pharmaceutical Research. 2016;11(6):1-10. DOI:10.9734/BJPR/2016/25608

16. Danmalam UH, Allahmagami PK, Ilyas N, Abdurahman AH, Ahmed A, Yaro, Magaji MG. Phytochemical and anticonvulsant studies on the aqueous ethanol extract of the root-back of *Ficus abutilifolia* (Miq.) Miq. (family: Moracea) Journal of applied pharmaceutical science. 2012;02(07):234-237.
DOI: 10.7324/JAPS.2012.2739.
17. Maiha BB, Magaji MG, Yaro AH, Ahmed ST, Hamza AH, Magaji RA. Anticonvulsant studies on Cochlospermum tinctorium andPaullinia pinnata extracts in laboratory animals. Nig J Pharm Sci. 2009;8(1):102-108.
18. Lhuillier A. Contribution à l'étude phytochimique de quatre plantes Malgaches: Agauria salicifolia Hook.f ex Oliver,Agauria polyphylla Baker (Ericaceae), Tambourissatrichophylla Baker (Monimiaceae) et Embelia concinna Baker (Myrsinaceae). Ph.D.Dissertation, Institut National Polytechnique de Toulouse, France; 2007.
19. Hanny BW, Gueldner RC, Bayou SM1 J. Surface lipids of the glabrous cotton (Gossypium hirsutum) strain, Agric. Food Chem.1976;24: 401–403.
20. Luhata Lokadi Pierre Munkombwe Namboole Moses. Isolation and Characterisation of Stigmasterol and B - Sitosterol from Odontonema Strictum (Acanthaceae). JIPBS. 2015;2(1): 88-95.
21. Ebajo Jr. V, Shen C-C, Ragasa CY. Terpenoids and Sterols from Hoya multiflora Blume. Journal of Applied Pharmaceutical Science. 2015;5(04):033-039.
DOI:10.7324/JAPS.2015.50406
22. Latifou Lagnika. Etude phytochimique et activité biologique de substances naturelles isolées de plantes béninoises. Thèse de Doctorat à l'Université Louis Pasteur Strasbourg en cotutelle avec l'université d'abomey-calavi Benin. 2005;280.
23. López-Mejía O, López-Malo A, Palou E. Antioxidant capacity of extracts from amaranth Amaranthus hypochondriandriacus L.) Seeds or leaves. Industrial Cops and Products. 2014;53:55-59.
24. Olorunnisola OS, Bradley G, Afolayan AJ. Antioxidant activity of acetone and ethanolic leaves extracts of Hippobromus pauciflorus (L.f.) Radlk. African Journal of Biotechnology. 2011;11(5):1206-1213.
DOI:10.5897/AJB11.1497
25. Oyaizu, M. Studies on products of browning reaction-Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition. 1986;44:307–315.
26. Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ. Polyphenolic contents and biology activities of Rumex ecklonianus. Pharma. Biol. 2008;46(5):333-340.
DOI:org/10.1080/13880200801887765
27. Akinpelu DA, Aiyegoro OA, Okoh AI. The in vitro antioxidant property of methanolic extract of Afzelia africana (Smith.). J .Med. Plants Res. 2010;4(19):2021-2027.
DOI:10.5897/JMPR10.484
28. Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ. Polyphenolic Contents and Biological Activities of Rumex ecklonianus., Pharmaceutical Biology. 2008;46(5):333-340.
DOI:10.1080/13880200801887765
29. Isaac K Amponsah , Theophilus C Fleischer, Rita A Dickson, Kofi Annan, Vera Thoss. Evaluation of anti-inflammatory and antioxidant activity of Furanocoumarins and Sterolin from the stem bark of Ficus exasperate Vahl (Moraceae). Journal of Scientific and Innovative Research. 2013;2(5): 880-887.
30. Talla E, Tamfu AN, Biyanzi P, Sakava P, Asobo FP, Mbafor JT, 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;3(5):321-329.
31. Kumaran A. et Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. Lebensmittel-Wissenschaft und Technologie. 2007;40:344–352.
DOI:10.1016/j.lwt.2005.09.011.
32. Jeong SM, Kim SY, Kim DR, Jo SC, Nam KC, Ahn DU, et Lee SC. Effects of and Food Chemistry. 2004;52: 3389–3393.
DOI:10.1021/jf049899k
33. Rahman MAA, Moon SS. Antioxidant polyphenol glycosides from the plant Draba nemorosa. Bull. Korean Chem. Soc. 2007;28(5):827-831.

34. 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.). 2012; 487–502.

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