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Original Research Article

Biological activities of the methanolic extracts and compounds from leaves and twigs of *Diospyros zenkeri* (Gürke) F. White (Ebenaceae)

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ABSTRACT

This study aimed for investigating chemical constituents and biological activities of *Diospyros zenkeri* (Gürke) F. White. 14 known secondary metabolites have been isolated from the leaves and the twigs of *D. zenkeri* such as 3-methoxy-7-methyljuglone (**1**), β-carotene (**2**), lupeol (**3**), mixture of β-sitosterol (**4**) and stigmasterol (**5**), betulin (**6**), ursolic acid (**7**), messagenin (**8**), 3β,28,30-lup-20(29)-ene triol (**9**), mixture of the glucosides of stigmasterol (**10**) and β-sitosterol (**11**), norbergenin (**12**), betulinic acid (**13**) and vanillic acid (**14**), respectively . The structures of the compounds were elucidated with the help of NMR and mass spectral studies. The compounds **8**, **9** and **14** are reported for the first time from the genus *Diospyros*. The biological screening of all the isolates and the crude methanolic extracts have been carried out including antiproliferative activity, antioxidant potential and inhibitory activity against the enzymes lipoxygenase and urease, respectively. Compound **1** exhibited significant antiproliferative activity against two cancer cell lines CAL-27 (IC_{50}=2.98 µM) and NCI-H460 (IC_{50}=5.57 µM). Methanolic extracts of the leaves and twigs of *D. zenkeri* presented low antiproliferative activity against these two cancer cell lines. Compounds **1**, **6**, **8**, **9**, **12** and the crude extracts exhibited moderate antioxidant activity with IC_{50} values of 76.5 µM, 65.8 µM, 55.3 µM and 51.2 µM respectively compared to BHA (IC_{50}=44.2 µM). Compounds **6**, **8** and **9** showed moderate lipoxygenase inhibition activity with IC50 values of 58.5 µM, 52.8 µM and 58.8 µM, respectively compared to baicalein (IC_{50}=22.6 µM).

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1. Introduction

The importance of African traditional medicines in the indigenous management of diseases is well-established (Sofowara, 1996). The greatest attention of researchers is attracted by natural compounds with reliably established biological activity. An attractive factor is availability of natural metabolites due to frequent occurrence of the sources, and technological reasonableness of the methods of isolation of natural substances (Mohammadhosseini et al., 2017, 2019; Ganesan and Xu, 2017; Nunes and Miguel, 2017; Frezza et al., 2017; Wansi et al., 2018, 2019) . In Cameroon, traditional medicine is increasingly solicited by herbalists particularly for the treatment of several diseases. Genus *Diospyros* contains more than 350 species, some of which are known for the treatment of diseases in traditional systems of medicines such as Ayurveda, the African folklore and Chinese medicine (Tangmouo et al., 2005; Chen et al., 2008). Recent studies show that some of

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Fig. 1. The photograph from the leaves of *Diospyros zenkeri* species (Burkil, 1985).

these species possess antitumor, hypocholesterolemic, antidiabetic and antioxidant effects (Chen et al., 2008; Dongmo et al., 2018). Previously, many species of *Diospyros* have been studied and diverse classes of compounds such as triterpenoids (betulin, betulinic acid), steroids (stigmastrol, glucoside of stigmasterol), naphthoquinones (7-methyl juglone dimer, plumbagin), hydrocarbons (hentriacontane, hentriacontanol) and lipids (palmitic acid, stearic acid) reported in literature (Jain and Yadava, 1994; Kuo et al., 1997; Mallavadhani et al., 1998; Feumo et al., 2016, 2017). One of the species of this genus is *Diospyros zenkeri* (Gürke) F. White, known in Cameroon as "ganda" (Letouzey, 1966). Survey of literature revealed that no phytochemical or pharmacological studies were so far been carried out on this Cameroonian species. This is also the first report of occurrence of compounds **8**, **9** and **14** from the genus *Diospyros*. The biological screening of the crude methanolic extracts and isolated compounds have also been reported.

2. Experimental

2.1. Apparatus

Melting points were determined on a Büchi M-560 melting point apparatus, UV spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. The IR spectra were taken on Shimadzu FT/IR-8900 spectrophotometer, the NMR spectra (^{1}H, ^{13}C and 2D) were measured on a Bruker DRX NMR spectrometer operating at 400 MHz. The chemical shifts are given in δ values in ppm with TMS as internal standard, and coupling constants (*J*) in Hz. The EIMS and HREIMS were screened on a JOEL MS 600-I mass spectrometer.

Silica gel (70-230 mesh, Merck) was used for column chromatography and thin layer chromatography was carried out on Merck pre-coated silica gel 60 F_{254} aluminum foils. Spots were visualized under UV light (254 and 365 nm) or by spraying with ceric sulfate followed by heating.

2.2. Plant material

The leaves and twigs of *D. zenkeri* were collected in the forest at Benakoumbe situated at 2º56.235' of latitude North and 9º54.459' longitude East in the Southern Region of Cameroon, in November 2015. Authentication was performed by M. Nana Victor at the National Herbarium, Yaounde, Cameroon where a voucher specimen (44702 HNC) has been deposited (Fig. 1).

2.3. Extraction and isolation

The collected air-dried and powdered plant materials of *D. zenkeri* (leaves 1.3 kg; twigs 5.5 kg) were separately subjected to extraction at room temperature with methanol for 72 hours. The extracts were concentrated under reduced pressure to afford 181.5 g crude extract from leaves and 228.3 g from the twigs. The methanolic extract of the leaves (176.5 g) was subjected to flash chromatography over silica gel, eluting with dichloromethane to obtain fraction A (96.8 g) followed by ethyl acetate to furnish fraction B (63.2 g).

The fraction A was subjected to column chromatography, successively eluting with *n*-hexane, mixtures of *n*-hexane-EtOAc in increasing order of polarity and finally EtOAc. Elution with *n*-hexane afforded 3-methoxy-7-methyljuglone (1; 6.2 mg; red crystals) (Budzianowski, 1995); β-carotene (**2**; 8.2 mg; red flakes) (Shinichi, 2014); *n*-hexane-EtOAc (39:1) provided lupeol (**3**; 22.4 mg; white powder) (Chi-Ren et al., 2014) and mixture of β-sitosterol and stigmasterol (**4** and **5**; 18.7 mg; white fiber) (Luhata and Munkombwe, 2015). Elution with *n*-hexane-EtOAc (19:1) yielded compound **1** (12.1 mg; red crystals) and betulin (**6**; 68.7 mg; white powder) (Tijjani et al., 2012). Elution with *n*-hexane-EtOAc (7:1) afforded ursolic acid (**7**; 6.1 mg; white powder (Werner et al., 2003). Further elution with *n*-hexane-EtOAc (17:3) furnished messagenin (**8**; 20.4 mg; white crystals) (Hee Rae et al., 2015) and 3β,28,30-lup-20(29)-ene triol (**9**; 15,9 mg; white powder) (Antonio et al., 1992). Finally, a mixture of the glucosides of stigmasterol and β-sitosterol (**10** and **11**; 37.3 mg; white powder) (Rail et al., 2006) was obtained through elution with EtOAc.

The fraction B, was also subjected to column chromatography elution with CH_2Cl_2-/EtOAc (19:5) and led to the isolation of betulin (**6**; 36.2 mg; white powder) (Tijjani et al., 2012) and further elution with CH_2Cl_2-EtOAc (1:1) provided norbergenin (**12**; 123 mg; white powder) (Reiko et al., 1990).

The extract of twigs was chromatographed, eluting with *n*-hexane, various mixtures of *n*-hexane-EtOAc, EtOAc and mixtures of EtOAc-MeOH. Elution with *n*-hexane-EtOAc (39:1) afforded lupeol (**3**; 46.5 mg; white powder) (Chi-Ren et al., 2014); mixture of β-sitosterol and stigmasterol (**4** and **5**; 12.9 mg; white powder) (Luhata and Munkombwe, 2015) and compound **1** (6.8 mg; red crystals). Elution with *n*-hexane-EtOAc (7:1) yielded betulinic acid (**13**; 16.7

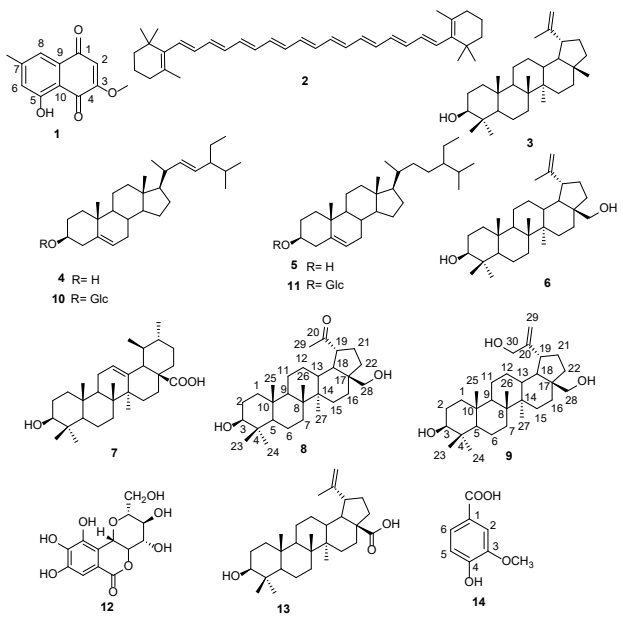

Fig. 2. Known compounds isolated from *Diospyros zenkeri*.

mg; white powder) (Chaturvedula and Indra, 2012) and *n*-hexane-EtOAc (2:3) led to the isolation of vanillic acid (**14**; 3.2 mg; colorless gum) (Sang Wook et al., 2009). Elution with EtOAc-MeOH (9:1) gave norbergenin (**12**; 32.7 mg; white powder) (Reiko et al., 1990). The known secondary metabolites were identified through comparison of their physical and spectral data with those reported in literature. The chemical structures of the isolated compounds are presented in Fig. 2.

3-Methoxy-7-methyljuglone (1): Red crystals; (+)-HREIMS *m/z*: 218.0566 $[M]^+(C_{12}H_{10}O_4)$; mp: 191-192 ºC. ^{1}H NMR (600 MHz; $CDCl_3$): 11.70 (1H, s, H-OH); 7.43 (1H, dq, *J*=0.5, 1, H-8); 7.02 (1H, dq, *J*=0.5, 1, H-6); 6.09 (1H, q, *J*=0.5, H-2); 3.89 (3H, d, *J*=0.5, H-OMe); 2.41 (3H, t, *J*=0.5, H-Me).^{13}C NMR (150 MHz; $CDCl_3$): 184.3 (C-4); 182.0 (C-1); 162.3 (C-5); 160.3 (C-3); 149.3 (C-7); 123.5 (C-9); 123.4 (C-6); 120.4 (C-8); 120.4 (C-10); 110.2 (C-2); 56.5 (OCH_3); 22.3 (CH_3).

Messagenin (8): White crystals; (+)-EIMS *m/z*: 444.3 $[M]^+(C_{29}H_{48}O_3)$; mp: 213-215 ºC. ^{1}H NMR (500 MHz; $CDCl_3$): 3.77 (1H, d, *J*=10.4, H-28); 3.23 (1H, d, *J*=10.8, H-28); 3.19 (1H, dd, *J*=11.2, 4.8, H-3); 2.62 (1H, ddd, 11.2, 11.2, 6.0, H-19); 2.12 (3H, s, H-29); 2.05 (1H, d, *J*=8.5, H-21); 1.91 (1H, d, *J*=1.6, H-22); 2.02 (1H, d, *J*=11.2, H-18); 1.63 (1H, d, *J*=11.2, H-1); 1.59 (1H, d, *J*=5.0, H-2); 1.54 (1H, ddd, *J*=12.4, H-13); 1.41 (1H, d, *J*=9.8, H-7); 1.40 (1H, d, *J*=12.2, H-11); 1.35 (1H, d, *J*=9.8, H-6); 1.27 (1H, dd, *J*=12.2, H-9); 1.26 (1H, d, *J*=2.7, H-16); 1.15 (1H, d, *J*=12.4, H-12); 1.04 (1H, d, *J*=2.7, H-15); 0.98 (6H, s, H-26, H-27); 0.95 (3H, s, H-23); 0.85 (1H, d, *J*=11.2, H-1); 0.80 (3H, s, H-25); 0.74 (3H, s, H-24); 0.68 (1H, d, *J*=10.8, H-5).^{13}C NMR (125 MHz; $CDCl_3$): 212.2 (C-20); 78.9 (C-3); 60.5 (C-28); 55.2 (C-5); 52.0 (C-19); 50.2 (C-9); 49.6 (C-18); 47.8 (C-17); 42.5 (C-14); 40.8 (C-8); 38.8 (C-4); 38.6 (C-1); 37.1 (C-10); 36.2 (C-13); 34.1 (C-7); 33.9 (C-22); 29.4 (C-29); 28.8 (C-16); 28.0 (C-23); 27.6 (C-21); 27.3 (C-2); 27.2 (C-12); 26.9 (C-15); 20.8 (C-11); 18.2 (C-6); 16.0 (C-25); 15.9 (C-26); 15.4 (C-24); 14.7 (C-27).

3β,28,30-lup-20(29)-ene-triol (9): White powder; (+)-EIMS *m/z*: 458.3 $[M]^+(C_{30}H_{50}O_3)$; ^{1}H NMR (400 MHz; $CDCl_3$):): 4.93 (1H, brs, H-29); 4.88 (1H, brs, H-29); 4.10 (2H, d, *J*=3.6, H-30); 3.79 (1H, d, *J*=10.4, H-28); 3.31 (1H, d, *J*=10.4, H-28); 3.19 (1H, dd, *J*=11.2, H-3); 2.31 (1H, ddd, 11.2, H-19); 2.28 (1H, d, 11.2, H-19); 2.09 (1H, ddd, *J*=11.2, H-21);1.96 (1H, d, *J*=12.6, H-16); 1.86 (1H, ddd, *J*=1.6, H-22); 1.67 (1H, d, *J*=11.2, H-18); 1.64 (1H, d, *J*=11.2, H-1); 1.62 (1H, d, *J*=12.4, H-13); 1.55 (1H, d, *J*=5.0, H-2); 1.51 (1H, d, *J*=9.8, H-6); 1.39 (1H, d, *J*=9.8, H-7); 1.29 (1H, d, *J*=12.2, H-11); 1.19 (1H, d, *J*=12.2, H-9); 1.06 (1H, d, *J*=2.7, H-15); 1.00 (3H, s, H-26); 0.96 (3H, s, H-27); 0.95 (3H, s, H-23); 0.88 (1H, d, *J*=12.4, H-12); 0.80 (3H, s, H-25); 0.74 (3H, s, H-24); 0.67 (1H, d, *J*=9.8, H-5). ^{13}C NMR (100 MHz; $CDCl_3$): 154.5 (C-20); 107.1 (C-29); 79.0 (C-3); 65.1 (C-30); 60.3 (C-28); 55.3 (C-5); 51.0 (C-9); 50.3 (C-18); 49.4 (C-19); 47.8 (C-17); 43.5 (C-14); 42.7 (C-8); 38.7 (C-1); 27.3 (C-2); 40.9 (C-4); 38.9 (C-13); 37.2 (C-10);34.2 (C-7); 33.8 (C-22); 31.7 (C-21); 28.8 (C-16); 28.0 (C-23); 27.4 (C-15); 27.1 (C-12); 20.9 (C-11); 18.3 (C-6); 16.1 (C-25); 16.0 (C-26); 15.4 (C-24); 14.7 (C-27).

Vanillic acid (14): Colorless gum, (+)-EIMS *m/z*: 375 $[2M+K]^+(C_8H_8O_4)$;^{1}H NMR (500 MHz; CD_3OH): 7.55 (1H, s, H-2); 7.54 (1H, dd, *J*=8.5, 2.0, H-6); 6.83 (1H, d, *J*=8.5, H-5); 3.88 (3H, s, OCH_3). ^{13}C NMR (125 MHz; CD_3OD):170.1 (COOH); 153.1 (C-3); 149.2 (C-4); 125.2 (C-1); 123.4 (C-6); 115.8 (C-2); 113.8 (C-5); 56.4 (OCH_3).

2.4. *In vitro* anti-proliferative assay

The anti-proliferative activities of crude extracts and isolated compounds were evaluated against two cancer cell line, namely CAL-27 (Human oral squamous cell carcinoma) and NCI-H460 (Human lung cancer cell line) using MTT assay (Hansen et al., 1989; Subhasree et al., 2009). Both the cell lines were seeded in 96 well plate with the density of 15.000 and 10.000 cells per well, respectively in DMEM supplemented with FBS (10.0%) and incubation for 24 h at 37 ºC in a humidified incubator with CO_2 (5.0%). After incubation, compounds were serially diluted to the plate in an incomplete medium. After 48 h of treatment, media was aspirated. MTT dye with concentration of 0.5 mg/mL was added to the plate and further incubated for 4 h. Subsequently, formazine crystals were dissolved with 100 µL DMSO per well after aspiration of media and absorbance was measured at 570 nm. According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in-vitro*. In our study, IC_{50} is a concentration of drug at which 50% of cell population die. For primary screening, a threshold of 50% cell growth inhibition as a cut off for compound toxicity against cell lines was used. IC_{50} values were determined from the plot of dose response curve between log of compound concentration and percentage cell growth inhibition. Graph was plotted by keeping log concentration

Table 1

IC_{50} values of crude extracts and pure compounds from leaves and twigs of *D. zenkeri* for cell lines cancerNCI-H460 and CAL-27.

Compounds	IC_{50} (µM)	
	NCI-H460	**CAL-27**
1	2.98±0.02	5.67±0.79
8	185.09±4.96	70.63±2.21
5-Flurouracil	97.76	
DZL	124.25±0.48	51.12±72
DZT	149.47±4.44	181.72
5-Flurouracil	12.7	

DZL: Extract of *Diospyros zenkeri* leaves; **DZT**: Extract of *Diospyros zenkeri* twigs.

of drug on X axis and cell growth inhibition(%) or cytotoxicity(%) on Y axis. IC_{50} values were estimated as a concentration of drug at 50% position on Y axis. The relationship should be sigmoidal, log concentration of the drug on the X axis and 'response/measurement' of the Y axis. The prism website has some good guides for this plot. Therefore, we have used this software. IC_{50} values were calculated using the non-linear regression program origin. The average of two (duplicates manner) were taken in determination. IC_{50} value has been derived using curve fitting methods with graph pad prism statistical software.

2.5. Determination of DPPH radical scavenging activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl hydrazil (DPPH) using the method described by Gulcin et al. (2005). The solution of DPPH (0.3 mM) was prepared in ethanol. Five microlitres of each sample with different concentrations (62.5 µg-500 µg) was mixed with 95 µL of DPPH solution in ethanol. The mixture was dispersed in 96 well plate and incubated at 37 ºC for 30 min. The absorbance was measured at 515 nm by microtitre plate reader (Spectramax Plus 384 Molecular Device, USA) and percent radical scavenging activity was determined in comparison with the methanol treated control (Subhasree et al., 2009). BHA was used as standard, as well.

$$\text{DPPH scavenging effect}(\%)=(A_c-A_s)/A_c\times 100 \quad \text{(Eqn. 1)}$$

Where A_c and A_s respectively account for absorbance of control (DMSO treated) and absorbance of the sample.

2.6. Lipoxygenase inhibition assay

Lipoxygenase inhibitory activity was measured by modifying the spectrophotometric method developed by Tappel (1963). Lipoxygenase enzyme solution was prepared so that the enzyme concentration in reaction mixture was adjusted to give rates of 0.05 absorbance/min. The reaction mixture contained 160 µL of sodium phosphate buffer (100 mM; pH=8), 10 µL of test solution and 20 µL of LOX buffer solution. The contents were mixed and incubated for 10 min at 25 ºC. The reaction was then initiated by the addition of 10 µL substrate solution involving linoleic acid, 0.5 mM and 0.12% (w/v) tween 20 in ratio of 1:2 and the change in absorbance was followed for 6 min at 234 nm. The concentration of the test compound that inhibited lipoxygenase activity by 50% (IC_{50}) was determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The IC_{50} values were calculated by means of EZ-Fit, Enzyme Kinetics Program (Perrella Scientific In., Amhherset, USA).

2.7. Urease Inhibition Assay

Reaction mixtures comprising 25 µL of enzyme (Jack bean urease) solution and 55 µL of buffers containing 100 mM urea were incubated with 5 µL of test compounds (1.0 mM concentration) at 30 ºC for 15 min in 96-well plates (Nalini et al., 2002). Urease activity was determined by measuring ammonia production using the indophenol method as described by (Weatherburn, 1967). Briefly, 45 µL each of phenol reagent (1.0% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH=8.2 (0.01 M $K_2HPO_4.3H_2O$, 1.0 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula:

$$\text{Percentage inhibition}=100-(OD_{testwell}/OD_{control})\text{x}100 \quad \text{(Eqn. 2)}$$

Thiourea was used as the standard inhibitor of urease.

3. Results and Discussion

3.1. Characterization of the isolated compounds

The methanolic extracts of the leaves and twigs were subjected to a series of chromatographic resolutions as described in the experimental section to obtain fourteen known secondary metabolites characterized as 3-methoxy-7-methyljuglone (**1**), β-carotene (**2**), lupeol (**3**), mixture of β-sitosterol (**4**) and stigmasterol (**5**), betulin (**6**), ursolic acid (**7**), messagenin (**8**), 3β,28,30-lup-20(29)-ene triol (**9**), mixture of the glucosides stigmasterol (**10**) and β-sitosterol (**11**), norbergenin (**12**), betulinic acid (**13**) and vanillic acid (**14**), respectively (Fig. 2). The structures of the isolated compounds, **1-14**, were unambiguously confirmed by spectroscopic methods including ^{1}H NMR, ^{13}C NMR,

Table 2
IC_{50} values of pure compounds of leaves and twigs of *D. zenkeri* for enzyme inhibition.

Compounds	IC_{50} (µM)		
	Antioxidant	Urease Inhibition	Lipoxygenase Inhibition
1	76.5±0.47	Nil	>500
3	85.2±0.25	Nil	88.8±0.27
6	65.8±0.54	Nil	58.5±0.17
8	55.3±0.36	Nil	52.8±0.69
9	51.2±0.47	Nil	58.8±0.24
12	62.8±0.27	Nil	55.2±0.24
DZL	86.8±0.09		
DZT	80.2±0.16		
BHA	44.2±0.06	-	
Baicalein		-	22.6±0.08
Thiourea		21.6±0.12	-

DZL: Extract of *Diospyros zenkeri* leaves; **DZT:** Extract of *Diospyros zenkeri* twigs.

2D NMR and MS analysis. From the leaves and twigs of *D. zenkeri* we obtained mainly sterols and triterpenes showing that they are predominent metabolites found in this genus (Feumo et al., 2016, 2017; Dongmo et al., 2018). This study explains why triterpenoids have been used for long time as chemotaxonomic makers of genus *Dyospyros* (Feumo et al., 2016).

3.2. Antiproliferative activity

The biological activities of methanolic extracts of leaves, twigs and pure compounds were studied against two cancer cell lines CAL-27 (Human Oral Squamous Cell Carcinoma) and NCI-H460 (Human Lung Cancer Cell Line) using MTT assay. Compounds **1** showed significant antiproliferative activity against these cell lines with IC_{50}=2.98 µM and IC_{50}=5.57 µM, respectively. Methanolic extracts of leaves and twigs of *D. zenkeri* exhibited low antiproliferative activity against these two cancer cell lines (Table 1). This result confirms that naphtoquinone derivatives have a broad spectrum of biological activities including *in vitro* cytotoxic activities (Baikar and Malpathak, 2010).

3.3. Antioxidant activity and lipoxygenase inhibition activity

Compounds **1**, **6**, **8**, **9**, **12** and the crude extracts exhibited moderate antioxidant activity with IC_{50} values of 76.5 µM, 65.8 µM, 55.3 µM and 51.2 µM, respectively compared to BHA (IC_{50}=44.2 µM). Compounds **6**, **8**, **9** and **12** showed moderate lipoxygenase inhibition activity with IC_{50} values of 58.5 µM, 52.8 µM, 58.8 µM and 55.2 µM, respectively compared to baicalein (IC_{50}=22.6 µM) (Table 2). These results suggest that the presence of the hydroxyl group at C-28 on lupane-type triterpenoids (betulin (**6**), messagenin (8) and 3β,28,30-lup-20(29)-ene triol (**9**)) seems to be necessary to exert a moderate antioxidant activity and lipoxygenase inhibition activity. 3-Methoxy-7-methyljuglone (**1**) and norbegenin (**12**) showed moderate antioxidant activity confirming that phenolic compounds are the known antioxidant from plant sources (Saikat et al., 2009).

4. Concluding remarks

The present study revealed the presence of three known compounds reported for the first time from the genus *Diospyros*: messagenin (**8**), 3β,28,30-lup-20(29)-ene triol (**9**) and vanilic acid (**12**). Compound **1**, 3-methoxy-7-methyljuglone exhibited significant antiproliferative activity against two cancer cell lines CAL-27 (IC_{50}=2.98 µM) and NCI-H460 (IC_{50}=5.57 µM). Therefore, further *in vivo* studies are recommended for this compound so that it may be used in drug development against cancer. This study confirms that *Diospyros* species exhibit interesting pharmacological activities. We will continue to investigate other Cameroonian *Diospyros* species in our undergoing projects to isolate more bioactive secondary metabolites.

Conflict of interest

The authors declare that there is no conflict of interest.

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