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Isolation and structural elucidation of cytotoxic compounds from the root bark of *Diospyros quercina* (Baill.) endemic to Madagascar

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PEER REVIEW

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Comments

This is a valuable research work in which the authors have demonstrated the cytotoxicity of three compounds of *Diospyros quercina in vitro*. The structural elucidation was determined based on chemical and spectroscopic studies. The bioactivity was assessed based on cell proliferation assay. *Diospyros quercina* is then a promising anticancer agent.
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ABSTRACT

Objective: To isolate and characterize the cytotoxic compounds from *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae).
Methods: An ethno-botanical survey was conducted in the south of Madagascar from July to August 2010. Bio-guided fractionation assay was carried out on the root bark of *Diospyros quercina*, using cytotoxicity bioassay on murine P388 leukemia cell lines as model. The structures of the cytotoxic compounds were elucidated by 1D and 2D NMR spectroscopy and mass spectrometry.
Results: Biological experiments resulted in the isolation of three bioactive pure compounds (named TR-21, TR-22, and TR-23) which exhibited very good *in vitro* cytotoxic activities with the IC_{50} values of (0.0175±0.0060) μg/mL, (0.089±0.005) μg/mL and (1.027±0.070) μg/mL respectively. Thus, they support the claims of traditional healers and suggest the possible correlation between the chemical composition of this plant and its wide use in Malagasy folk medicine to treat cancer.
Conclusions: The ability of isolated compounds in this study to inhibit cell growth may represent a rational explanation for the use of *Diospyros quercina* root bark in treating cancer by Malagasy traditional healers. Further studies are, therefore, necessary to evaluate the *in vivo* anti-neoplastic activity of these cytotoxic compounds as effective anticancer drugs.

KEYWORDS

Diospyros quercinia, Cytotoxic activities, Isodiospyrin, 6′-Ethoxy-1′, 3′-dihydroxy-4, 6-dimethyl-1,2′-binaphthyl-2,5′,8,8′tetraones, (*E*)-5,6-Dimethyl-2-(2-methyl-3-(prop-1-enyl) phenyl)-2H-Chromene, Madagascar

1. Introduction

Madagascar is reputed for the extraordinary richness of its flora (biodiversity) and boasts a wide variety of indigenous species[1]. These plants species represent an enormous reservoir of new molecules with potential therapeutic activity waiting to be discovered.

In Madagascar as well as in the others parts of African

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continent, the majority of people rely on traditional medicine for their health care needs because the costs of conventional drugs are unaffordable[2-4]. In addition, this big island is unique because of the endemicity of its flora which makes 85% of Malagasy biodiversity[5]. During an ethno-botanic survey conducted in the south-west part of Madagascar, it was reported that the aerial part of the plant known under the vernacular name Maintifototse (Malagasy name), and scientifically named *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae) is used by the local communauties to treat malaria and incurable wounds. The genus *Diospyros* is represented in Madagascar by 21 endemic species and the majority of species are mostly distributed in the south and southwest parts of Madagascar[6,7]. This plant is a well-known and very important recipe in these regions because of its therapeutic values in the Malagasy traditional medicine.

The aim of the present study was to isolate and characterize the cytotoxic compounds from *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae). Thus, if admitted that *Diospyros quercina* contains such cytotoxic compounds, the use of this plant as antimalarial or antidiabetic by traditional healers should be carefully adjusted or discouraged because recent findings have shown that many plants used in traditional medicine are potentially toxic, mutagenic and carcinogenic[8]. Such information would be useful in preventing the wide use of the plant by local communities and will guide laboratory research in identifying new anticancer lead compounds.

In this present study, the crude extract of the root bark of *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae) exhibited a good activity with the IC_{50} value of 0.85 μg/mL at 5 μg/mL concentration. This interesting preliminary result has conducted us to undertake further analyses in order to purify and to elucidate the chemical structure(s) of the bioactive compounds using a combination and spectroscopic techniques (1D and 2D NMR and mass spectrometry in positive mode IES.SM-TOF 6.97Ev).

2. Materials and methods

2.1. Selection and collection of plant material

Ethnobotanical information about plant species selected for this study was obtained by interviewing traditional healers during field work which was conducted in the south of Madagascar from July to August 2010. *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae) was collected in the *Izombitse Sakaraha* National Park at nearly 165 km from Toliara town, in the south of Madagascar. The plant was identified by comparison with reference specimen available at the Department of Botany; *"Parc Botanique et Zoologique de Tsimbazaza"* in Antananarivo, Madagascar. Voucher specimen assigned sample number MQ-01 was deposited at the herbarium of the Applied Chemistry Laboratory of the University of Toliara, Madagascar.

2.2. Extraction and bioguided isolation

The air-dried and powdered root barks of *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (2 kg) were repeatedly extracted by cold percolation with ethanol 90° (3×3 h, 15 L) at room temperature on a shake. Pooled organic solvents were dried over Na_2SO_4 and evaporated until dryness at 40 °C, under reduced pressure to yield 30 g of crude extract. Twenty-five grams of the ethanol crude extract was suspended in water and partitioned successively with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol to yield the corresponding soluble extract fractions. Bioassay-guided extraction revealed interesting activity only with the dichloromethane extract fraction, which exhibited an inhibition rate of leukemia P388 cell growth of 94.67% at 2 μg/mL. Ten grams of the dichloromethane crude extract was first subjected to fractionation, using a silica gel column chromatography eluted with *n*-hexane and gradient of ethyl acetate (9:1 to 6:4) resulting into eight fractions (F_1-F_8). Two fractions, F_5 and F_6, showed strong cytotoxic activities with an inhibition rate of 96.08% and 92.43% respectively at 0.5 μg/mL. These fractions were checked for purity by analytical TLC, and the zone was detected with a UV lamp at 254 and 365 nm and by spraying with sulfuric vanillin acid, followed by heating at 120 °C for 1-5 min. F_5 and F_6 were combined on the basis of TLC profile similarity and resubmitted to seperation by silica gel column chromatography eluting with a mixture of diethyl ether/chloroform/methanol (2/7.5/0.5); the column was in isocratic regime and at the end, it resulted into seven fractions.

The fraction F_{63} showed cytotoxic activity with an inhibition rate of 99.012% at 0.25 μg/mL. Then 100 mg of F_{63} was subjected to further purification, using a silica gel column chromatography, eluting with *n*-hexane and gradient of ethyl acetate furnished a pure compound (6 mg) and a mixture of two compounds (40 mg) which were further separated by preparative TLC using *n*-hexane/ethyl acetate/acetone (2/6/2) as the solvent system affording compounds 2 (4 mg) and 3 (5 mg). The three pure compounds showed

strong cytotoxic activities against murine P388 leukemia cell lines after biological test. The purity of compounds was proved by HPLC analysis. The mobile phase consisted of a $CHCl_3$/MeOH (1/1) mixture of chloroform and methanol; the chromatography was performed with isocratic regime during 25 min. Eluted compounds were detected on the basis of their UV absorption in the wavelength range from 190 nm to 315 nm. The purity of each product was respectively 99.92% at λ=202 nm, 98.99% at λ=198 nm, and 99.42% at λ=245 nm.

2.3. Cytotoxicity assay

The crude extract, the fractions (*n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and aqueous extracts) and the pure compounds were systematically submitted to cytotoxicity test. In that way, murine P388 leukemia cells were grown in RPMI 1640 medium containing 0.01 nmol/L of β-mercapto-ethanol, 10 mmol/L of L-glutamine, 100 IU/mL of G-penicillin, 100 μg/mL of streptomycin, 50 μg/mL of gentamycin, and 50 μg/mL of nystatine, supplemented with 10% of fetal calf serum. Cells were maintained at 37 °C in a moisturized atmosphere with 5% CO_2. The inoculums seeded at 10^4 cells/mL at an optimal volume of 0.1 mL per well, were introduced into flat-bottomed 95-well plates containing a serie of concentrations of compounds (0.001, 0.01, 0.1 and 1 μg/mL for compound/extract having IC_{50}<1 μg/mL or ranging from 0.976 to 50 μg/mL for extract/compound having IC_{50}<50 μg/mL), alongside untreated controls. Culture was then incubated at 37 °C for 71 h in the required atmosphere. Thereafter, cells were incubated at 37 °C with 0.02% neutral red dissolved in methanol/water (1/9), 0.1 mL per well for 1 h and then washed with 1 mol/L PBS and finally lyzed with 1% SDS. After a brief agitation on a micro-culture plate shaker, the plates were transferred to a Titertek Twin reader equipped of a 540 nm filter to measure absorbance of the extracted dye. Cell viability was expressed as the percentage of cells incorporating dye relative to the untreated controls, and IC_{50} values were determined by linear regression method.

2.4. Statistical analysis

All statistical calculations were carried out with GraphPadPrism4 software. The results are expressed as the mean±standard error of mean (SEM) of n independent experiments with individual values. Unpaired Student's *t*-test was used for statistical comparison, *P* values less than 0.01 were considered as significantly different against the control.

3. Results

3.1. Phytochemical studies

The structures of three isolated compounds named TR-21, TR-22, and TR-23 are summarized in Figure 1.

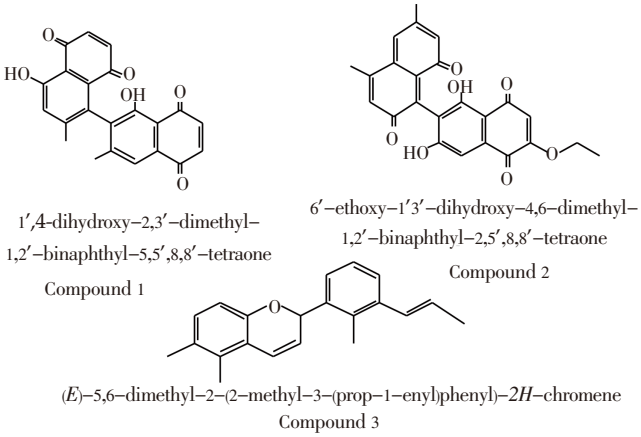

Figure 1. Structures of cytotoxic compounds 1, 2 and 3.

The bioassay-guided fractionation of the crude extract of the root bark of *Diospyros quercina* using repeated silica gel column chromatography resulted in the isolation of three pure compounds as evidenced by analytical TLC and HPLC analysis.

The isolated compound 1 (TR-21), showed a quasi-molecular ion at *m/z*= 375.1942 $[M+H]^+$ in the ESI.TOF.MS spectrum which corresponds to the molecular formula $C_{22}H_{14}O_6$. The ^{1}H-NMR spectrum showed characteristic singlet attributed to two methyl groups δ1.99, δ2.00 and six protons δ6.75(d), δ6.89(d), δ6.92(d), δ6.93(d), δ7.27(s) and δ7.60 typical for benzenes skeleton, and at the end two protons acid attributed to hydroxyl protons of phenol at δ12.00 and δ12.43. Examination of the ^{13}C NMR (*broad brand* and *DEPT*), and the HSQC spectra data of the pure compound revealed the presence of a four carbonyls carbons at δ_C 184.4 (C-1), δ_C 184.6(C-4), δ_C 190.1(C-4′), δ_C 190.3(C-1′) and sixteen alcens carbons (C=C) double bond typical for benzenes skeleton at δ_C 113.1(C-1a); δ_C 114.2(C-1b′); δ_C 121.3(C-8); δ_C 125.7(C-6′); δ_C 128.6(C-6′b); δ_C 128.7(C-6a); δ_C 130.2(C-8′), δ_C 135.0(C-6), δ_C 137.7(C-3′); δ_C 138.8(C-3); δ_C 139.5(C-2); δ_C 140.1(C-2′); δ_C 145.5(C-7); δ_C 148.3(C-7′); δ_C 158.5(C-5); δ_C 161.5(C-5′) and two of carbons methyl groups (Table 1). The ^{1}H and ^{13}C chemical shift values of individual spin systems were determined by correlation in the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by the ^{1}H-^{1}H COSY spectrum and 2D HSQC and

HMBC correlation spectra, respectively (Table 1).

Table 1

^{1}H and ^{13}C chemical shift, the ^{1}H-^{1}H COSY, and important HMBC correlations of compound 1 (TR-21).

Position	δ ^{1}H, multiplicity coupling constants	δ ^{13}C	COSY	HMBC
1′		190.3		
2′	6.75(d)	140.1	H-3′	C-6′b, C-4′
3′	6.89(d)	137.7	H-2′	C-1b′, C-1′
4′		190.1		
1b′		114.2		
5′	12.4(s)	161.5		C-1b′, C-6′
6′	7.27(s)	125.7		C-1b′, C-8′, 7′-CH3
7′		148.3		
8′		130.2		
6b′		128.6		
1		184.4		
2	6.93(d)	139.5	H-3	C-4, C-6a
3	6.92(d)	138.8	H-2	C-1a, C-1
4		184.6		
1a		113.1		
5	12.00(s)	158.5		C-1a, C-6
6		135.0		
7		145.5		
8	7.60(s)	121.3		C-1a, C-8, 7-CH3
6a		128.7		
7′-CH_3	2.00(s)	20.4		C-6′, C-7′, C-8′
7-CH_3	1.99(s)	20.6		C-6, C-7, C-8

Compound 2 (TR-22) had as molecular formula $C_{24}H_{18}O_7$ from ESI.TOF.MS (*m/z*=419.0814 $[M+H]^+$ calculated). Its ^{1}H-NMR spectrum exhibited two singlets at δ2.21, δ2.35 and a doublet at δ1.22 characteristic of the three methyl groups, one triplet at δ3.85 was attributed by the protons in the OCH_2 group and thereafter others singlets at δ6.92, δ6.98, δ7.16, δ7.52 and δ7.59 characteristic attributed to the benzenic protons and at the end two hydroxyls protons between δ11.85, δ12.52 typical for a phenol. The 1D ^{13}C (Broad Band) NMR spectrum contained twenty-four (24) signals including the carbonyls between δ_C 184.20(C-5′), δ_C 184.40(C-8), δ_C 188.13(C-2), δ_C 190.00(C-8′). Examination of the 1D ^{13}C (DEPT), and the 2D HSQC spectra data of the compound 2 revealed the presence of a fourteen alcens carbons (C=C) double bond typical for benzenes skeleton at δ_C 117.58(C-1); δ_C 121.21(C-3); δ_C 148.70(C-4); δ_C 142.76(C-4b′); δ_C 120.72(C-5); δ_C 146.60(C-6); δ_C 124.21(C-7), δ_C 113.60(C-8b), δ_C 159.95(C-1′); δ_C 112.51(C-2′); δ_C 162.04(C-3′); δ_C 139.95(C-4′); δ_C 132.45(C-4′a); δ_C 117.89(C-6′); δ_C 138.2(C-7′); δ_C 1124.41(C-8′a) and two of carbons methyl groups, and at the end, one ethoxy group (OCH_2CH_3) (Table 2). The ^{1}H and ^{13}C chemical shift values of individual spin systems were determined by correlation in the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by the ^{1}H-^{1}H COSY spectrum and 2D HSQC and HMBC correlation spectra, respectively (Table 2).

Table 2

^{1}H and ^{13}C chemical shift, the ^{1}H-^{1}H COSY, and important HMBC correlations of compound 2 (TR-22).

Position	δ ^{1}H, multiplicity coupling constants	δ ^{13}C	COSY	HMBC
1′	12.52 (s)	159.95		C-8′a, C-2′
2′		112.51		
3′	11.85 (s)	162.04		C-2′, C-4′
4′	6.92 (s)	139.95		C-2′, C-5′, C-8′a
4′a		132.45		
5′		184.20		
6′		117.80		
7′	6.98 (s)	138.20		C-8′a, C-5′, C-8′
8′		190.00		
8′a		124.41		
1		117.58		
2		188.13		
3	7.52 (s)	121.21		4-CH_3, C-4a, C-2
4		148.70		
4a		142.76		
5	7.59 (s)	120.72		6-CH_3, C-7, C-8a
6		146.60		
7	7.16 (s)	124.21		6-CH_3, C-5, C-8a
8		184.40		
8a		113.60		
4-CH_3	2.21(s)	21.07		C-3, C-4, C-4a
6-CH_3	2.35(s)	22.26		C-5, C-6, C-7
O-CH_2-	3.85(q)	58.41	CH3	C-6, CH_3
CH_3	1.22(t)	18.41	O-CH2-	O-CH_2-

The molecular formula of compound 3 (TR-23) was determined to be $C_{21}H_{22}O$ by ESI.TOF.MS and NMR experiments. The ^{1}H-NMR spectrum showed singlet characteristic attributed to four methyl groups δ1.62, δ2.03, δ2.21, δ2.35 and eleven signals between δ8.19(d), δ8.16(d), δ8.13(t), δ8.09(t), δ7.73(dd), δ7.53(t), δ7.45(t), δ6.40(d), δ6.14(d), δ5.57(d), δ5.43(dd), attributed to characteristic of signals alcens proton typical for benzenes skeleton. The 1D ^{13}C (Broad Band) NMR spectrum contained twenty one (21) signals of the carbons. Examination of the 1D ^{13}C (DEPT), and the 2D HSQC spectra data of the compound 2 revealed the presence of about ten (10) alcens carbons (C=C) double bond typical for benzenes skeleton at δ_C 125.94; δ_C 133.51; δ_C 126.71; δ_C 125.21; δ_C 123.93; δ_C 126.39; δ_C 122.29, δ_C 117.50, δ_C 128.05; δ_C 123.10, six quaternary C at δ_C 135.50; δ_C 112.8; δ_C 143.00; δ_C 148.00; δ_C 110.04; δ_C 1142.01 and four of carbons methyl groups (Table 3). The ^{1}H and ^{13}C chemical shift values of individual spin systems were determined by correlation with the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by the ^{1}H-^{1}H COSY spectrum and 2D HSQC and HMBC correlation spectra,

respectively (Table 3).

Table 3

^{1}H and ^{13}C chemical shift, the $^{1}H-^{1}H$ COSY, and important HMBC correlations of of compound 3 (TR-23).

Position	δ ^{1}H,multiplicity coupling constants	δ ^{13}C	COSY	HMBC
1				
2	6.68(d)	67.8	H-3	C-2, C-1′
3	6.39(d d)	126.5	H-2, H-4	C-4a, C-1
4	6.62(d)	121.7	H-3	C-8a, C-1
4a		122.8		
5		134.8		
6		129.0		
7	6.96(d)	129.10		C-5, C-8, 6-CH_3
8	6.67 (d)	113.4		C-6, C-4a
8a		154.5		
1′		137.7		
2′		133.7		
3′		136.4		
4′	7.19(d)	125.2	H-5′	C-2′, C-5′
5′	7.04(dd)	125.8	H-4′, H-6′	C-1′, C-3′
6′	7.12(d)	126.2	H-5′	C-2′, C-4′
2′-CH_3	2.21(s)	22.26		
3′a	6.64(d)	127.4	H-3b	C-2′, C-4′, 3′b-CH_3
3′b	5.89(m)	133.3	H-3a, 3′b-CH_3	C-3′, 3′b-CH_3
3′b-CH_3	2.05(d)	18.3	H-3b	C-3′a
5-CH_3	2.22(s)	25.43		C-4a, C-5, C-6
6-CH_3	2.34(s)	26.0		C-5, C-6, C-7

3.2. Biological screening

Crude extract, five fractions (*n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and aqueous extracts), and the three pure compounds, from the root bark of *Diospyros quercina* were screened for cytotoxicity assay against P388 cell lines using a serie of concentrations. The results for the cytotoxic bioassay are dose-response curves for each fractions/compounds. These curves are plots of P388 cells survival percentage against logarithm of concentration. The IC_{50} values from these graphs are summarized in Table 4.

Table 4

In vitro cytotoxic activities of crude extract, different fractions and the pure compounds from the root bark of *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (*n*=6).

Fractions	IC_{50} (μg/mL)
Crude extract	0.851±0.050
Hexane	35.87±2.54
Dichloromethane	0.641±0.060
Ethyl acetate	26.04±2.06
n-Butanol	43.07±3.65
Aqueous	40.089±1.980
F5 and F6	0.351±0.080
F63	0.291±0.043
Compound 1	0.0175±0.0060
Compound 2	0.0895±0.0050
Compound 3	1.27±0.07

The results indicate that crude extract, dichloromethane soluble fraction, F_5, F_6, F_{63}, Compounds 1, 2 and 3 can be accepted as potent cytotoxic agents because their IC_{50} values are below 20 μg/mL[9].

It can also be seen from the Table 4 that compound 1 displayed a very good activity similar to that of known anticancer actinomycin (0.018 μg/mL) and camptothecin (0.016 μg/mL) than taxol (0.032 μg/mL) and vincristin (0.023 μg/mL). Compounds 2 and 3 are less active than all positive control cited[10].

4. Discussion

The goal of ethnopharmacology is to preserve the cultural heritage by documenting information on medicinal plants and their isolates compounds. This is due to the fact that the first line of treatment for poor people in the developing countries is the use of medicinal plants at home. In the present study, three cytotoxic compounds were isolated and characterized from the medicinal plant *Diospyros quercina* endemic to Madagascar.

The lower activity of compound 3 compared to that of compound 1 or 2 may be linked to the difference in their chemical structures and the difference in biological activity of naphtylquinone derivatives compounds could be linked to the ethoxy side chain which could reduce the toxicity of these leads. The quinone reductive functions are able to react with amine function of nucleotides to form imines' derivatives responsible for their probable cytotoxic properties as intercalating agents[11].

There are several cancerous cell lines used in different academic and pharmaceutical laboratories of the world for cytotoxicity evaluation. Among them P388 lymphocytic leukemia cell lines which have been used in this study. The nuclear proto-oncogenes c-myc in such cells is frequently overexpressed and constitutes an indication of early response of cell proliferation[12]. We postulated in this study that cytotoxic compounds could induce apoptosis in P388 cells by down-regulating/suppressing c-myc expression. This study revealed that herbal and plants based traditional medicine are good source of chemical structures that might be effective in attacking invading cancer cells. Indeed, the plant kingdom offers a way of hope because of its enormous chemical diversity[13]. Several known anticancer drugs have been derived from medicinal plants and some of these include vincristine, vinblastine and taxol[10].

The chemical structures of the active compounds isolated from the root bark of *Diospyros quercina* (Baill.) G.E. Schatz & Lowry revealed that these leads are closely linked to *Diospyros* genus. Indeed, recent findings showed that

phenolic complex compounds such as naphtoquinone could play a key role in chemotaxonomic classification of plants species (chemical systematic) or in ecological biochemistry research[14]. The compound 1 was obtained as pure major component and had a red color. According to spectroscopic data, its molecular formula $C_{22}H_{14}O_4$ was elucidated as 1′,4-dihydroxy-2′, 3-dimethyl-2′binaphtyl-5, 5′, 8, 8′ tetraone or isodiospyrin. This compound exhibited a very good cytotoxic activity with the IC_{50}<0.1 μg/mL. Isodiospyrin, a naphtoquinone derivative, was previously reported in the literature to have a broad spectrum of biological activities including *in vitro* 12(S)-HETE inhibitory effects[15], antitermitic[16], analgesic, antipyretic and anti-inflammatory properties[17] and cytotoxic activities[18]. *Diospyros* genus elaborated a large number of 1, 4-naphtoquinone secondary metabolites belonging to the juglone class[19]. About 75% of *Diospyros* phytochemicals reported in the literature are 1,4-naphtoquinones which include several monomers, dimmers and derivatives. In fact, these secondary metabolites can serve as specific markers of *Diospyros* genus.

The molecular formula $C_{24}H_{18}O_7$ for compound 2 was established by positive ESI.TOF.MS (*m/z*=419.0814 for $[M+H]^+$ calculated). The NMR spectral data resembled closely to those of compound 1, except for the presence of ethoxy group. From our knowledge, this molecule was not previously reported in the literature.

Compounds 1 and 2 were identified as 1,4′-dihydroxy-2,3′ dimethyl-1,2′binaphthyl-5,5′,8,8′tetraone and 6′ethoxy-1′,3′dihydroxy-4,6 dimethyl-1,2′binaphthyl-2,5′,8,8′tetraones respectively. They had the same binaphtylquinone ring and differed by their substituent on the hydroxyl group at position 3′ and by the carbonyls carbons at position 2, at the end presence of ethoxy group at position 6 in the compound 2.

Compound 2 also exhibited a very good cytotoxic activity with an IC_{50}<0.1 μg/mL.

The compound 3 has got as molecular formula $C_{21}H_{22}O$ with molecular mass *m/z*=291.386 $[M+H]^+$ according to spectroscopic data, this compound was elucidated as a derivative of cyanidin: (E)-5, 6-dimethyl-2-(2-methyl-3-(prop-1-enyl) Phenyl)-*2H*-Chromene. From our knowledge, this molecule is also reported for the first time in the *Diospyros* genus. The compound 3 exhibited good to moderate cytotoxic activity with an IC_{50} value of 1.27 μg/mL. Others chemical investigations of the plant genus *Diospyros* also yielded many compounds with interesting and exciting biological activities[15-19].

This study focused on the isolation and characterization of the cytotoxic compounds from *Diospyros quercina* which have been selected through ethno-botanical survey. At the end of this study, we have demonstrated that ethnobotanical survey and ethnopharmacology are powerful tools for selecting and validating the biological activity of an appropriate medicinal plant species traditionally used by indigenous community to treat commonly diseases. The combination of biological experiments *in vitro*, chromatographic and spectroscopic modern techniques (CC/HPLC/MS/NMR) has led to the isolation and structural elucidation of three cytotoxic compounds from *Diospyros quercina*: Isodiospyrin (compound 1), 6′ethoxy-1′, 3′-dihydroxy-4, 6-dimethyl-1,2′-binaphthyl-2,5′,8,8′tetraones (compound 2), and (*E*)-5,6-dimethyl-2-(2-methyl-3-(prop-1-enyl) phenyl)-*2H*-Chromene (compound 3). Compounds 2 and 3 are reported for the first time in the *Diospyros* genus. All lead compounds displayed very interesting cytotoxic properties thus justifying the wide use of *Diospyros quercina* (Baill.) G.E. Schatz & Lowry (Ebenaceae) in Malagasy folk medicine to treat cancer. Further studies on these cytotoxic compounds involving *in vivo* anti-neoplastic activity are necessary for promoting these molecules as anticancer new lead compounds. It can also be concluded that the use of this plant as antimalarial or antidiabetic by traditional healers should be carefully adjusted or discouraged because of the presence of potentially toxic compounds for healthy human.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In recent years, cancer has threatened human living and life and thrown human into a panic. However, modern medicine does not work effectively for cancers and they exhibit side effects. Thus, there is need of new and potent anticancer agents without side effects.

Research frontiers

The present research study describes the anti-proliferative

effect and the structure of three compounds isolated from the alcohol extract of *Diospyros quercina* (Baill).

Related reports

Recent findings showed that phenolic compounds such as naphtoquinone could play a key role in chemotaxonomic classification of plants species or biochemistry research. Isodiospyrin, a naphtoquinone derivative, is reported to have a broad spectrum of bioactivities including *in vitro* 12(S)-HETE inhibitory effects, antitermitic, analgesic, antipyretic and anti-inflammatory and cytotoxicity.

Innovations and breakthroughs

An ethnobotanic survey conducted in Madagascar reported that the aerial part of *Diospyros quercina* (Baill.) is used as medicinal plant to treat malaria and incurable wounds. In the present study, the authors have demonstrated the anti-proliferative effect of three purified compounds isolated from *Diospyros quercina* (Baill.).

Applications

From the ethnobotanical survey it has been found that *Diospyros quercina* is safe to humans. This scientific research support and suggest the use of this plant as an anticancer agent.

Peer review

This is a valuable research work in which the authors have demonstrated the cytotoxicity of three compounds of *Diospyros quercina in vitro*. The structural elucidation was determined based on chemical and spectroscopic studies. The bioactivity was assessed based on cell proliferation assay. *Diospyros quercina* is then a promising anticancer agent.

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