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RESEARCH ARTICLE

Isolation, Quantification and Antioxidant Activity of Extracts and Compounds from the Aerial Parts of *Archidendron Bauchei* (Jack) I. Niels

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Abstract: In this study, the chemical constituents of the aerial parts of *Archidendron bauchei* (Jack) I. Niels have been investigated for the first time. Ten antioxidant compounds have been isolated and identified from the extracts, in which the amounts of methyl gallate and quercetin are fairly large with around 1.588 ± 0.014 and 3.145 ± 0.049 mg/g, respectively. In addition, the antioxidant potential of all extracted fractions and the isolated compounds were evaluated through DPPH radical scavenging. Moreover, the antioxidant capacity of some selected phenolic compounds was also computationally predicted using density functional theory. It was found that there is a consistency in both theoretical and experimental approaches for evaluating the antioxidants of the phenolic compounds extracted from *A. bauchei*.

Keywords: *Archidendron bauchei,* HPLC, DPPH, Total antioxidant capacity, Bond dissociation enthalpies, Ionization energies.

1. INTRODUCTION

The reactive oxygen species (ROS), which are chemically reactive molecules containing oxygen such as $HO^\bullet$, $HOO^\bullet$, $O_2^\bullet$… are the high-energy and unstable molecules [1-3]. They tend to attach to macromolecules in the body such as lipid, DNA, protein to cause diseases like cancer, cardiovascular disease, diabetes, obesity, and accelerated ageing [1]. Scientific evidences suggest that antioxidants reduce the risk for diseases by using antioxidants to trap and eliminate the free radicals in body [4-7]. Most of the antioxidant compounds are derived from plant sources and belong to various classes of compounds with a variety of physical and chemical properties. *Archidendron* plants are known as the plant sources of antioxidant compounds such as kaempferol, rutin, quercetin, and have been investigated in many studies [8, 9].

A. bauchei is a small plant or an evergreen shrub of the genus *Archidendron*, *Mimosaceae*. The Pako ethnic minority in Quang Tri province of Viet Nam has traditionally used *A. bauchei* to treat some infections of upper respiratory tract, pharyngitis, laryngitis, tonsillitis, burns, scalds and other types of wounds, scabies, and so on. Thus, it is promising that *A. bauchei* will have potential antioxidant activity and contain medicinal compounds.

However, up to now, the study of chemical constituents and evaluation of biological activity of *A. bauchei* has not been reported yet. In the purpose of regarding *A. bauchei* as a traditional medicine, in this work, a study of isolation, structural elucidation, quantification analysis of fractions and extracted compounds from *A. bauchei* collected in Quang Tri province of Viet Nam has been performed. The DPPH assay of the fractions and the isolated compounds from *A. bauchei* and the antioxidant capacities based on the computational methods [10-12] of the important isolated compounds (ArOHs) has been studied.

2. RESULTS AND DISCUSSION

2.1. The Chemical Structure of Compounds Isolated from the Extract of *A. bauchei*

Using combined chromatographic methods, five compounds from chloroform fraction, three compounds from ethyl acetate fraction and two compounds from water fraction of the *Archidendron bauchei* were isolated. Their structures were elucidated to be betulinic acid [13], lup – 20 (29) – en – 3 – one [14], α – tocospiro A [15], spinasterol [16], stigmasterol [17], daucosterol [8], methyl gallate [18], quercitrin, rutin [19] and quercetin [20] by the data of NMR, MS and the literature.

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2.2. *In vitro* Antioxidant Activity of Methanol Extraction and Fractions

It was hypothesized in the previous studies that the antioxidant performance of a substance or a mixture of substances may follow the mechanism of hydrogen atom or electron transfer or both simultaneously take place [3]. However, one model only evaluates one side of the antioxidant capacity. Therefore, we investigated the antioxidant activity of *A. bauchei* regarding both hydrogen atom and electron transfer.

2.2.1. Total Antioxidant Capacity in Terms of Electron Transfer

The total antioxidant capacity was determined by assessing the electron-donating capacity of the sample using the phospho-molybdenum method. In principle, this method based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex at a low pH with a maximal absorbance at 695 nm. A high absorbance value indicates that the sample exhibits high antioxidant activity [21]. A comparison of the antioxidant activity of five *A. Bauchei* fractions and curcumin was presented in Fig. (**1**).

As can be seen from the Fig. (**1**), the fractions of *A. bauchei* have the significant antioxidant ability based on the electron transfer mechanism. The ethyl acetate and water fractions have high values of antioxidant activity while these of *n*-hexane, chloroform, and *n*-butanol fractions are lower at all testing concentrations. Especially, at high concentrations (0.4 ÷ 0.5 mg / mL), total antioxidant capacity of the water fraction is higher than that of curcumin.

2.2.2. DPPH Radical Scavenging Activity in Terms of Hydrogen Transfer

The antioxidant capacity of *A. bauchei* fractions was evaluated using the DPPH radical scavenging method and the results were summarized in Table **1**. As shown from the Table **2**, the antioxidant capacity of five fractions of *A. bauchei, (n*-hexane, chloroform, ethyl acetate, *n*-butanol and water) is found to be much higher than that of the positive control-curcumin with the IC_{50} values at 17.25, 13.17, 3.01, 12.68, 2.61 μg/mL, respectively. Especially, the water fraction demonstrates the greatest activity with the lowest IC_{50} value of 2.61 μg/mL, which is 17 times lower than the IC_{50} of curcumin. Thus, based on the two *in vitro* models, the five fractions extracted from *A. bauchei* are considered as antioxidants with high activity, particularly in the water fraction.

2.3. DPPH Radical Scavenging Activity of Compounds Isolated from Fractions of *A. bauchei*

The antioxidant activity of the isolated compounds was tested in a DPPH radical scavenging assay. The IC_{50} values for the compounds **1-10** are showed in Table **3**. This assay involved a comparison with curcumin (IC_{50} = 38.50) which is used as a positive control.

It is clear from the Table **2**, the four isolated compounds (quercetin, methyl gallate, quercitrin, rutin) possess high antioxidant activity while this activity is low for the rest six isolated compounds (daucosterol, betulinic acid, lup-20(29)-en-3-one, α-tocospiro A, α-spinasterol and stigmasterol). That is because quercetin, methyl gallate, quercitrin, rutin contain phenolic hydroxyl groups which have been known as high antioxidant sources [22]. Based on the IC_{50} values, their antioxidant performance can be arranged in the sequence: quercetin > methyl gallate > quercitrin, rutin > curcumin that is matched with the results (methyl gallate (2.80 μg/mL) [23], rutin (from 5.43 to 10.26 μg/mL) [24-26] and quercitrin (6.16 μg/mL) [27]).

In addition, as can be seen from the Table **3**, computational study was also performed to prove and demonstrate the antioxidant capacity of the compounds **7, 8, 9** and **10**. Label and numbered structures of methyl gallate **7** and three other selected phenolic compounds namely **8, 9, 10** identified in the experimental part were shown in Fig. (**2**). In order to estimate the antioxidants *via* HAT mechanism, the bond dissociation enthalpies of the O−H bond need to be

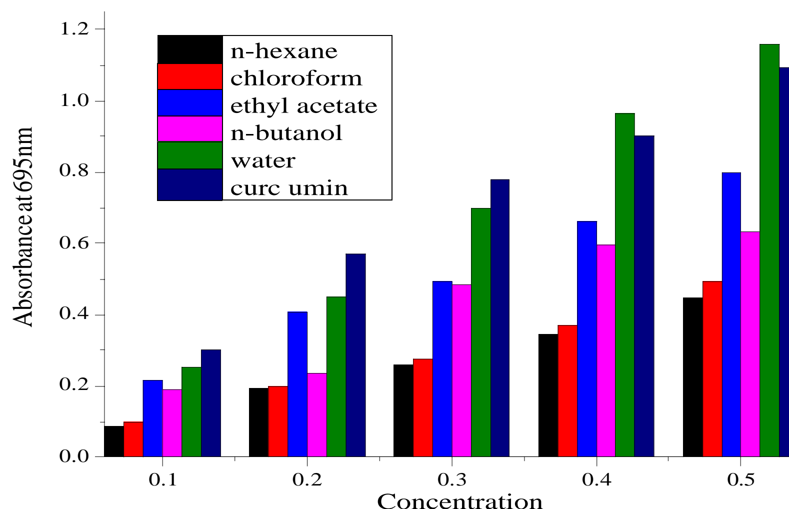

Fig. (1). Total antioxidant capacity of extracted fractions from *A. bauchei*.

Table 1. The DPPH radical scavenging activity rates of fractions of *A. bauchei.*

Concentration (µg/mL)	DPPH radical scavenging activity of fractions (%)					
	H	**C**	**E**	**B**	**W**	**Curcumin**
100	62.98	94.36	82.08	75.83	97.56	81.26 ± 0.21
20	52.16	85.70	70.83	72.15	96.58	40.64 ± 0.25
4	39.59	66.02	53.81	23.75	72.30	29.07 ± 0.37
0.8	28.98	28.47	41.45	11.71	21.12	20.19 ± 0.45
IC_{50} (µg/mL)	17.25	13.17	3.01	12.68	2.61	35.08 ± 0.31

Table 2. The DPPH radical scavenging activity of the studied compounds isolated from fractions of *A. bauchei.*

Serial Number	Compounds	DPPH radical scavenging activity at concentration				
		100	**20**	**4**	**0.8**	**IC50 (µg/mL)**
1	Betulinic acid	20.25	18.26	10.89	5.29	> 100
2	Lup-20(29)-en-3-one	24.78	21.48	18.26	15.38	> 100
3	*α*-Tocospiro A	28.45	24.08	15.34	10.72	> 100
4	*α*-Spinasterol	22.23	17.45	14.87	9.68	> 100
5	Stigmasterol	25.48	21.49	13.45	7.28	> 100
6	Daucosterol	30.54	20.47	15.12	8.56	> 100
7	Methyl gallate	99.08	97.16	80.12	48.48	1.95 ± 0.05
8	Quercitrin	95.41	81.58	69.86	31.23	2.35 ± 0.14
9	Rutin	93.12	85.14	40.23	20.14	7.48 ± 0.23
10	Quercetin	98.72	85.16	75.52	48.23	1.93 ± 0.02
Position control	Curcumin	81.26	40.64	29.07	20.19	38.50 ± 0.14

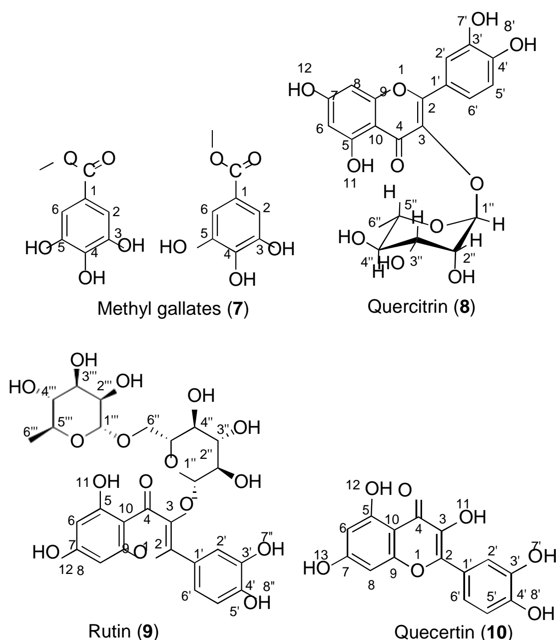

Fig. (2). Structures of compounds **7, 8, 9, 10**.

accurately calculated. It should be noted that there are two conformations of methyl gallate **7,** the structure on the right of (Fig. **2**) is stable than the preceding one about -11.99 kcal/mol. Therefore, all calculations for BDE(O−H) in compound **7** were based on this structure.

Concerning to BDE(O−H) calculations, two computational methods were proposed to compare with each other. In this context, the structural optimization combined with frequency calculation for the first and second computational methods were carried out at the PM6 and B3LYP/6-31g(d), respectively. Then, based on these optimized structures, the energy at higher level of chemistry *i.e.* B3LYP/6-311++G(2d,2p) and B3LYP/6-311++G(2df,2p) methods was calculated.

The Cartesian coordinates of the optimized structures of **7**, **8**, **9**, and **10** are given in Table **S1**. The calculated bond lengths and bond angles of methyl gallate **7** at the B3LYP/6-31G(d) and PM6 and are given in Tables **S2**, **S1-1** and **S3**. The atom by atom superimposing of the molecular skeletons obtained from B3LYP/6-31G(d) and PM6 is drawn in Fig. (**3**). The results showed that there is a slight deviation of the geometrical parameters between two methods. The mean

deviation (MD) for the bond lengths and bond angles are 0.007 Å and 1.4^0 respectively.

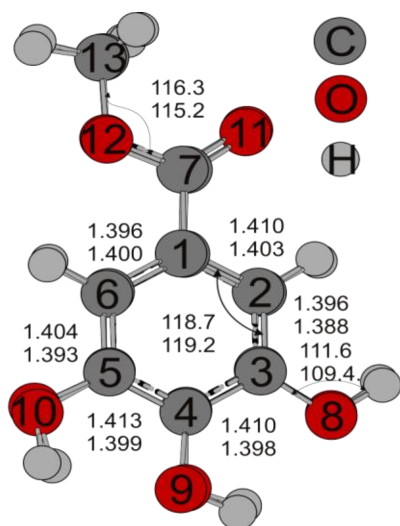

Fig. (3). Atom and atom superimposition of the optimized structures obtained at the PM6 and B3LYP/6-31G(d).

From the data on Table **3**, it is said that the differences of the calculated BDE(O–H)s using two computational methods are insignificant and in the range of 1.63 ÷ 1.99 kcal/mol, but the computer time for the B3LYP/6-311++G(2d,2p)//PM6 is less than for the latter one. Therefore, for further calculations, the first method is favored to apply on the larger phenolic compounds, labelled by **7, 8, 9** and **10**.

The accurate estimation of adiabatic ionization energy using the semiempirical PM6 method has been systematically proved by Thong *et al*. [28]. Hence, this method was used to calculate the IE values for the selected phenolic compounds in this work.

The lowest BDE(O–H) of compounds **7, 8, 9 and 10** are found at the *para* position of the phenolic ring, *e.g.* at O4-H, O8'-H, O8'-H and O8'H respectively (Fig. **2**). It should be noted that among three compounds **8, 9, 10**, the BDE(O–H) values are quasi the same with very slight differences. In terms of HAT mechanism, it can be stated that three molecules **8**, **9**, **10** display the same antioxidant ability and better than molecule **7**. This conclusion is completely adapted with the experimental results given above regarding the IC_{50} of a DPPH antioxidant ability assay shown in Table **2** (IC_{50} values refer to µmol/mL). Obviously, the sequence when comparing the IC_{50} for four phenolic compounds is listed as follows **7** ($IC_{50} = 1.95 \pm 0.05$) ≈ **10** ($IC_{50} = 1.93 \pm 0.02$) ≤ **8** ($IC_{50} = 2.35 \pm 0.14$) < **9** ($IC_{50} = 7.48 \pm 0.23$).

2.4. Qualification of High Antioxidant Compounds from the Extracts by HPLC

There has been great effort to find candidates from natural products to effectively control radicals. Extracts of various plants containing phenolic compounds and tocopherols have also been previously reported to possess antioxidant activity. For instance, methyl gallate [29], rutin [30], quercetin [31] and quercitrin [32] have been reported to exhibit radical scavenging activity.

The ten compounds were tested for their antioxidant activity in a DPPH radical scavenging assay. Five compounds namely methyl gallate, rutin, quercetin and quercitrin were chosen to determine content (with value of IC_{50} is low).

Single peaks at specific retention times of methyl gallate, quercetin, rutin, and quercitrin were indicated in the HPLC profile (Table **5**). The mean for methyl gallate, rutin, quercetin and quercitrin content in *Archidendron bauchei* is given in Table **6**.

The total content of compounds owning the antioxidant activity in *A. bauchei* is 4.798 mg/g while the amount of methyl gallate and quercetin is 1.588 ± 0.014 mg/g and 3.145 ± 0.049 mg/g, respectively. The study of twenty-two plant species including fifteen vegetables and seven medicinal herbs showed that the highest quercetin content was 0.359 mg/g [33]. Thus, the *A. bauchei* contains nine times the amount of active ingredients compared to the highest value in this publication. Phenolic and flavonoid compounds significantly contribute to the antioxidant activity obeying the hydrogen donor mechanism. The results of quantification analysis by HPLC were cleaned antioxidant activity of *A. bauchei*. These results also demonstrate that the compounds exhibit antioxidant activity by using DPPH assay and computational method, which agree with the hydrogen donor mechanism.

3. EXPERIMENTAL

3.1. Plant Materials

The aerial parts of *Archidendron bauchei* (Jack.) Nielsen were collected in March 2015 in Quang Tri province of Vietnam, then were taxonomically identified by the Institute of Marine Biochemistry, Viet Nam (IMBC). A voucher

Table 3. The predicted BDE(O–H)s of methyl gallate 7 (kcal/mol) calculated at B3LYP/6-311++G(2d,2p)//PM6 and B3LYP/6-311++G(2df,2p)//B3LYP/6-31G(d).

BDE(O-H)			
B3LYP/6-311++G(2d,2p)//PM6		B3LYP/6311++G(2df,2p)//B3LYP/6-31G(d)	
O3–H	83.24	O3–H	81.25 (–1.99)
O4–H	81.81	O4–H	80.20 (–1.61)
O5–H	89.89	O5–H	88.26 (–1.63)

Table 4. Calculated BDE(O–H) of the studied compounds 7, 8, 9 and 10 at the B3LYP/6-311++G(2d,2p)//PM6 and calculated IE values at PM6 method.

Compound 7		Compound 8		Compound 9		Compound 10	
BDE(O–H), kcal/mol							
O3–H	81.59	O7'–H	82.87	O7'–H	83.97	O7'–H	81.16
O4–H	81.81	O8'–H	77.20	O8'–H	79.90	O8'–H	78.73
O5–H	89.89	O11–H	90.69	O11–H	90.07	O11–H	82.26
		O12-H	100.44	O12-H	93.4	O12–H	98.15
						O13–H	89.92
IE (kcal/mol)							
192.43		172.45		171.75		178.79	
IC_{50} (µmol/ mL)							
0.011		0.005		0.012		0.006	

Table 5. Regression equation and recovery of methyl gallate, quercetin, rutin, and quercitrin.

N^0	Compounds	Regression Equation	Regression Coefficient R^2	Recovery (%)
1	methyl gallate	y = 59648698.76x + 20722.99	1.000	96.23 ± 0.89
2	rutin	y = 27371495,33x + 15425.25	0.999	98.54 ± 0.12
3	quercetin	y = 48417026.11x + 10,733.17	0.999	95.59 ± 1.50
4	quercitrin	y = 42424607.90x – 373749.32	0.999	96.99 ± 1.37

Table 6. Methyl gallate, quercetin, rutin, and quercitrin contents from *Archidendron bauchei.*

Sample	Methyl Gallate (mg/g)	Rutin (µg/g)	Quercetin (mg/g)	Quercitrin (µg/g)
A. bauchei	1.588 ± 0.014	45.976 ± 0.054	3.145 ± 0.049	0.017 ± 0.001

specimen was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

3.2. Preparation of Methanol Extracts and Fractions

A dried sample of *A. bauchei* (5.0 kg) was extracted with 15 L methanol (MeOH) three times at room temperature. The solutions were combined and evaporated under reduced pressure at 50 ºC, resulting in 760 g of the crude methanol extract. The MeOH extract was distributed in water and then extracted with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The solvents were then distilled to get five fractions named H (*n* -hexane, 100 g), C (chloroform, 145 g), E (ethyl acetate, 250 g), B (*n*-butanol, 105 g) and W (water, 145 g).

3.3. Extraction and Isolation

The chloroform fraction was fractionated into seven fractions (C1 to C6) by silica-gel column chromatography using *n*-hexane: acetone (100: 0, 40: 1, 20: 1, 10: 1, 5: 1, 1: 1, v/v) as mobile phase, respectively.

The C4 fraction was further fractionated using another silica-gel column with *n*-hexane: acetone (10: 1, v/v) as mobile phase to obtain five fractions (C4.1 to C4.5). The C4.2 fraction was separated into five fractions (C4.2.1 to C4.2.5) by reversed-phase column chromatography using acetone: water (3:2, v/v) for elution. The C4.2.2 fraction was subjected to be separated on column with chloroform: methanol (20: 1, v/v) to obtain other four fractions (C42.2.1 to C4.2.2.4). The C4.2.2.2 fraction was processed by a reversed-phase column using YMC (YMC RP-18 resins (30÷ 50µm) and acetone: water (6:1, v/v) to yield compound **1** (m = 17 mg).

The C2 fraction was chromatographed on YCM column using acetone: water (4: 1) as mobile phase to obtain eight fractions (C2.1 to C2.8). The C2.4 fraction was chromatographed on a silica gel column and eluting with *n*-hexane: acetone (20: 1, v/v) obtained five fractions (C2.4.1 to C2.4.5). The C2.4.1 fraction was chromatographed on an YCM column using acetone: methanol: water (3: 1: 0.1, v/v/v) obtain five fractions (C2.4.1.1 to C2. 4.1.5). The

C2.4.1.1.3 fraction was finally chromatographed on a Sephadex LH-20 column eluted with methanol: water (2: 1, v/v) to yield compound **2** (m = 10 mg).

The C2.5 fraction was chromatographed on a silica gel column and eluting with *n*-hexane: acetone (5: 1, v/v) obtained two fractions (C2.5.1 and C2.5.2). The C2.5.1 fraction was separated by reversed-phase column chromatography using methanol: water (3:1, v/v) to yield compound **3** (m = 10 mg).

The C5 fraction was further fractionated using another silica-gel column with *n*-hexane -chloroform (8: 1, v/v) as mobile phase to obtain four fractions (C5.1 to C5.4). The C5.2 fraction was fractionated using silica-gel column with *n*-hexane: chloroform: methanol (2: 1: 0.1, v/v/v) to yield compound **4** (m = 10 mg).

The C5.3 fraction was separated into four fractions (C5.3.1 to C5.3.4) by reversed-phase column chromatography using methanol: water (3:1, v/v). The C5.3.1 fraction was chromatographed on a Sephadex LH-20 column eluted with methanol to yield compound **5** (m = 10 mg).

The ethyl acetate fraction was fractionated into eight fractions (E1 to E8) by silica-gel column chromatography using *n*-hexane: acetone (100:0, 40:1, 20:1, 10:1, 5:1, 1:1, 0:100, v/v) and methanol as mobile phase, respectively. The E4 fraction was further fractionated using another silica-gel column with chloroform: acetone (10:1, v/v) as mobile phase to obtain five sub-fractions (E4.1 to E4.5). The E4.2 fraction was separated into four fractions (E4.2.1 to E4.2.4) by reversed-phase column chromatography using acetone: water (3:2, v/v) for elution. The E4.2.3 fraction was subjected to be separated on a column with chloroform: ethyl acetate: methanol (15:1:0.1, v/v/v) solvent system to obtain other five fractions (E4.2.3.1 to E4.2.3.5). The E4.2.3.4 fraction was processed by a reversed-phase column using YMC and methanol: water (3:1, v/v) to obtain three fractions. The E4.2.3.4.2 fraction was finally chromatographed on a Sephadex LH-20 column eluted with methanol to yield compound **6** (25 mg).

The E4.4 fraction was chromatographed on a Sephadex LH-20 column eluted with methanol to obtain four fractions (E4.4.1 to E4.4.4). The E4.4.2 fraction was fractionated using YMC and methanol: water (8:1, v/v) as a stationary phase and mobile phase, respectively. Four fractions (E4.4.2.1 to E4.4.2.4) were obtained and among them, the fraction E4.4.2.2 was purified by preparative TLC developed with chloroform: methanol: formic acid (5:1:0.1, v/v/v) and identified as compound **7** (12 mg).

The E6 fraction was further fractionated using another silica-gel column with chloroform: acetone (1: 1, v/v) as mobile phase to obtain five fractions (E6.1 to E6.5). The E6.2 fraction was separated into five fractions (E6.2.1 to E6.2.5) by reversed-phase column chromatography using methanol: water (2:1, v/v) for elution. The E6.2.2 fraction was then subjected to be separated on Sephadex column with methanol: water (4:1, v/v) to obtain other four fractions (E6.2.2.1 to E6.2.2.4). The E6.2.2.1 fraction was processed by a reversed-phase column eluted with chloroform: methanol: water (6: 1: 0.1, v/v/v) to yield compound **8** (95 mg).

The water fraction was then fractionated into four fractions (W1 to W4) by Dianion HP-20 column chromatography using methanol: water (1:3, 1:1, 3:1, 100:0, v/v) as mobile phase, respectively. The W2 fraction was further fractionated using another silica-gel column with chloroform: methanol (5:1, 3:1, 2:1, 1:1, 0:100, v/v) as mobile phase to obtain five sub-fractions (W2.1 to W2.5). The W2.2 fraction and the W2.3 fraction combined into one fraction (W2.2-3) by chromatography similar. The W2.2-3 fraction was separated into five fractions (W2.2-3.1 to W2.2-3.5) by a reversed-phase column using YMC and methanol: water (1:1, v/v) for elution. The W2.2-3.5 fraction was then subjected to be separated on a column with chloroform: methanol (15:1, v/v) solvent system to obtain other five fractions (W2.2-3.5.1 to W2.2-3.2.5). The W2.2-3.5.2 fraction was finally chromatographed on reversed-phase column eluted with methanol: water: *n*-butanol (1:1.5:0.1, v/v/v) to yield compound **9** (150 mg).

The W2.5 fraction was chromatographed on an YCM column using acetone: water: formic acid (5:15:0.2, v/v/v) obtain four smaller fractions W2.5.1 to W2.5.4). The W2.5.2.2 fraction was chromatographed on a Sephadex LH-20 column eluted with methanol to yield compound **10** (35 mg).

3.4. HPLC conditions

3.4.1. Preparation of Standard Solutions

Methyl galate standard solutions were prepared in 10 mL methanol at 5 levels varied from 5 to 50 mg, rutin from 0.5 to 20 mg, quercetin from 5 to 20 mg, and quercetin from 0.5 to 10 mg.

3.4.2. Preparation of Sample Solutions

One hundred milligrams of given sample was accurately weighed and put into 10 mL volumetric flask. The sample was then dissolved by adding 10 mL of methanol to obtain 10 mg/mL sample solution.

3.4.3. Chromatographic Conditions

Chromatographic analysis was carried out by C_{18} reversed phase Inertsil ODS-3 column (150 x 4.6 mm) packed by 5μm diameter particles, detector UV-Vis. The HPLC specification and chromatographic conditions are given in Table **8**.

All of the solutions and the mobile phases were filtered through a 0.45 μm membrane cellulose filter before used and all chromatographic operations were carried out at ambient temperature.

3.5. Evaluation of the Total Antioxidant Activity Using the Phospho-molybdenum Method

The total antioxidant activity of studied samples was assessed using the phospho-molybdenum method, which determines the electron transfer capability of the antioxidant compounds in the test sample. The total antioxidant activity of studied samples was determined according to the method described in the literature [21] with certain modifications. In brief, a 0.3 mL aliquot of the sample was mixed with 3 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium

Table 7. The NMR and MS data of the isolated compounds.

Comp. No.	Name	Data of NMR and MS	Refs.
1	betulinic acid	^{1}H-NMR (500 MHz, CDCl$_3$) δ_H (ppm): 3.17 (*dd*, 10.5, 6.0, H-3), 0.96 (*s*, H-23), 0.75 (*s*, H-24), 0.82 (*s*, H-25), 0.94 (*s*, H-26), 0.97 (*s*, H-27), 4.60 (*s*, H-29), 4.73 (*s*, H-29), 1.69 (*s*, H-30). ^{13}C-NMR (125 MHz, CD-Cl$_3$) δ_C (ppm): 38.9 (C-1), 27.2 (C-2), 79.0 (C-3), 38.8 (C-4), 55.5 (C-5), 18.4 (C-6), 34.5 (C-7), 40.8 (C-8), 50.7 (C-9), 37.3 (C-10), 21.0 (C-11), 25.7 (C-12), 38.4 (C-13), 42.6 (C-14), 30.7 (C-15), 32.4 (C-16), 56.4 (C-17), 47.1 (C-18), 49.2 (C-19), 150.9 (C-20), 29.8 (C-21), 37.3 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.8 (C-27), 179.4 (C-28), 109.6 (C-29), 19.4 (C-30).	[13]
2	lup-20 (29) -en-3-one	^{1}H-NMR (500 MHz, CDCl$_3$) δ_H 1.08 (*s,* H-23/H-26), 1.03 (*s,* H-24), 0.93 (*s,* H-25), 0.96 (*s,* H-27), 0.80 (*s,* H-28), 4.57 (*s*, H-29a), 4.70 (*s*, H-29b), 1.69 (*s*, H-30). ^{13}C-NMR (125 MHz, CDCl$_3$) δ_C (ppm): 39.6 (C-1), 34.1 (C-2), 218.2 (C-3), 47.4 (C-4), 55.0 (C-5), 19.3 (C-6), 33.6 (C-7), 40.8 (C-8), 49.8 (C-9), 36.9 (C-10), 21.5 (C-11), 25.2 (C-12), 38.2 (C-13), 42.9 (C-14), 27.5 (C-15), 35.5 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 150.9 (C-20), 29.9 (C-21), 39.6 (C-22), 26.7 (C-23), 21.1 (C-24), 16.0 (C-25), 15.8 (C-26), 14.5 (C-27), 18.0 (C-28), 109.4 (C-29), 19.7 (C-30).	[14]
3	α-tocospiro A	^{1}H-NMR (500 MHz, CDCl$_3$) δ_H (ppm): 2.02 (*s*, H-3a), 1.82 (*s*, H-5a), 1.83 (*s*, H-6a), 1.05 (*s*, H-9a), 0.85 (*d*, 7,0, H-13), 0.84 (*d*, 6.5, H-17a) 0.87 (*d*, 7.0, H-21a/H-22), 4.17 (*s*) (-OH). ^{13}C-NMR (125 MHz, CDCl$_3$) δ_C (ppm): 204.9 (C-1), 92.2 (C-2), 207.1 (C-3), 24.9 (C-3a), 89.1 (C-4), 163.0 (C-5), 11.8 (C-5a), 139.3 (C-6), 8.7 (C-6a), 32.9 (C-7), 36.2 (C-8), 87.0 (C-9), 25.5 (C-9a), 41.5 (C-10), 22.5 (C-11), 37.3 – 37.6 (C-12, C-14, C-16, C-18), 32.7 (C-13), 32.8 (C-17), 19.7 (C-13a), 19.8 (C-17a), 24.8 (C-15), 24.5 (C-19), 39.4 (C-20), 28.0 (C-21), 22.7 (C-21a), 22.6 (C-22).	[15]
4	spinasterol	^{1}H-NMR (500 MHz, CDCl$_3$) δ_H (ppm): 5.15 (*br.s*, H-7), 1.03 (*d*, 6.5, H-21), 0.85 (*d*, 6.5, H-26),0.81 (*d*, 6.0, H-27), 0.81 (*t*, 7.0, H-29), 0.80 (*s*, H-19), 0.55 (*s*, H-18), 3.59 (*m*, H-3), 5.17 (*dd*, 9.0, 15.0, H-22), 5.03 (*dd*, 8.5, 15.0, H-23). ^{13}C-NMR (125 MHz, CDCl$_3$) δ_C (ppm): 37.2 (C-1), 31.5 (C-2), 71.1 (C-3), 38.0 (C-4), 40.3 (C-5), 29.7 (C-6), 117.5 (C-7), 139.6 (C-8), 49.5 (C-9), 34.2 (C-10), 21.6 (C-11), 39.5 (C-12), 43.3 (C-13), 55.1 (C-14), 23.0 (C-15), 28.5 (C-16), 55.9 (C-17), 12.1 (C-18), 13.0 (C-19), 40.8 (C-20), 21.4 (C-21), 138.2 (C-22), 129.5 (C-23), 51.3 (C-24), 31.9 (C-25), 21.1 (C-26), 19.0 (C-27), 25.4 (C-28), 12.3 (C-29).	[16]
5	stigmasterol	mp: 155-157 ºC. ^{1}H-NMR δ_H 5.18 (*m*, H-6), 5.16 (*m*, H-22), 5.03 (1H, *dd*, H-23).	[17]
6	daucosterol	^{1}H-NMR (CDCl$_3$ + CD$_3$OD, 500 MHz) δ_H (ppm): 4.41 (*d*, 7.5, H-1’), 3.26 (m, H-2’), 3.30 – 3.47 (m, H-3’/ H-4’/ H-5’), 3.76 (*dd*. 12.0, 5.0 H-6’a), 3.84 (*dd*, *J* = 12.0, 3.0 H-6’b), 3.58 (*m, H-3*), 5.37 (*d*, 5.0, H-6), 0.69 (*s,* H-18), 1.01 (*s,* H-19), 0.93 (*d,* H-21), 0.82 (*d,* H-26), 0.83 (*d,* H-27), 0.85 (*t,* H-29)	[8]
7	methyl gallate	mp: 201.2-202.5 ºC, M= 184.0. ^{1}H-NMR (500 MHz, CD$_3$OD) δ (ppm): 7.07 (2H, s, H-2 và H-6), 3.83 (3H, s, H-8).^{13}C-NMR (125 MHz, CD$_3$OD) δ (ppm): 169.0 (s, C-7), 146.4 (s, C-3&C-5), 139.7 (s, C-4), 121.5 (s, C-1), 110.1 (d, C-2&C-6), 52.2 (q, C-8).	[18]
8	Quercitrin	M= 448.1. ^{1}H-NMR (500 MHz, CD$_3$OD) δ_H (ppm): 6.21 (*d*, 2.0, H-6), 6.38 (*d*, 2.0, H-8), 7.35 (*d*, 2.0, H-2’), 6.93 (*d*, 8.0, H-5’), 7.32 (*dd*, 8.0, 2.0, H-6’), 5.37 (*d*, 1.5, H-1’’), 4.24 (*m*, H-2’’), 3.77 (*dd*, 9.5, 3.5, H-3’’), 3,36 (*d*, 9.5, H-4’’), 3.44 (*m*, H-5’’), 0.96 (*d*, 6.5, H-6’’). ^{13}C-NMR (125 MHz, CD$_3$OD) δ_C (ppm): 159.3 (C-2), 136.2 (C-3), 179.64 (C-4), 163.2 (C-5), 99.8 (C-6), 165.8 (C-7), 94.7 (C-8), 158.5 (C-9), 105.9 (C-10), 123.0 (C-1’), 117.0 (C-2’), 146.8 (C-3’), 149.8 (C-4’), 116.4 (C-5’), 122.9 (C-6’), 103.5 (C-1’’), 71.9 (C- 2’’), 72.1 (C- 3’’), 73.3 (C- 4’’), 72.0 (C- 5’’), 17.6 (C- 6’’).	
9	Rutin	M= 610.16. ^{1}H-NMR (DMSO-d6, 500 MHz) δ_H (ppm): 7.55 (1H, *d*, 2,0 Hz, H-6’); δ_H 7.53 (1H, *d*, 2.5, H-5’); δ_H 6.83 (1H, *d*, 2.0, H-2’); δ_H 6.36 (1H, *s*, H-8) and δ_H 6.16 (1H, *d*, 1.0 Hz, H-6), 5.32 (*t*, 3.5, H-1’’), 4.39 (*s*, H-1’’’), 0.99 (*d*, 6.0, H-6’’’). ^{13}C- NMR (125 MHz, DMSO-d6) δ (ppm): 156.2 (C-2), 133.2 (C-3), 177.2 (C-4), 161.1 (C-5), 98.9 (C-6), 165.1 (C-7), 93.7 (C-8), 156.2 (C-9), 103.5 (C-10), 121.0 (C-1’), 115.2 (C-2’), 144.8 (C-3’), 148.6 (C-4’), 116.1 (C-5’), 121.6 (C-6’), 101.3 (C-1”), 74.1 (C-2”), 76.5 (C-3”), 70.5 (C-4”), 75.9 (C-5”), 67.0 (C-6”), 100.7 (C-1”’), 70.3 (C-2”’), 70.0 (C-3”’), 71.9 (C-4”’), 68.2 (C-5”’), 17.7 (C-6”’).	[19]
10	Quercetin	M= 302.05. ^{1}H-NMR (DMSO-d6, 500 MHz) δ: 12.48 (1H, *s*, 5-OH), 7.68 (1H, *d*, 2.5, H-2'), 7,54 (1H, *dd*, 6.5, 2.0, H-6 '), 6.89 (1H, *d*, 8.0, H-5'), 6.41 (1H, *d*, 2.0, H-8), 6.19 (1H, *d*, 2.0, H-6). ^{13}C-NMR (125MHz, DMSO-d6) δ (ppm): 146.9 (C-2), 135.8 (C-3), 175.9 (C-4), 160.8 (C-5), 98.3 (C-6), 163.9 (C-7), 93.4 (C-8), 156.3 (C-9), 103.1 (C-10), 122.1 (C-1’), 115.2 (C-2’), 145.2 (C-3’), 147.8 (C-4’), 115.7 (C-5’), 120.1 (C-6’).	[20]

Table 8. HPLC specifications for phytochemical analysis

Compounds	Methyl Gallate	Rutin	Quercetin	Quercitrin	α-tocopherol
Mobile phase (v/v)	0.5% orthophosphoric acid (A): Methanol (B) (0 ~ 10 min, 10 → 25% A; 10 ~ 60 min, 25 → 47% A)			Water (A) : acetonitrile (B) (0 ~ 20 min, 15% B → 25% B, 20 ~ 30 min, 25% B → 70%)	Methanol: water (97:3)
Flow rate (mL/min)	1.0			1.0	1.2
Injection volume (µL)	20			10	20
Standard Rt (Min)	15.48 ± 0.12	38.33 ± 0.23	55.62 ± 0.42	15.03 ± 0.18	11.21 ± 0.11
Detection wavelength (nm)	370	370	370	370	295

phosphate and 4 mM ammonium molybdate), and then the mixture was incubated at 95ºC for 90 min. The mixture was then cooled down to 25ºC and the absorbance was measured at the wavelength of 695 nm against a blank that contained 3 mL of the reagent solution without the sample. The total antioxidant activity was expressed as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity. Curcumin was used for comparison as the reference [34].

3.6. Evaluation of DPPH Radical Scavenging Activity

The DPPH radical scavenging activity determines the hydrogen atom transfer capability of the antioxidant compounds in the test samples. The DPPH free radical scavenging activity of each sample was determined using the Jasco V-630 Spectrophotometer according to the method described by Gopi and coworkers [21] and Wong et al. [35] with certain modifications. The samples were dissolved in 1.5 mL methanol at various concentrations (25, 50, 75 and 100 µg/mL) and mixed with 1.5 mL of 100 µM DPPH (100 µM DPPH dissolved in methanol before using). The reaction mixture was shaken for 1 minute and incubated at room temperature for 30 minutes. The absorbance was then measured at a wavelength of 517 nm. Three milliliters of methanol was used as a blank sample. The DPPH radical scavenging activity (%) of the sample was calculated using the following formula.

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Where SA_{DPPH} (%) is the inhibition of DPPH activity; A_c is the optical density of the blank; A_s is the optical density of the sample.

All experiments were repeated three times to avoid errors. Radical scavenging activity was evaluated using the IC_{50} value [36, 37].

3.7. Computational Method

All computational calculations were carried out using the Gaussian 09 software [38]. The geometry optimization and the vibrational frequency calculations of the studied compounds and their radicals were primarily performed using the PM6 method. On the basis of the PM6-optimized structures, single point electronic energies were computed using density functional theory at the restricted-open shell (RO)B3LYP/6-311++G(2d,2p) level of theory. Thermoparameters like bond dissociation enthalpies and ionization energies were calculated based on the data of the output files of each phenolic compound (ArOH) and can be expressed as the following:

$$BDE = H_f(ArO^\bullet) + H_f(H^\bullet) - H_f(ArOH) \quad (1)$$

$$IE = H_f(ArOH^{\bullet+}) + H_f(e^-) - H_f(ArOH) \quad (2)$$

Where H_f is the total enthalpy of the studied species at the temperature of 298.15 K. The enthalpy of $H_f(H^\bullet)$ is taken from the experimental value of –0.5 Hartree. Vibrational frequencies obtained at the PM6 method were scaled by a factor of 1.078 [39].

CONCLUSION

From aerial parts of *A. bauchei*, chemical structures of the ten compounds were identified. Four compounds including betulinic acid, lup-20(29)-en-3-one, α-tocospiro A, and α-spinasterol were isolated from *Archidendron* for the first time. Moreover, for the first time, A. *bauchei* was investigated for its chemical composition. The five fractions of *A. bauchei* exhibited a good antioxidant activity obeying the hydrogen donor mechanism in the DPPH with IC_{50} values from 2.61 to 17.25 µg/mL, which are better than curcumin (38.50 µg/mL). Importantly, the IC_{50} values from the water fraction of *A. bauchei* showed the highest activities, which are approximately 14 times lower than that of curcumin. Furthermore, at high concentrations (0.4 ÷ 0.5 mg/mL), total antioxidant capacity of the water fraction was higher than that of curcumin. Among ten isolated compounds, four compounds showing strong antioxidant property are quercetin, methyl gallate, quercetin -3-O-α-L-rhamnopyranoside and rutin. In addition, the computational calculations using B3LYP/6-311++G(2d,2p)//PM6 for **7**, **8**, **9** and **10** also indicate that these phenolic compounds display good antioxidants. The BDE(O–H) and IE have been computed in the gas phase to confirm again the antioxidant capacity of the four selected compounds. These results also demonstrate that the compounds exhibit antioxidant activity in DPPH and computational method, and content of some compounds having antioxidant activity, which agree with the hydrogen donor mechanism. The total content of compounds owning the an-

tioxidant activity in *A. bauchei* is 4.798 mg/g while the amount of methyl gallate and quercetin is 1.588 ± 0.014 and 3.145 ± 0.049 mg/g, respectively. These results play an important role in further research regarding the discovery of new therapeutic compounds from nature. As far as we know, this is the first report on the antioxidant activity and component structures of *A. bauchei* species using both experimental and computational approaches.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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