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Baccaurea ramiflora: Isolation of Aldehydes and *in Vitro* Biological Investigations

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Abstract

The stem bark of *Baccaurea ramiflora* was studied. Four aldehydes named as 3 methoxy 4 hydroxy-cinnamaldehyde (coniferyl aldehyde); 3, 4, 5 trimethoxy cinnamaldehyde; 3, 4, 5 trimethoxy benzaldehyde and 3,4 dimethoxy benzaldehyde) (veratraldehyde) have been isolated and then identified by NMR spectroscopy. All of them are first time reported for this plant. Here *in vitro* biological investigations include antioxidant and cytotoxicity study. Among all fractions, the chloroform soluble fraction exhibited strong free radical scavenging activity having IC_{50} value of 12.87 μg/ml compared to BHT (IC_{50} value 5.64 μg/ml). On the contrary, aqueous soluble fraction exhibited most toxicity towards brine shrimp compared with vincristine sulphate having LC_{50} value of 1.44 and 0.9258 μg/ml respectively.

Keywords

Baccaurea ramiflora, Aldehyde, NMR Spectroscopy, Antioxidant, Cytotoxicity

1. Introduction

World Health Organization (WHO) claimed that 80% of people still rely on plant-based traditional medicines for their primary health care. Natural origins lead to development of many drugs [1]. So phytochemical research is of paramount importance especially for third world countries where synthetic drug research is highly barricaded due to lack of resources and infrastructures.

Baccaurea is a genus of flowering plant belonging to the family Phyllanthaceae. The term "*Baccaurea*" is derived from Latin and it refers to the golden yellow color of the fruits [2]. 80 species of this genus have been reported around the world. Based on the fruit character, this is divided into following classes: [3]

- Rambai-Thin skinned fruits.
- Tampoi-Thick skinned fruits.

Baccaurea ramiflora belongs to Phyllanthaceae family, which is a slow growing, evergreen, short to medium height shade loving plant. *Baccaurea ramiflora* is distributed mostly in tropical areas like South East Asia region, the sub-Himalayan tract and Andaman Islands [4]. Locally it is known as latkan and bhubi [4].

According to a report published by Digital Herbarium of Crop Plants, they have the following features:

Root: Tap root system.

Leaf: Leaves are papery, oblong to obovate-oblong, measuring 9 - 18 cm long and 3 - 8 cm wide. Adaxial (upper) surface of leaf is green and abaxially (lower) surface is yellowish-green. The base of leaf is cuneate.

Flowers: Flowers are small, borne in clusters on old branches or trunk. Flowers are yellowish-white.

Fruits: Fruits are ovoid or sub-globose, about 2.5 cm in diameter, reddish-yellow or purple when mature.

A wide range of compounds e.g. phenols, esters, sterols etc. have been isolated from different parts of *Baccaurea ramiflora*. 6'-O-vanilloylisotachioside, 6'-O-vanilloyltachioside, icariside B_5, (-)-epicatechin, bis(8-catechiny1)methane, aviculin, 3-O-caffeoyl-4-O-methylquinic acid, 5-O-caffeoylquinic acid methyl ester, tuberonic acid glucoside methyl ester, erigeside B and β-sitosterol were isolated from the leaves of *Baccaurea ramiflora* [5]. 4'-O-(6-O-vanilloyl)-β-D-glucopyranosyl tachioside D, 6'-O-vanilloylpicraquassioside D and 6'-O-vanilloylicariside B_5 were isolated from the stems of *Baccaurea ramiflora* [6].

As well as analgesic activity from seeds [7], anthelmintic from the whole plant [8], antioxidant activity from fruits [9], cytotoxicity from fruits [10] and hypoglycemic and hypolipidemic activity from the leaves [11] of *Baccaurea ramiflora* were mentioned. Further research can identify whether there are any unidentified bioactive principles.

Phytochemical profiling of the stem bark of *Baccaurea ramiflora* has not done extensively. So in this investigation, we have tried to focus on this part, which lead to isolation of aldehydes from the stem bark of *Baccaurea ramiflora* for the very first time. *In vitro* antioxidant and cytotoxicity activity of this plant has been also checked.

2. Materials and Method

2.1. Collection and Preparation

The stem bark of *Baccaurea ramiflora* was collected in April 2019 from Kishoreganj district. Later it was identified by an expert from Bangladesh National Herbarium (BNH) and a voucher specimen was deposited (DACB Accession number-55316). After cleaning and shade drying for two weeks, they were crushed into coarse powder using high capacity grinding machine.

2.2. Extraction

About 1500 gm of powdered plant material was taken in an amber-colored bottle and soaked with distilled methanol for 15 days with occasional shaking and stirring. The mixture was therefore filtered using a fresh cotton plug. The solvent of the mixture was evaporated using Buchii Rotavapour rotary evaporator at 40˚C temperature and low pressure and the extract was prepared.

2.3. Chromatographic Separation

After evaporation we obtained ethyl acetate and methanolic extract which was then subjected to vacuum liquid chromatography (VLC) and it yielded 40 fractions of different polarity [12]. Selected VLC fractions were taken and gel permeation chromatography was done using Sephadex LH 20 for further separation [13]. Later these column fractions were analyzed by thin layer chromatography [14] and compounds of interest were isolated using preparative layer chromatography (PLC) [15].

2.4. Structure Elucidation

Finally their structures were elucidated using ^{1}H NMR spectroscopy (400 MHz, $CDCl_3$).

2.5. Determination of DPPH Scavenging Activity

The free radical scavenging activities of the plant extracts on 1,1-diphenyl-2picrylhydrazyl (DPPH), a stable radical, were estimated [16]. 2.0 mL of a methanol solution of the extract at different concentration from 400.0 to 1.5625 µg/mL were mixed with 2.0 mL of a DPPH methanol solution (20 µg/mL). After 30 minutes reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer.

Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$I\% = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blank}}\right) \times 100\%$$

Where, Absorbance of blank is the absorbance of control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

2.6. Brine Shrimp Lethality Bioassay

Brine shrimp eggs were hatched in simulated sea water to get nauplii. By the addition of calculated amount of dimethylsulphoxide (DMSO), desired concentration of the test samples were prepared. The nauplii were counted by vis-

ual inspection and were taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations were added to the pre-marked vials through micropipette. The vials were then left for 24 hours. Survivors are counted after 24 hours [16]. The median lethal concentration (LC_{50}) value was calculated from the graph plotted percentage mortality rate against extract concentration.

3. Results and Discussion

Four aldehydes has been identified as 3 methoxy 4 hydroxy cinnamaldehyde (coniferyl aldehyde) (**1**); 3, 4, 5 trimethoxy cinnamaldehyde (**2**); 3, 4, 5 trimethoxy benzaldehyde (**3**) and 3, 4 dimethoxy benzaldehyde (veratraldehyde) (**4**) by ^{1}H NMR spectroscopic studies (**Figure 1**).

Strong free radical scavenging activity has been showed by the chloroform soluble fraction of the plant extract having IC_{50} value of 12.87 μg/mL with compared to BHT (IC_{50} value 5.64 μg/mL) while petroleum ether soluble fraction exhibited good antioxidant activity (IC_{50} = 15.47 μg/mL). Aqueous soluble fraction exhibited most toxicity towards Brine shrimp while petroleum ether soluble fraction exerted moderate toxicity compared with vincristine sulphate having LC_{50} value of 1.44, 1.831 and 0.9258 μg/mL respectively.

3.1. Characterization of Compound 1

VLC fraction of 15 yielded compound **1** by PLC as colorless liquid and molecular formula was found to be $C_{10}H_{10}O$. ^{1}H NMR spectrum (400 MHz, $CDCl_3$)

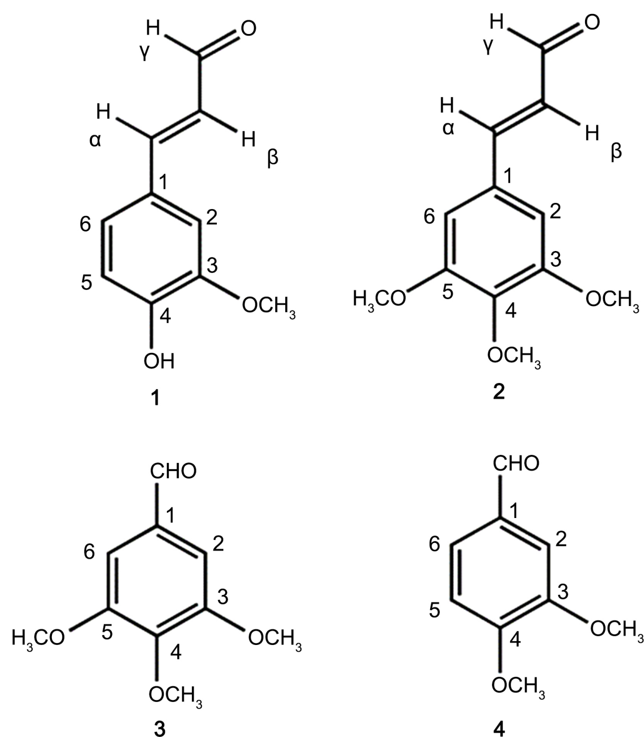

Figure 1. Chemical structure of compound **1**, **2**, **3** and **4**.

(Table 1) of **1** showed two one proton signals at δ 7.09 (d, J = 1.6Hz) and 6.99 (d, J = 8.0 Hz), which were assigned to aromatic protons H-2 and H-5 respectively; another two proton signals at δ 7.42 (d, J = 16.0 Hz) and δ 7.15 (dd, J = 8.0 Hz, 1.6 Hz) were assigned to α and β protons respectively. They showed *trans* coupling (J = 16.0 Hz) with each other and the β proton showed additional coupling (J = 8.0 Hz) with the aldehyde proton (γ). The most deshielded one proton doublet at δ 9.68 was accounted for the aldehydic proton. The three proton singlet at δ 3.98 was characteristic for a methoxy group, located at 4 of the benzene ring. The spectral data confirmed compound **1** as 3 methoxy 4 hydroxy cinnamaldehyde (coniferyl aldehyde) [16].

3.2. Characterization of Compound 2

VLC fraction of 15 yielded compound **2** by PLC as light yellow liquid. Molecular formula was determined to be $C_{12}H_{14}O_4$. In ^1H NMR spectrum (400 MHz, $CDCl_3$) (Table 1) of **2**, two protons singlet at δ 6.84 protons was assigned to H-2 and H-6. Two one proton signals at δ 7.42 (d, J = 16.0 Hz) and δ 6.63 (dd, J = 16.0 Hz, 8.0 Hz) were assigned to α and β protons respectively. The most deshielded one proton doublet at δ 9.78 was indicated aldehydic proton (H-γ). The nine proton singlet at δ 3.97 was characteristic for three methoxy groups located at 3, 4, 5 of the benzene ring. So the compound **2** was identified as 3, 4, 5 trimethoxy cinnamaldehyde [17].

Table 1. ^1H NMR (400 MHz, $CDCl_3$) spectroscopic data of compound **1**, **2**, **3** and **4**.

Position	δ_H, J in Hz			
	1	**2**	**3**	**4**
H-2	7.09 (d, J = 1.6 Hz)	6.84 (s)	7.178 (s)	7.45 (d, J = 1.6 Hz)
H-5	6.99 (d, J = 8.0 Hz)	-	-	7.07 (d, J = 8.4 Hz)
H-6	7.15 (dd, J = 8.0, 1.6 Hz)	6.84 (s)	7.178 (s)	6.95 (dd, J = 8.4, 1.6 Hz)
H-α	7.42 (d, J = 16.0 Hz)	7.42 (d, J = 16.0 Hz)	-	-
H-β	6.62 (dd, J = 16.0, 8.0 Hz)	6.63 (dd, J = 16.0 Hz, 8.0 Hz)	-	-
H-γ	9.68 (d, J = 8.0 Hz)	9.68 (d, J = 8.0 Hz)	-	-
OCH_3-3	3.98 (s)	3.97 (s)	3.99 (s)	4.00 (s)
OCH_3-4	-	3.97 (s)	3.99 (s)	4.00 (s)
OCH_3-5	-	3.97 (s)	3.99 (s)	-
-CHO	-	-	9.845	9.857

3.3. Characterization of Compound 3

Compound **3** was also isolated from the VLC fraction of 15 by PLC as colorless liquid and molecular formula was determined as $C_{10}H_{12}O_4$. ^1H NMR spectrum (400 MHz, $CDCl_3$) (**Table 1**) of **3** displayed a two protons singlet at δ 7.718, which were assigned to aromatic protons H-2 and H-6. The most deshielded one proton singlet δ 9.845 was accounted for the aldehydic proton. The nine protons singlet at δ 3.99 was characteristic for three methoxy group located at 3, 4, 5 of the benzene ring. Based on the above features, the compound **3** was identified as 3, 4, 5 trimethoxy benzaldehyde [18].

3.4. Characterization of Compound 4

VLC fraction of 17 + 18 yielded compound **4** by PLC as yellow liquid and its molecular formula was found to be $C_9H_{10}O_3$. Three proton signals at δ 7.45 (d, J = 1.6 Hz), δ 7.07 (d, J = 8.4 Hz) and δ 6.95 (dd, J = 8.4 Hz, 1.6 Hz) were displayed in ^1H NMR spectrum (400 MHz, $CDCl_3$) (**Table 1**) of **4**, accounted for an *ortho* & *para* substituted aromatic ring assigned as H-2, H-5 and H-6 respectively. H-2 and H-6 showed *meta* coupling (J = 1.6 Hz) to each other while H-5 and H-6 showed *ortho* coupling (J = 8.4 Hz) to each other. The most deshielded one proton singlet at δ 9.857 was characteristic for aldehyde proton while the six protons singlet at δ 4.00 indicated presence of two methoxy groups located at 3, 4 of the benzene ring. The compound **4** was identified as 3, 4 dimethoxy benzaldehyde (veratraldehyde) [19].

3.5. Free Radical Scavenging Activity

Antioxidant activity of plant extracts can be accurately measured using DPPH assay method [15]. **Table 2**, **Figure 2** showed % inhibition values of different solvent fractions of *Baccaurea ramiflora* stem bark at variable concentration, while **Table 3**, **Figure 3** depicted their IC_{50} value. **Table 4** provided their summative antioxidant activity. Probably phenolic compounds are responsible for their antioxidant property. Their antioxidant activity was also previously mentioned [5] [9].

3.6. Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay has been utilized as a primary screening method of lethality of different plant extracts. All the samples having LC_{50} value < 1000 μg/mL are considered for further pharmacological analysis [16]. **Table 4**, **Figure 4** depicted mortality rate of different fractions of *Baccaurea ramiflora* while **Table 5**, **Figure 5** showed different degree of lethality of plant extracts of *Baccaurea ramiflora* to Brine shrimp. Among the fractions, chloroform soluble fraction was found to be most toxic to brine shrimp and petroleum ether soluble fraction showed moderate toxicity compared to anticancer drug vincristine sulphate, which support the previous data about this [9] [10] [20]. Further *in vivo* acute oral toxicity study can confirm whether this toxicity level is harmful for susceptible biological systems or not.

Table 2. % Inhibition of different fractions of *Baccaurea ramiflora.*

Conc.(μg/mL)	BHT	BRA	BRE	BRC	BRP
400	96.36%	80.80%	83.76%	75.15%	85.09%
200	95.03%	77.14%	78.46%	72.17%	80.45%
100	91.39%	67.86%	68.20%	67.20%	74.16%
50	87.08%	62.89%	37.40%	65.21%	68.19%
25	77.47%	57.26%	28.76%	59.91%	59.25%
12.5	64.21%	37.71%	24.78%	55.60%	44.67%
6.25	51.95%	33.40%	24.15%	41.02%	27.80%
3.125	37.71%	16.20%	15.18%	36.05%	18.80%
1.5625	26.77%	4.90%	11.20%	28.76%	10.90%

BHT = Tert-Butyl-1-hydroxytoluene, BRA = Aqueous soluble fraction, BRE = Ethyl acetate soluble fraction, BRC = Chloroform soluble fraction, BRP = Petroleum ether soluble fraction, BR = *Baccaurea ramiflora*; Absorbance of blank = 0.3018.

Table 3. Antioxidant activity of different fractions of *Baccaurea ramiflora.*

Different fractions	IC_{50} value	Regression equation	R^2
BHT	5.64	$y = 0.1327 \ln(x) + 0.2705$	0.9412
BRA	27.49	$y = 0.1396 \ln(x) + 0.0375$	0.9666
BRE	46.48	$y = 0.1397 \ln(x) + 0.0363$	0.9017
BRC	12.87	$y = 0.0856 \ln(x) + 0.2813$	0.952
BRP	15.47	$y = 0.1253 \ln(x) + 0.1568$	0.9571

Table 4. % Mortality rate of different fractions of *Baccaurea ramiflora.*

Conc. (μg/mL)	VS	BRA	BRE	BRC	BRP
400	90	40	30	90	80
200	90	40	30	80	70
100	80	40	30	70	40
50	70	30	20	60	40
25	70	30	10	40	40
12.5	60	20	10	30	30
6.25	50	10	10	30	20
3.125	40	10	0	20	10
1.5625	20	10	0	10	10

VS = Vincristine sulphate.

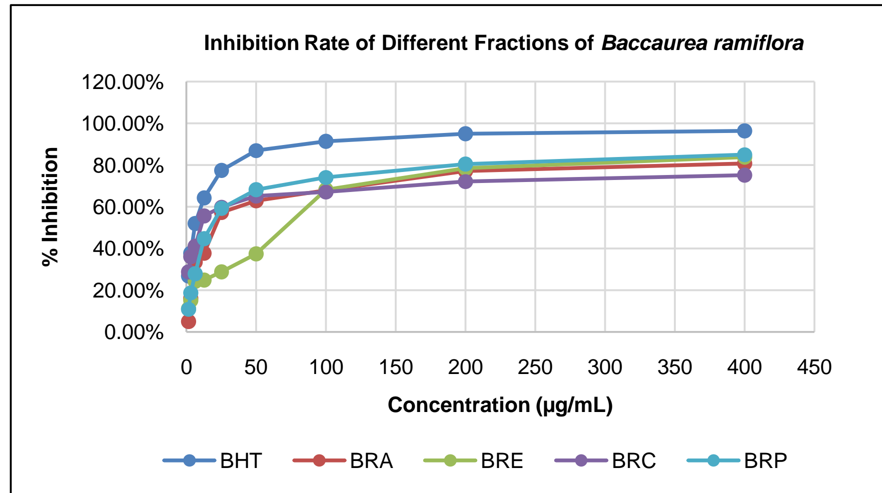

Figure 2. Inhibition rate of different fractions of *Baccaurea ramiflora.*

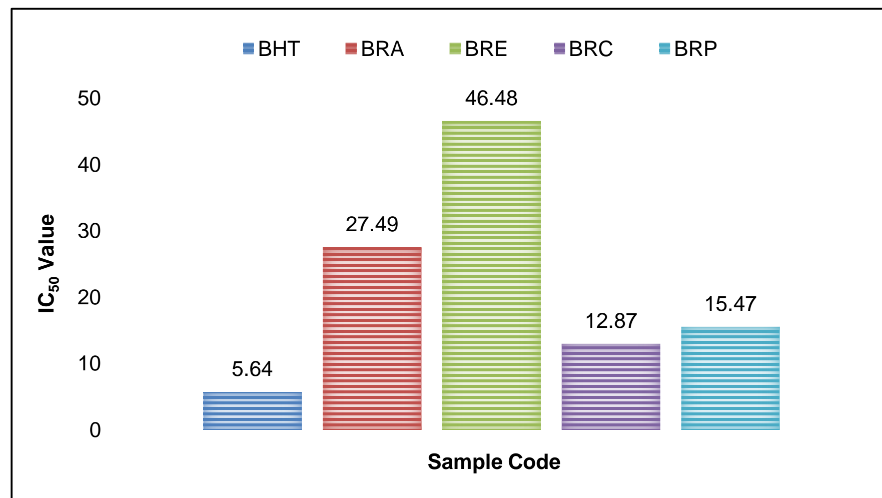

Figure 3. IC_{50} value of different fractions of *Baccaurea ramiflora.*

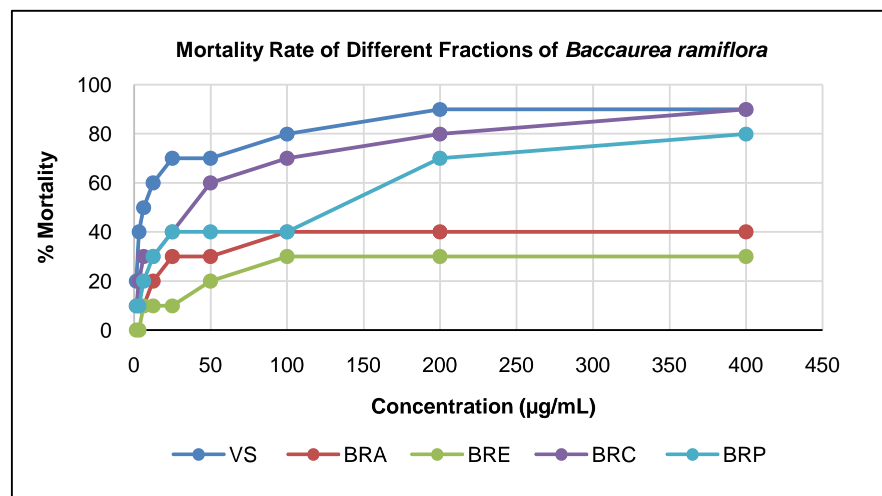

Figure 4. Mortality rate of different fractions of *Baccaurea ramiflora.*

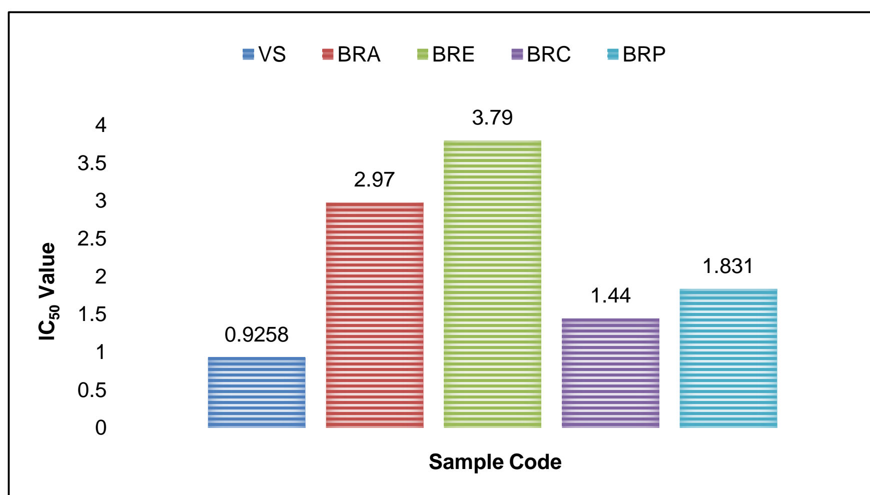

Figure 5. LC_{50} value of different fractions of *Baccaurea ramiflora.*

Table 5. Cytotoxicity of different fractions of *Baccaurea ramiflora.*

Different fractions	LC_{50} value	Regression equation	R^2
VS	0.9258	y = 27.985x + 24.091	0.9614
BRA	2.97	y = 15.502x + 3.8843	0.9187
BRE	3.79	y = 14.394x − 4.5679	0.9218
BRC	1.44	y = 33.773x + 0.5649	0.9758
BRP	1.831	y = 28.236x − 1.695	0.9116

4. Conclusion

From the spectral data compound 1, 2, 3 and 4 can be confirmed as 3 methoxy 4 hydroxy cinnamaldehyde (coniferyl aldehyde); 3, 4, 5 trimethoxy cinnamaldehyde; 3, 4, 5 trimethoxy benzaldehyde and 3, 4 dimethoxy benzaldehyde (veratraldehyde). Some of the fractions can be potential source for *in vitro* antioxidant and cytotoxic property. Further investigation can identify the *in vivo* activities.

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Conflicts of Interest

The authors have no conflict of interest.

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