

## Full Length Research Paper

# Evaluation of phytochemical components from ethyl acetate fraction of *Vernonia calvoana* using gas chromatography-mass spectrometry analysis and its antioxidants activities

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Gas chromatography-mass spectrometry (GC-MS) analysis and antioxidants activity of phytochemical constituents from ethyl acetate fraction of *Vernonia calvoana* (V.C) was evaluated. The fraction was subjected to GC-MS analysis using 5% diphenyl 95% dimethylpolysiloxane (30 m × 0.25 mm ID × 0.25 µm film thickness), the compounds were separated and identified by Turbo gold mass detector. The antioxidants activity was investigated employing the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and Ferric reducing antioxidant power (FRAP) scavenging method. Eighteen different phytochemical compounds namely: Etylobenzene (22.13%), isopropylidenecyclopentadiene (8.24%), 1-ethyl-2-methylbenzene (8.64%), decane (15.24%), 1,2,3-trimethylbenzene (12.51%), 2,7-Dimethyloctane (5.79%), 4-methyltridecane (0.72%), tetrahydro-2-furanmethanol (2.44%), 3,3-Dimethylhexane (0.54%), 5-methylundecane (0.74%), 1-Nonyne (2.16%), 1-pentadecane carboxylic acid (4.70%), 2,6-Dimethyl-1,7-octadien-3-ol (4.00%), cis-9-octadecanoic acid (5.49%), n-Nonadecanoic acid (1.53%), Nonyl propyl ester (1.84%), 5,8-octadecadienoic acid (1.79%), and 9,12,15-octadatrienal (1.53%) were identified. Increase in activity was observed in both the DPPH and FRAP analysis, and were concentrations dependent and closely related to ascorbic acid. The phytochemical components using GC-MS and antioxidant activity of ethyl acetate extract of V.C have been evaluated. In addition to this, further isolation and characterization of individual components would provide more insight to its use in the production of new drugs of therapeutic value.

**Key words:** 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), Ferric reducing antioxidant power (FRAP), gas chromatography, *Vernonia calvoana*, antioxidants.

## INTRODUCTION

Before the advent of synthetic drugs, man depended on the curative properties of medicinal plants. The value for

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the use of these plants originate from ancient belief which says God created plants to provide food, medical treatment, and other effects for man (Ahvazi et al., 2012). Medicinal plant serves as the first line for primary health care to the rural populace and “backbone” of traditional medicine; this suffice that, more than 3.3 billion people in the less developed countries make use of medicinal plants almost on a regular basis for curative purposes (Davidson-Hunt, 2000).

*Vernonia* (Asteraceae) is the largest genus in the tribe Vernoniae, with over 1000 species (Keeley and Jones, 1979). The genus is known for having several species with both nutritional (Igile et al., 1995) and industrial importance (Perdue et al., 1986). The genus, overtime, have been popularly used in the management of different disorders including inflammation, malaria, fever, worms, pain, diuresis, cancer, abortion, and several gastrointestinal problems (Alves and Neves 2003). Also based on pharmacological use, *Vernonia* species have been documented to possess hypotensive activity (Achola et al., 1996), phototoxic, antibacterial and anti-inflammatory (Perez-Amador et al., 2008), immunomodulatory (Nergard et al., 2004), antioxidants (da Silva et al., 2013) and anti-histaminic activity (Laekeman et al., 1983). These activities reported may be attributed to diverse secondary metabolites such as flavonoids, anthraquinones, polyphenols, tannins, alkaloids, sesquiterpene lactones, phenol, steroidal saponins, cardiac glycosides, carotenoids and high nutritive components present in this species (Ejoh et al., 2005; Igile et al., 2013).

*Vernonia calvaona* Hook F (Asteraceae) is popularly called “Ekeke leaf” by the people of Ugep in Yakurr Local Government Area of Cross River State of Nigeria. It is a small plant of about 1 m in height and leaves of 10.0 mm wide. It is eaten raw with pepper sauce, leaving a sweet taste in the tongue. It is also used to prepare native soups (melon soup) in place of its sister plant *Vernonia amygdalina* to provide slight bitter taste. Documented reports have shown that extracts from this plant possess hypoglycemic, hypolipidemic, and *in vivo* antioxidant activity in diabetic animal which could make available the needed information to choose the extract from this plant for further biochemical investigations (Iwara et al., 2015 and 2017). Also report by Egbung et al. (2016) showed that the inflorescences from the plant possess a strong antioxidant activity compared to ascorbic acid.

Antioxidants are compounds that have the potentials of delaying or inhibiting the oxidation of other molecules by blocking the oxidizing chain reactions. The molecule have the ability of neutralizing free radical by donating electrons and further prevent damages that may be caused by this radical to cell and tissue (da Silva et al., 2013). Fruits, vegetables and herbs are good sources of these compounds which are known as phytochemicals including flavonoids, ascorbic acids etc. These phytochemicals are documented to have corresponding

characteristics with antioxidant potentials (Katalinic et al., 2004). In this study, the phytochemical components of *V. calvaona* would be evaluated using GC-MS with further investigation of its *in-vitro* anti-oxidant activity using Ferric reducing antioxidant power (FRAP) and 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging method.

## MATERIALS AND METHODS

### Sample collection and preparation

Fresh leaves of *V. calvaona* were harvested from a farm in Ugep, in Yakurr L.G.A of Cross River State, Nigeria. The leaves were collected in the early hours of the day, cleaned and air dried for 7 days after which they were ground into powder form. A measured quantity of 5 kg of powder leaves were extracted via cool maceration in 8 L of 80% ethanol for 48 h. The extract was further double filtered with chess cloth, then with filtered paper (Whatman 4 filtered paper) and the residue obtained was further extracted with 4 L of 80% ethanol. The filtrate was then concentrated at 45°C in rotary evaporator (RE-52A, Shanghai Ya Rong Biochemistry Instrument Company, China) to 10% volume and then to complete dryness using water bath yielding 310.3 g (6.2%) of crude extract. The obtained crude extract was subjected to fractionation.

### Fractionation of crude extract

The crude extract (251.8 g) was chromatographically eluted with ethyl acetate in a column packed with silica gel of mesh 60 to 120. The fractions were collected and evaporated in rotary evaporator at 50°C to 10% of its original volume and further evaporated to paste form in a water bath at 50°C. The percentage yield for the fraction was 20 g (8.10%) ethyl acetate fraction. The fraction and the remaining crude extract were stored in a freezer at -4°C for further experiments.

### Gas chromatography–mass spectrum (GC-MS) analysis

Analysis of fractionated samples was carried out using GC-MS - QP2010 plus (SHIMADZU-JAPAN), comprising of AOC-20i auto sampler and gas chromatograph interfaced with a mass spectrometer. The assay conditions were as follows: fused silica capillary column (Rastek RT × 5Ms; 30 m × 0.25 mm ID × 0.25 µm film thickness) composing of 5% diphenyl 95% dimethylpolysiloxane; column oven temperature at 80°C, injection temperature maintained at 250°C; injection mode split; pressure of 108 kpa; total flow of 6.2 ml/min at 1 ml/min; column flow of 1.58 ml/min; split ratio of 1.0 and solvent cut time of 2.50 min. Mass spectra were taken at start time of 3.0 min and end time of 27.0 min; while the ACQ mode- Scan was carried out at event time (0.50 s), with scan speed 1250 m/s.

### Identification of component

Identification of components on the chromatogram was carried out using the database of National Institute Standard and Technology (NSIT) containing over 62,000 patterns. The spectrum of the unknown sample was compared with that of the known components present in the library. Molecular weight, name and the structural formula of the unknown sample were then ascertained.

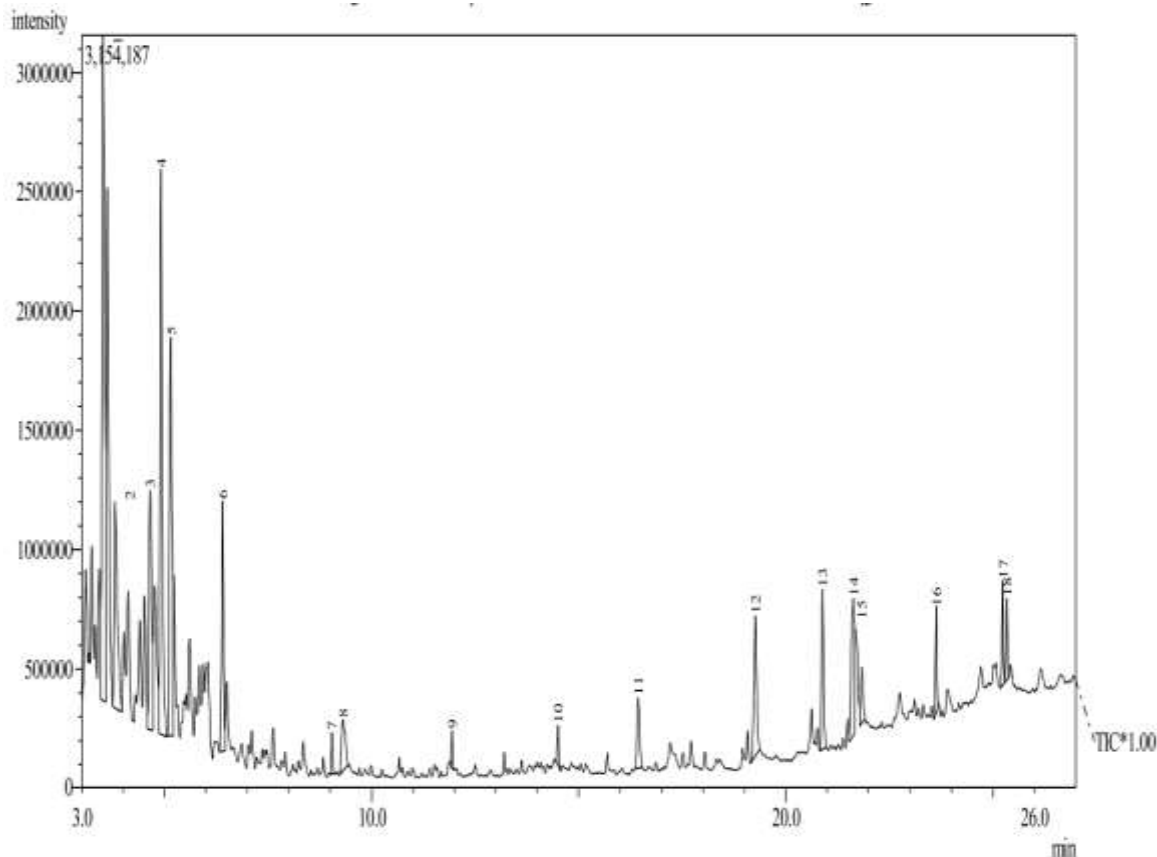


Figure 1. Chromatogram of ethyl acetate fraction of *Vernonia calvoana*.

### *In vitro* antioxidant estimation

#### Estimation of antioxidant capacity using the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) technique

Standard method according to Mensor et al. (2001) was employed in elucidation of scavenging activity of extracts of *Vernonia calvoana*. Two mls of the extract at different concentration ranges 10 to 400  $\mu\text{g/ml}$  was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance was measured at 517 nm after 30 min incubation for 30 min in the dark at room temperature. Methanol (1.0 ml) mixed with 2.0 ml of the sample was used as blank while ascorbic acid was used as standard. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ Antioxidant Activity [AA]} = 100 - \left[ \frac{(\text{Abs sample} - \text{Abs blank}) \times 100}{\text{Abs control}} \right]$$

### Ferric reducing antioxidant power (FRAP) assay

The scavenging capacities of extract were elucidated using FRAP assay method according to Benzie and Strain (1999) with modification by Udeh et al. (2011). "FRAP assay determines the change in blue colored  $\text{Fe}^{\text{II}}$ -triipyridyltriazine compound at absorbance of 593 nm from a colorless oxidized  $\text{Fe}^{\text{III}}$  by the reaction of antioxidants. Standard curve was prepared using different concentrations (100 to 1000  $\mu\text{mol/L}$ ) of  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ . Solution (working solution) for the assay was freshly prepared by mixing 2 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . The temperature of the solution was 37°C before use. In the assay, the antioxidant capacity of the extracts was calculated with reference to the reaction signal given by a  $\text{Fe}^{2+}$  solution of known concentration. Ascorbic acid was measured within 30 min after preparation, and calculations were made using the formula.

$$\text{FRAP value } (\mu\text{M}) = \frac{\text{Changes in absorbance from 0 - 4 min} \times \text{FRAP value OF Std (1000}\mu\text{M)}}{\text{Changes in absorbance of STD 0 min - 4 min}}$$

### Statistical analysis

The statistical analysis was carried out using SPSS (21.0) and results were presented as mean  $\pm$  SD. The degree of freedom was determined using student t-test and values of  $P < 0.05$  are considered significant.

## RESULTS AND DISCUSSION

The results of the phytochemical screening of the ethyl acetate fraction of *V. calvoana* leaves is presented in Figure 1 and Table 1 present the chromatogram and

**Table 1.** Various Phytochemical compounds identified in the ethyl acetate leaf extract of *Vernonia calvoana* by GC-MS analysis.

RT	Name of compound	Molecular formula	Molecular weight	Peak area %
3.516	Etylo benzene	C <sub>8</sub> H <sub>10</sub>	106	22.13
3.810	Isopropylidenecyclopentadiene	C <sub>8</sub> H <sub>10</sub>	106	8.24
4.654	1-ethyl-2-methyl benzene	C <sub>9</sub> H <sub>12</sub>	120	8.64
4.917	Decane	C <sub>10</sub> H <sub>22</sub>	142	15.24
5.147	1,2,3-trimethyl benzene	C <sub>9</sub> H <sub>12</sub>	120	12.51
6.403	2,7-Dimethyl Octane	C <sub>10</sub> H <sub>22</sub>	142	5.79
9.046	4-methyltriDecane	C <sub>14</sub> H <sub>30</sub>	198	0.72
9.311	2-furanoMethanol	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	102	2.44
11.943	3,3-DimethylHexane	C <sub>8</sub> H <sub>18</sub>	114	0.52
14.502	5-methyl Undecane	C <sub>12</sub> H <sub>26</sub>	170	0.74
16.437	1-Nonyne	C <sub>9</sub> H <sub>16</sub>	124	2.16
19.265	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	4.70
20.885	Phytol	C <sub>20</sub> H <sub>40</sub> O	296	4.00
21.630	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	5.49
21.835	n-Nona Decanoic acid	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	1.53
23.630	Oxalic acid	C <sub>14</sub> H <sub>26</sub> O <sub>4</sub>	258	1.84
25.235	5,8- Octadecadienoic acid	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	1.79
25.336	9,12,15-Octadecatrienal	C <sub>18</sub> H <sub>30</sub> O	262	1.53

RT= Retention time.

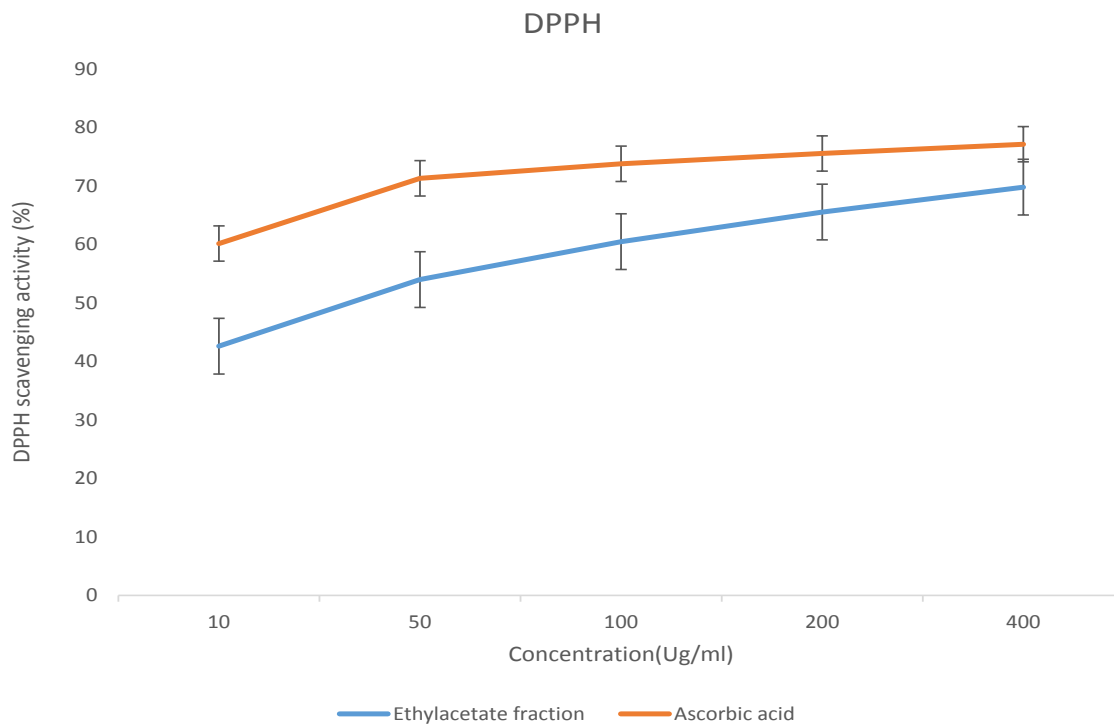
identified compounds of ethyl acetate of *V. calvoana*, their names, percentage peak area, molecular weight, retention time, and molecular formula. The results indicated the presence of eighteen compounds in ethyl acetate fraction of *V. calvoana* leaves. Etylobenzene was observed to be highest present, followed by decane, 1,2,3-trimethylbenzene, 1-ethyl-2-methylbenzene and isopropylidenecyclopentadiene, other compounds were found to be present in trace concentrations.

Oleic (linolenic acid -5.69%) acid and hexadecanoic acid (palmitic acid -4.70%) have been reported to have hypocholesterolemic, antioxidant and lubricating activity (Selvamangai and Bhaska, 2012). Oleic acid is commonly found in diet. It is a monounsaturated fat which on consumption has been linked with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol (Teres et al., 2008). Palmitaldehyde which is a derivative of palmitic acid has been reported to function in the stabilization of human erythrocyte membrane (Zavodnik et al., 1996).

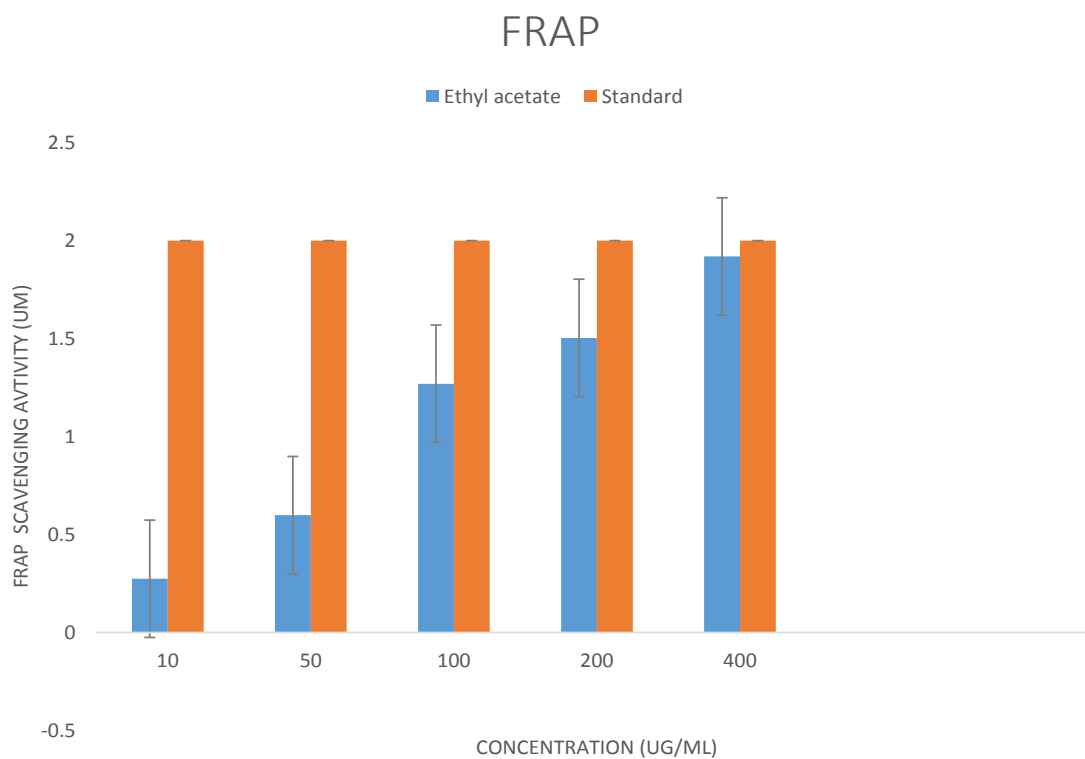
Assay for free radical scavenging activity falls among the most widely used methods in establishing activity of medicinal plant extract and their bioactive components. DPPH has the ability to remove labile hydrogen, and the capacity to scavenge the DPPH radical correlates the inhibition of lipid peroxidation (Matsubara et al., 1999). DPPH radical has odd electron, which is responsible for the deep purple coloration observed when it accepts an electron donated by an antioxidant. From Figure 2, it was

observed that, the extract inhibited DPPH activity in a dose-dependent manner, with significant potency observed in 400 ug/ml concentration which compared closely to ascorbic acid. Data from the study clearly shows that *V.C* leaves has high electron/hydrogen-donating secondary metabolites and may function as active antioxidants; "Least amount of activity in the least polar extract and highest amount of activity in the most polar extract" (Atangwho et al., 2013). Although, ethyl acetate is known to be a moderately polar solvent, relatively non-toxic and volatile chemical, it was noted that the antioxidant activity was dose dependent which may further be attributed to the polarity of the extraction solvent. The observation is in agreement with the report of Oboh et al. (2008), which showed that the polar extracts of leafy vegetables are better DPPH scavengers than the non-polar extract.

The ability of *V. calvoana* extract to reduce ferric ions was estimated using FRAP assay (Figure 3). The change in color was from colorless oxidized Fe<sup>+3</sup> in absorbance at 593 nm owing to the formation of blue colored Fe<sup>+2</sup>-tripirydyltriazine (TPTZ) by the action of electron donating antioxidant. The FRAP values of extract was found to be higher as compared to ascorbic acid (standards) (Figure 3). This observed activity was dose dependent with significant (P<0.05) activity observed at does level of 400 ug/ml. Findings from this study is consistent with earlier report by Egbung et al. (2016), indicating that the inflorescences from *V. calvoana* possess a high antioxidant activity that is comparable to



**Figure 2.** DPPH scavenging activity of ethyle acetate fraction of V.C leaves compared to the standard antioxidants ascorbic acid. Value expressed as mean $\pm$  SD (n=3), where determination were in triplicate.



**Figure 3.** Free radical reducing potential (FRAP) of ethyl acetate V.C extracts compared to the standard antioxidants ascorbic acid. Value expressed as mean $\pm$  SD (n=3), where determination were in triplicate.

that of ascorbic acid.

## Conclusion

In this study, the phytochemical constituents present in ethyl acetate fraction of *V. calvoana* and their ability to function as an antioxidant in neutralizing DPPH and FRAP, producing free radical into its non-reactive forms was investigated. Data from the study clearly shows that *V. calvoana* leaves has high electron/hydrogen-donating secondary metabolites and may function as active antioxidants. Thus, these findings further suggest that this plant is a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing down oxidative stress related degenerative diseases, such as cancer and various other human ailments. However, more studies are needed for the isolation and characterization of this active biocomponents and also further biochemical studies are needed for understanding their possible mechanism of action as better antioxidants.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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