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(RESEARCH ARTICLE)



## *In vitro* antioxidant analysis and quantitative determination of phenolic and flavonoid contents of *Emilia sonchifolia* (L) D.C (Asteraceae) leaf extract and fractions

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### Abstract

*Emilia sonchifolia* (L.) D.C. is a medicinal plant from the family Asteraceae known for a wide range of ethnomedicinal uses such as management of inflammatory diseases, pains, cancer, diabetes, cataract, asthma and liver disease. This research was aimed at assessing the antioxidant potential and quantitative determination of phenolic and flavonoid contents of the extract and fractions of *Emilia sonchifolia* leaves. The leaves of the plant were processed, extracted and partitioned successively and exhaustively with dichloromethane (DCM) and ethyl acetate. Phytochemical screening was carried out on the methanol extract using standard methods. Antioxidant evaluation of the methanol extract and fractions of *Emilia sonchifolia* leaves was carried out using Ferric Reducing Antioxidant Power (FRAP) assay and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Quantitative determination of total phenolic content and total flavonoid content was done. The extraction and partitioning yielded the crude extract and respective fractions. The phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids and cardiac glycosides. Antioxidant evaluation of the crude extract and fractions of the plant exhibited significant ( $p < 0.001$ ) antioxidant activity in both assay though not comparable with the standard agent, ascorbic acid. In DPPH assay, the highest percentage inhibition was observed in ethyl acetate fraction (62%) followed by the extract (61%), DCM fraction (55%) and aqueous fractions (54%) at 100  $\mu\text{g/mL}$ . In FRAP assay, the highest reducing power was exhibited by ethyl acetate fraction (0.457 nm) followed by the extract (0.444 nm), DCM fraction (0.441 nm) and aqueous fraction (0.439 nm). Although these activities were significant, they were not comparable to that of the standard drug ascorbic acid (0.914 nm). In total phenolic content assay ethyl acetate fraction had the highest phenolic content (5.804 mg/g), followed by crude extract (2.500 mg/g). The total flavonoid content was observed to be highest in ethyl acetate fraction (10.556 mg/g), followed by crude extract (4.444 mg/g). From this research work it was observed that *Emilia sonchifolia* leaves have a good antioxidant activity which could be responsible for its wide range of ethnomedicinal activities and this lends scientific credence for the use of this plant in the management of disease conditions.

**Keywords:** Antioxidant; Flavonoids; Phenolic Asteraceae; *Emilia sonchifolia*

### 1. Introduction

The use of medicinal plants to manage various ailments is gaining wide acceptance in both developed and developing countries. This is as a result of the abundance and ease of availability of these natural products. Several studies have shown that plants provide a good source of remedy against several diseases and ailments [1]. The use of herbal medicine as a primary source for healthcare is highest in the developing countries, which constitute about 80% of the world population [2]. Plants still remain the basis for development of modern drugs hence complementary science has acknowledged the actions of plants that are included in modern pharmacotherapy [3].

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Natural antioxidants occurring in plants can protect the human body from free radicals and retard the lipid oxidative rancidity in foods [4]. Recent studies have investigated the potential of employing plant products as antioxidants against various diseases induced by free radicals [5].

It has been determined that the antioxidant effect of plants products is mainly attributed to phenolic acids and flavonoids [6]. The medicinal plants that possess antioxidant activities are screened by means of various *in vitro* assays like nitric oxide scavenging assay, DPPH radical scavenging assay, total phenolic content, reducing power assay; and via various *in vivo* animal models using rats and mice. Some common antioxidant plants include: *Moringa oleifera* Lam. (Moringaceae), *Prosopis cineraria* (L.) Druce (Fabaceae) and *Aspergillus candidus* Link (Trichocomaceae), among others. Plants with antioxidant activity are rich in polyphenols, flavonoids, Beta Carotene, Lutein, Lycopene, Vitamin C, Vitamin A and Vitamin E [7].

*Emilia sonchifolia*, a member of Asteraceae family commonly named liliac tassel flower is a shrub with sparingly hairy soft stem and usually grows to about 20-70 cm with a branched tap root. The plant occurs frequently as a weed in grassy places by roadsides and thick forest [8]. *Emilia sonchifolia* occurs in the tropics and sub-tropics.



**Figure 1** *Emilia sonchifolia* plant in its natural habitat

Source: Field data

The herbs *Emilia sonchifolia* are used in folklore medicine for the treatment of tumor, inflammation, cough, rheumatism, cuts and wounds. The aerial parts of the plant have been reported to contain alkaloids, flavonoids and terpenes [9].

The plant has been documented in ethno medicine to have medical benefits in treating diarrhoea, night blindness, sore throat, chest pain, liver disease, eye inflammation, stomach tumor, rashes, measles, ear ache and asthma. *Emilia sonchifolia* is also used by traditional midwives and other birth attendants in Africa during child delivery. Africans usually eat the leaves as vegetables for its laxative property and it has been documented in the Nigerian folk medicine for the treatment of epilepsy in infants [8].

A study carried out on the plants showed that the aqueous leaf extract of *Emilia sonchifolia* has anti-inflammatory and analgesic activity.[10].

The anti-diabetic activity of the aqueous extract of *Emilia sonchifolia* was assessed and the results demonstrated that the aqueous extract could decrease the blood glucose level and also exhibits hepato protective effects in experimental diabetic rats [11] The anticonvulsant activity of the ethanol, chloroform and aqueous extracts of the plants in chicks and mice through maximal electro shock and strychnine induced seizures has been reported [12]

The above mentioned pharmacological studies and more indicate that the plant possesses notable biological activities such as analgesic, anti-inflammatory, antioxidant, anti-diarrheal anti diabetic, hepato protective, anti-anxiety, anti-convulsion and anti-cataract [8]. However, detail antioxidant and quantitative evaluations involving the fractions of the plant leaves have not been reported. Hence, this research targets the antioxidant evaluation and quantitative determinations of the extract and fractions of *Emilia sonchifolia* leaves.

## 2. Material and methods

### 2.1. Collection and identification of plant

The leaves of *Emilia sonchifolia* were collected from Obio Offot Village in Uyo Local Government Area of Akwa Ibom State, Nigeria in the month of July, 2018. The plant sample was identified and authenticated by Professor Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo and the voucher specimen number was UUH 3812.

### 2.2. Preparation and Partitioning of the Extract

*Emilia sonchifolia* leaves were washed, air-dried and pulverized. The powdered plant material was weighed (580 g) and extracted with methanol by maceration method at room temperature (26°C-33°C) for 72 hours with intermittent shaking. After 72 hours the liquid extract was filtered and concentrated in vacuo at 30°C. The dried extract obtained was weighed and stored in a refrigerator at -4°C for further studies. Part of the extract (27 g) was dispersed in 200 mL of distilled water and partitioned successively and exhaustively with dichloromethane, and ethyl acetate using separating funnel (Pyrex, England) to obtain their respective fractions. The fractions were concentrated in vacuo at 30°C to dryness.

### 2.3. Phytochemical Screening

The qualitative phytochemical screening was carried out on the methanol extract of *Emilia sonchifolia* leaves according to standard methods [13],[14].

### 2.4. Antioxidant Evaluation

The antioxidant evaluation was carried out using Ferric ion Reducing Antioxidant Power (FRAP) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

### 2.5. Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH free radical scavenging activity of *Emilia sonchifolia* extract, fractions and ascorbic acid prepared in methanol at various concentrations (20, 40, 60, 80 and 100 µg/mL) were evaluated according to the method of [15]. DPPH (0.1 M, 1 mL) was added to 3 mL of the solutions prepared with extract, fractions and ascorbic acid and stirred for 1 minute, each mixture was incubated in the dark for 30 minutes and the absorbance was measured at 517 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard error of mean. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The percentage DPPH scavenging effect was calculated using the following equations:

$$\text{DPPH percentage scavenging effect} = [(A_0 - A_s) / A_0] \times 100$$

Where  $A_0$  is the absorbance of control reaction, and  $A_s$  is the absorbance of the test sample or standard sample (ascorbic acid) [15].

### 2.6. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power activity of the plant was determined using the method of Oyaizu [16]. Various concentrations (20, 40, 60, 80, 100 µg/mL) of extract, fractions and ascorbic acid (2 mL) were mixed individually with the mixture containing 2 mL of 0.2 M sodium phosphate buffer (pH 6.6.) and 2 mL of 1% w/v potassium ferricyanide ( $K_3Fe(CN)_6$ ). The mixture obtained was incubated at 50°C for 20 minutes then 2 mL of trichloroacetic acid (10% w/v) was added and the mixture was centrifuged at 650 rpm for 10 minutes. The upper layer of the solution 5 mL was mixed with 5 mL of distilled water and 1 mL of ferric chloride (0.1% w/v).

### 2.7. Quantitative Assay

The quantitative evaluation of the extract and fractions of the plant sample was carried out using total phenolic and total flavonoid content determination.

## 2.8. Determination of Total Phenolic Content

The total phenolic contents determination of the extract and fractions of the plant were determined spectrophotometrically with Folin-Ciocalteu reagent (Sigma-Aldrich) according to the method of Kaur and Kapoor [17].

Gallic acid (5 mg) was dissolved in 5 mL of distilled water. Serial dilution was done to obtain a concentration of 0.1-0.5 mg/mL of gallic acid for developing a standard curve for total phenolic content determination. The extract, fractions and gallic acid (0.5 mL; 1 mg/mL) were mixed individually with 2.5 mL of 10% Folin-Ciocalteu reagent (Sigma-Aldrich) and 2 mL of Na<sub>2</sub>CO<sub>3</sub> (7%). The resulting mixture was then vortexed for 15 seconds and incubated at 40°C for 30 minutes in a water bath for colour development. The absorbance of the samples and gallic acid was measured at the wavelength of 765 nm. A standard calibration curve was plotted using the mean absorbance of gallic acid. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per gram dry weight. These were performed in triplicates [17].

## 2.9. Determination of Total Flavonoid Content

The total flavonoids content was estimated using the procedure described by [18]. The stock solution (1 mg/mL) of the plant extract and fractions were prepared, and 1 mL of each sample was diluted with 200 µL of distilled water followed by the addition of 150 µL of sodium nitrate solution (5%). The mixture was incubated for 5 min and 150 µL of aluminium chloride solution (10%) was added and allowed to stand for 6 minutes. Then 2 mL of sodium hydroxide solution (4%) was added and the mixture made up to 5mL with distilled water. The mixture was shaken well and left to stand for 15 minutes at room temperature. The procedure was carried out in triplicate and absorbance was measured at 570 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as milligram rutin equivalent (mg RE/g) of extract and fractions on a dry weight basis using the standard curve.

## 2.10. Statistical Analysis

Data were expressed as mean ± SEM and were analyzed using two-way analysis of variance (ANOVA), followed by Bonferroni post-tests. Values of  $p < 0.001$  were considered significant.

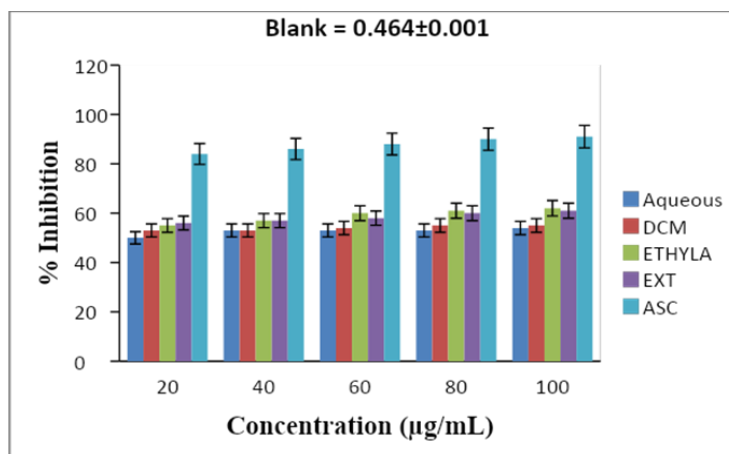
## 3. Results and discussion

### 3.1. Phytochemical Screening

The phytochemical screening revealed the presence of tannins, flavonoids, saponins, alkaloids and cardiac glycosides, while anthraquinone was absent.

**Table 1** DPPH Radical Scavenging Activity of *Emilia sonchifolia* Leaves Crude Extract and Fraction. Blank = 0.464±0.001

Conc. µg/mL	Mean Absorbance ± SEM				
	Extract	Dichloromethane	Ethyl acetate	Aqueous	Ascorbic Acid
20	0.204±0.001 (56)	0.218±0.001 (53)	0.208±0.002 (55)	0.223±0.001 (50)	0.079±0.001 (84)
40	0.199±0.002 (57)	0.217±0.001 (53)	0.198±0.001 (57)	0.220±0.002 (53)	0.067±0.001 (86)
60	0.199±0.001 (58)	0.213±0.001 (54)	0.187±0.001 (60)	0.217±0.001 (53)	0.056±0.002 (88)
80	0.187±0.001 (60)	0.211±0.001 (55)	0.181±0.000 (61)	0.216±0.001 (53)	0.048±0.002 (90)
100	0.184±0.002 (61)	0.210±0.001 (55)	0.178±0.001 (62)	0.213±0.002 (54)	0.046±0.001 (91)

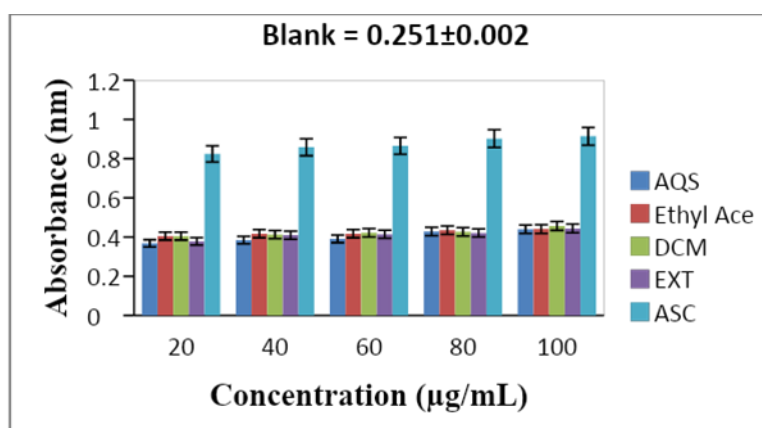


**Figure 2** Bar chart of DPPH Scavenging Activity of *Emilia sonchifolia* Leaves Crude extract and Fractions

**Table 2** FRAP assay of *Emilia sonchifolia* Leaves Crude extracts and Fraction Blank = 0.251±0.002

Conc. µg/mL	Mean Absorbance ± SEM				
	Extract	Ethyl acetate	Dichloromethane	Aqueous	Ascorbic Acid
20	0.378±0.002	0.405±0.0001	0.405±0.001	0.368±0.000	0.824±0.002
40	0.410±0.001	0.417±0.001	0.413±0.000	0.385±0.001	0.858±0.001
60	0.414±0.001	0.417±0.001	0.422±0.001	0.391±0.001	0.865±0.001
80	0.421±0.001	0.435±0.001	0.427±0.001	0.429±0.001	0.902±0.001
100	0.444±0.001	0.457±0.002	0.441±0.001	0.439±0.001	0.914±0.001

Values are expressed as mean ± SEM (n = 3)  
Significance relative to blank; \*P < 0.001

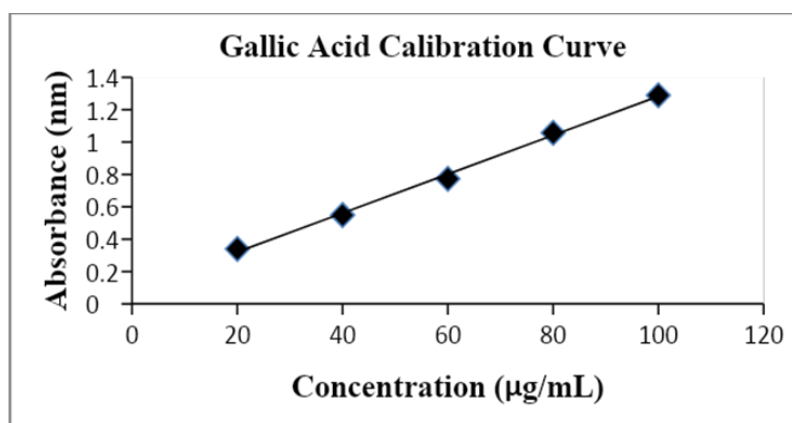


**Figure 3** Bar chart of ferric ion reducing antioxidant power activity of *Emilia sonchifolia* leaves extract and fractions

**Table 3** The Absorbance of Gallic Acid for Standard Calibration Curve

Conc. µg/mL	Mean ABS±SEM
20	0.340±0.001
40	0.549±0.001
60	0.773±0.002
80	1.057 ±0.000
100	1.289±0.001

ABS = Absorbance; Values are expressed as mean ± SEM

**Figure 4** Standard Calibration Curve for Total Phenolic Content As**Table 4** Total Phenolic Content of *Emilia sonchifolia* Leaves Crude extract and Fractions

Conc. 1 mg/MI	ABS 1	ABS 2	ABS 3	Mean±SEM	Total Phenolic Content (x) mg/g
Extract	0.098	0.098	0.098	0.098±0.001	2.500
DCM	0.075	0.076	0.076	0.076±0.002	0.536
Ethyl Acetate	0.134	0.134	0.136	0.135±0.001	5.804
Aqueous	0.061	0.061	0.061	0.061±0.001	-0.804

ABS – Absorbance; DCM – dichloromethane;  $Y = 0.0112x + 0.070$  (from the graph);  $X = Y - 0.070/0.0112$ ; When  $Y = 0.098$ ;  $X = 2.500$  (crude extract);  $Y = 0.076$ ;  $X = 0.446$  (dichloromethane fraction);  $Y = 0.135$ ;  $X = 5.804$  (ethyl acetate fraction);  $Y = 0.061$ ;  $X = -0.804$  (aqueous fraction)

**Table 5** The Absorbance of Rutin for Standard Calibration Curve

Conc. µg/mL	Mean ABS±SEM
20	0.094±0.001
40	0.123±0.001
60	0.169±0.002
80	0.204±0.000
100	0.230±0.001

Key: ABS = Absorbance  
Values are expressed as Mean ± standard Error of Mean

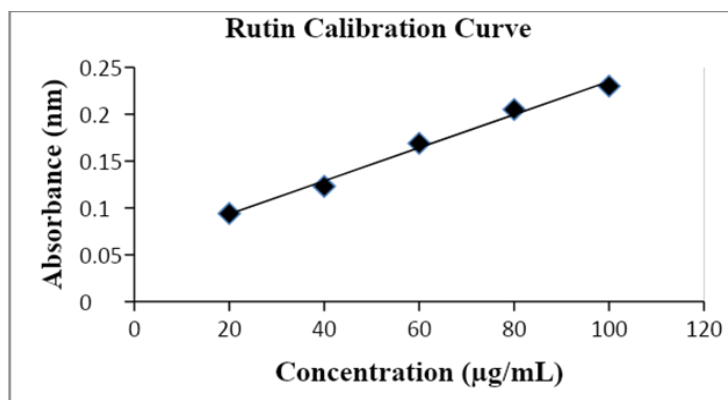


Figure 6 Standard Calibration Curve for Total Flavonoid

Table 6 Total Flavonoid Content of *Emilia sonchifolia* Leaves Crude extract and Fractions

Conc. 1mg/mL	ABS 1	ABS 2	ABS 3	Mean±SEM	Total Flavonoid Content (x) mg/g
Extract	0.073	0.073	0.074	0.073±0.001	4.444
Dichloromethane	0.068	0.068	0.069	0.068±0.001	1.667
Ethyl Acetate	0.084	0.083	0.085	0.084±0.001	10.556
Aqueous	0.037	0.038	0.039	0.038±0.002	-15.555

ABS = Absorbance;  $Y = 0.0018x + 0.065$  (from the graph);  $X = Y - 0.065 / 0.0018$ ; When  $Y = 0.073$ ;  $X = 4.444$  (Crude Extract);  $Y = 0.068$ ;  $X = 1.667$  (Dichloromethane fraction);  $Y = 0.084$ ;  $X = 10.556$  (Ethyl Acetate fraction);  $Y = 0.037$ ;  $X = -15.555$  (Aqueous fraction)

#### 4. Discussion

Antioxidant evaluation of the extract and fractions of *Emilia sonchifolia* leaves revealed significant ( $p < 0.001$ ) antioxidant activity in both assays, though the fractions and extract had lower antioxidant activity compared to the standard drug ascorbic acid. The reduction of the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is followed by its passage from purple colour radical (DPPH) to yellow colour (DPPH-H). This reduction in capacity is determined by a decrease in absorbance induced by the antioxidant substance [19]. Therefore, decreased absorbance indicates increase in free radical scavenging activity [20]. The extract and fractions were found to possess concentration-dependent inhibitory activity against the DPPH radical as shown in table 1 and figure 1. In DPPH assay ascorbic acid exhibited the highest antioxidant activity (91%) followed by ethyl acetate fraction (62%), extract (61%), dichloromethane fraction (55%) and aqueous fraction (54%) at 100 µg/ml. Activity increased as the concentration increased because lower absorbance indicates higher antioxidant capacity.

In the ferric reducing antioxidant power (FRAP) assay, ferric ions ( $Fe^{3+}$ ) are reduced to ferrous ions ( $Fe^{2+}$ ) in the presence of antioxidants agent [21]. The reducing capacity occurs in a concentration dependent manner, where yellow colour changes to pale green and blue colour depending on the concentration of the antioxidants in the samples [22]. In FRAP assay the highest reducing power was exhibited by ascorbic acid which is the standard (0.914 nm) followed by ethyl acetate fraction (0.457 nm), extract (0.444 nm), DCM fraction (0.441 nm) and aqueous fraction (0.439 nm) as shown in table 2 and figure 2. Activity was observed to increase proportionately as the absorbance increased due to increase in concentration. Higher absorbance implies higher activity.

In total phenolic assay ethyl acetate fraction had the highest phenolic content (5.804 mg/g), followed by extract (2.500 mg/g) then DCM fraction (0.446 mg/g); however aqueous fraction (-0.804 mg/g) was devoid of phenolic content (table 4). While total flavonoid content was also observed to be highest in ethyl acetate fraction (10.556 mg/g), followed by extract (4.444 mg/g), DCM fraction (1.667 mg/g); however aqueous fraction (-15.555 mg/g) was devoid of flavonoid content (table 6).

In both DPPH and FRAP assay ethyl acetate fraction exhibited higher antioxidant activity than other fractions and the extract. This implies that purification enhances activity; hence ethyl acetate fraction had improved activity over the extract. This also means that most of the secondary metabolites with antioxidant potentials such as flavonoids and tannins in the extract were distributed into the ethyl acetate fraction. This correlates with ethyl acetate fraction having more phenolic and flavonoids content as shown in table 4 and table 6.

Phytochemical screening revealed the presence of saponins, tannins, alkaloids, cardiac glycosides and Flavonoids. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. Flavonoids and tannins are known to exhibit a wide range of biological activity partly due to their antioxidant effect. These phytochemicals no doubt contribute to the high antioxidant potential of the extract and ethyl acetate fraction as well as other pharmacological activity of *Emilia sonchifolia* leaves.

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## 5. Conclusion

From the research carried out it can be concluded that *Emilia sonchifolia* leaves exhibit significant antioxidant activity which could be due to the presence of the phytochemical constituents such as flavonoids and tannins. The antioxidant property of this plant due to polyphenolic content supports the ethnomedicinal use of this plant in the management of many disease conditions.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

There is no conflict of interest among all authors.

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