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Research Article

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In vitro antioxidant properties of the methanol extracts of the whole plant and fruit of *Momordica foetida* (Cucurbitaceae)

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Abstract *Momordica foetida (*Cucurbitaceae) is a plant mainly used in Cameroon's folk medicine for management of many diseases including malaria, cancers and diabetes. This study aimed at evaluating the antioxidant content and activity of the methanol extracts of the whole plant and fruit of *M. foetida*. The results revealed the presence of antioxidants in whole plant (MEMfP) and fruit (MEMfF) extracts of *M. foetida*. Both extracts had comparable amount of flavonoids (19.160 ± 1.589 µg QE/ g of extract vs. 22.121 ± 4.044 µg QE/ g of extract) and vitamin C (1.586 ± 0.320 µg/ g of extract vs. 1.133 ± 0.320 µg/ g of extract), but MEMfP contained higher phenolic compounds than the fruit portion (27.648 ± 0.558 µg GAE/g of extract vs. 8.220 ± 0.472 µg GAE/g of extract). The MEMfP showed higher inhibition of lipid peroxidation (IC_{50} = 1.275 ± 0.007 µg/mL vs. 2.135 ± 0.106 µg/mL), reduction of ferric ions (EC_{50} = 1.160 ± 0.010 µg/mL vs. 1.610 ± 0.020 µg/mL) and stimulation of catalase activity than MEMfF. Conclusively, both whole plant and fruit methanol extracts of *M. foetida* displayed antioxidant content and activity with the whole plant extract having higher antioxidant properties. These results support the use of *M. foetida* in the management of antioxidant linked diseases such as infectious and metabolic diseases.

Keyword: antioxidant property, fruit, *Momordica foetida*, oxidative stress, whole plant

1. Introduction

Oxidative stress is a significant contributing factor for unset and development of many diseases affecting humans including atherosclerosis, diabetes mellitus, cardiovascular disease, cancer, neurodegenerative disorders and infertility [1,2]. Oxidative stress can be defined as disruption of the homeostasis between oxidants and antioxidants in favor of the oxidants, and this imbalance may lead to oxidation of biological molecules by the highly reactive oxidants. Oxidation of key cell components such as lipids, proteins, and DNA is detrimental, and the cell thus needs adequate amounts of the antioxidant molecules to prevent of alleviate this adverse effect [3]. Indeed, cellular antioxidants are compound that can delay or inhibit the oxidation of biomolecules. Antioxidants include enzymes (superoxide dismutase, glutathione peroxidase and catalase) and naturally occurring compounds (vitamins vitamin C and E, and phytochemical antioxidants such as polyphenols and carotenoids) [1,4]. Antioxidants reduce or inactivate reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals hydroxyl radical and

non free-radical species such as H_2O_2 and singled oxygen [5,6]. Reactive oxygen species are exacerbating factors in different cellular dysfunctions and aging process [4].

One of the majors sources of antioxidants are plants which largely available in sub-equatorial countries including Cameroon. Plants are rich in natural antioxidants such as phenolics, tocopherols, carotenoids, ascorbic acid, flavonoids and tannins [6,7]. *Momordica foetida* is a perennial climbing vine medicinal plant from Cucurbitaceae family, native to tropical Africa. The plant is used traditionally against various ailments including malaria, stomachache, nose bleeding, and diabetes as well as treat snakebites [8]. Moreover pharmacological study on *M. foetida* showed potential blood glucose lowering effects [9].

Previous studies have shown the ability of *M. foetida* leaf extracts to inhibit plasma lipid peroxidation, scavenge superoxide anion and nitric oxide radical, and reduce ferric [10,11]. However in Cameroon different parts of *M. foetida* and even the whole plant are commonly extracted and used for various treatments [12,13]. Also the ability of given medicinal plant to exhibit antioxidant potential may vary with the methods used for extraction of the plant material [14]. This study aimed to evaluate the antioxidant content and the *in vitro* antioxidant activity of the methanol extracts of *M. foetida* whole plant and fruit through determination of antioxidant compounds (total phenols, flavonoids and vitamin C), inhibition of lipid peroxidation, ferric scavenging potential and catalase activity.

2. Materials and Methods

2.1. Materials

2.1.1. Collection of the plant material and preparation of the methanol extracts

The plant *Momordica foetida* (Cucurbitaceae) and fresh fruits were harvested in Awai (Mfoundi division, Cameroon) in August 2015. Sample of the plant was identified and a specimen kept at the Cameroonian National Herbarium (No 33420 HNC). The fresh plants of *M. foetida* was cleaned, dried, chopped and finely ground. The fruits were dried at room temperature and then finely crushed. Four hundred grams and 1 kg of the dry powder from the whole plant and fruits, respectively, were macerated into 2L of methanol and allowed to stand for 48 hours, and then filtered using Whatman #1 filter paper. The solvent was evaporated using a rotary evaporator under reduced pressure at 65^{o}C, and 9.57g and 95 g of whole plant and fruit extracts were obtained, respectively.

2.1.2. Animal used and liver homogenate

A 2.5 months old male Wistar albino rat weighing 150 g was obtained from the Department of Biochemistry's animal house of the University of Bamenda. The study protocol complied with ethical guidelines for handling of the animals from the Cameroon National Veterinary Laboratory. It was sacrificed and the liver used to prepare a 20% (W/V) homogenate in phosphate buffer (pH 7.4, 50 mM).

2.1.3. Chemicals

Vitamin E (α-tocopherol) and trichloroacetic acid (TCA) were obtained from Sigma (St Louis, MO, USA). Quercitin, gallic acid and thiobarbituric acid (TBA) were gotten from Griffin and George (Wembly Middlesex, England). Methanol was purchased from Loba Chemie Pvt. Ltd.107, Woodehouse (Mumbai, India). Folin Ciocalteu's (FC) reagent was procured from Qualigens Fine Chemicals (Bombay, India). Other chemicals were of high quality grade.

2.2. Methods

2.2.1. Quantification of the total phenolic compounds

The total phenolic content of the methanol extract of *M. foetida* plant or fruits was determined by Folin Ciocalteu's method as described by Nantia et al. [15] with slight modification. A volume (0.1 mL) of plant extract was pipetted into different test tubes and 0.9 mL of Folin Ciocalteu reagent was added. After homogenization the mixture was incubated (at room temperature) for 10 min, and 1 mL of 20% Na_2CO_3 and 2.6 mL of distilled were added. The mixture was homogenized and incubated for 2 hours for colour development. The absorbance was read at 750 nm using a UV-visible spectrophotometer. The total phenolic content of the extract was expressed as mg of gallic acid

equivalent per mass of extract (mg GAE/ 100 g of extract) using standard gallic acid calibration graph (concentrations: 2.0 to 22.0 µg/mL).

2.2.2. Quantification of flavonoids

The total flavonoid content was determined in the methanol extract of *M. foetida* plant or fruits by the aluminum chloride colorimetric assay as described by Nantia et al. [15] using quercetin as the standard. Briefly, 0.1 ml of plant extract and o.1 mL of 1 M potassium acetate were introduced successively into tubes, and 1.1 mL de methanol was added. The mixture was homogenized and incubated at room temperature for 5 min. Then, 0.1 mL of 10% aluminum chloride and 2.1ml of distilled water were added. The mixture was homogenized, incubated for 15 min at room temperature for color development and absorbance was measured at 415 nm using a UV – visible spectrophotometer. The total flavonoids content of samples were expressed as µg of quercetin equivalents per mass of extract (µg QE/ 100 g of extract) from quercetin calibration curve (concentrations: 0.3 to 3.4 µg/mL).

2.2.3. Estimation of vitamin C

The total vitamin C content in the extracts from *M. foetida* was determined using redox titration by iodine as reported previously [16]. Summarily, 16 mL of distilled water and 5 drops of starch solution (0.5%) were added into a conical flask containing 4 mL of plant extract. The mixture was homogenized, and thereafter titrated with 5 mM iodine solution until appearance of a permanent blue- black colouration. The titer volume of iodine and the concentration of vitamin C standard (5 mg/mL) were used to calculate vitamin C concentration in the analyzed plant extracts.

2.2.4. Antioxidant reducing power assay

The antioxidant reducing power of the methanol extract of *M. foetida* plant or fruits was evaluated using the ferric reducing antioxidant power method as described by Kamtekar et al. [17]. The standard vitamin E or methanol extracts of *M. foetida* (50 - 300 µg/mL) was introduced into the tubes, then 0.4 mL of phosphate buffer (pH 6.6, 0.2 M) and 0.4 mL of 1% potassium ferrocyanide were successively added. After homogenization, the mixture was incubated at 50^{o}C for 20 min, then cooled and centrifuged (3000 rpm, 10 min). To 1 mL of supernatant was added 1 mL of 10% TCA, 1 mL of distilled water and 0.2 mL of 0.1% $FeCl_3$. After homogenization, the absorbance was measured at 593 nm using a UV-Vis spectrophotometer.

2.2.5. Inhibition of lipid peroxidation

A volume (0.8 mL) of phosphate buffer (pH 7.4, 50 mM) and 0.1 mL of liver homogenate were added to standard vitamin E or the methanol extract of *M. foetida* plant or fruits (50 - 300 µg/mL), followed by 0.1 mL of $Fe_2(SO_4)_2$. After homogenization, the mixture was incubated at 37^{o}C for 15 min, then 1 mL of 20% TCA and 1 mL of 0.67% TBA were added. The mixture was incubated at 90^{o}C for 10 min, cooled and centrifuged (3000 rpm, 15 min). The supernatant was collected and the optical density read at 530 nm [18]. The percentage inhibition (%I) of the standard and the methanol extract of *M. foetida* plant or fruits were computed.

2.2.6. Catalase activity

Vitamin E or the methanol extract of *M. foetida* plant or fruits (50 - 300 µg/mL) was introduced into test tubes containing 0.4 mL of 9% H_2O_2 and 2.3 mL of phosphate buffer (pH 7.2, 0.1 M), and 0.4 mL of the liver homogenate was added. The absorbance was recorded for one min at 240 nm using a UV-Vis spectrophotometer. Catalase activity was expressed as IU/mg protein [19].

2.2.7. Statistical analyses

For certain parameters the fifty percent inhibitory (IC_{50}) or efficient (EC_{50}) concentration of the tested compound determined. One factor analysis of variance (ANOVA) followed by the Student-Newman-Keuls test were used to asses statistical differences between treatments. Analyses were done using Graphpad Instat software Version 3.0.

3. Results

3.1. Antioxidant content of the methanol extract of *M. foetida*

The content of phenols, flavonoids and vitamin C quantified in the methanol extracts of *M. foetida* whole plant and fruit are summarized in Table 1. The whole plant extract of *M. foetida* contained higher phenolic compounds than the fruit portion. Both plant extracts however contained comparable amount of flavonoids and vitamin C.

Table 1: Phenolic, flavonoid and vitamin C content in *M. foetida* extracts

	***M. foetida* whole plant extract**	***M. foetida* fruit extract**
Phenolic compounds (µg GAE/g of extract)	27.648 ± 0.558[a]	8.220 ± 0.472
Flavonoids (µg QE/ g of extract)	19.160 ± 1.589	22.121 ± 4.044
Vitamin C (µg/ g of extract)	1.586 ± 0.320	1.133 ± 0.320

Values are mean ± SEM of 3 independent experiments, [a] $p < 0.05$ Student-Newman-Keuls test.

3.2. Lipid peroxidation

Using liver homogenate the inhibitory concentrations fifty (IC_{50}) were obtained with *M. foetida* extracts and vitamin E (α – Tocopherol) on lipid peroxidation (Figure 1). The whole plant extract of *M. foetida* (MEMfP) showed comparable inhibitory activity with the reference molecule vitamin E (IC_{50} = 1.275 ± 0.007 µg/mL vs. 1.230 ± 0.098 µg/mL). The vitamin E and MEMfP effects on lipid peroxidation were significantly higher ($P < 0.001$) as compared to that of *M. foetida* fruit (MEMfF) extract (IC_{50} = 2.135 ± 0.106 µg/mL).

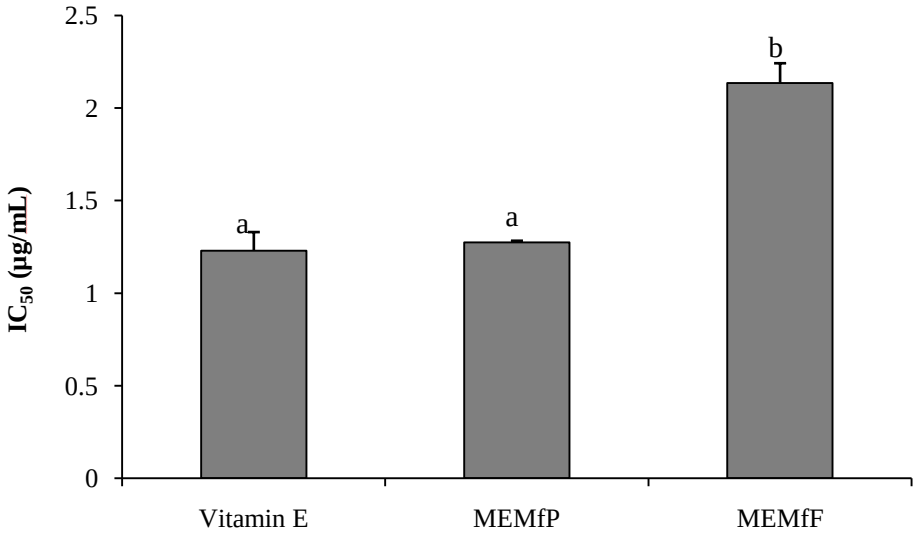

Figure 1: Inhibition of liver lipid peroxidation by vitamin E and M. foetida extracts. Data are mean ± SEM of 3 independent experiments; a, b: groups affected with the same letter do not differ significantly at p > 0.05 (Student-Newman-Keuls test). MEMfF: methanol extract of M. foetida fruit, MEMfP: methanol extract of M. foetida plant.

3.3. Antioxidant reducing power

The ferric reducing power of the *M. foetida* extracts and vitamin E is presented in Figure 2. Both the reference antioxidant compound, vitamin E and the whole plant methanol extract of *M. foetida* (MEMfP) showed comparable reducing activity (EC_{50} = 1.220 ± 0.070 µg/mL vs. 1.160 ± 0.010 µg/mL), that was significantly higher ($P < 0.001$) than that of the fruit extract of *M. foetida* (MEMfF) (EC_{50} = 1.610 ± 0.020 µg/mL).

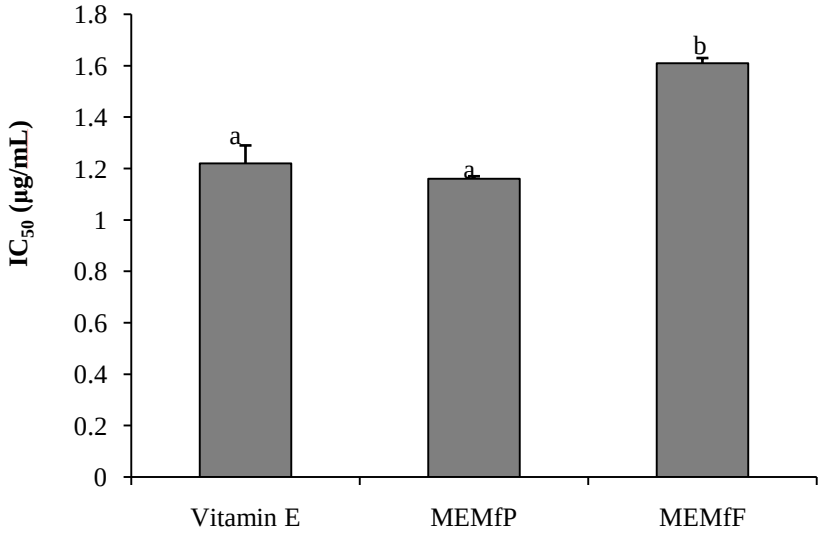

Figure 2: Ferric reducing activity of vitamin E and M. foetida extracts. Data are mean ± SEM of 3 independent experiments; a, b: groups affected with the same letter do not differ significantly at p > 0.05 (Student-Newman-Keuls test). MEMfF: methanol extract of M. foetida fruit, MEMfP: methanol extract of M. foetida plant.

3.4. Catalase activity

The catalase activity in the presence of vitamin E and *M. foetida* extracts showed a general concentration dependent trend (Figure 3). At all concentrations, the effect of the whole plant methanol extract of *M. foetida* (MEMfP) on catalase activity was comparable to that of the reference compound vitamin E. In general, the effect of the fruit extract of *M. foetida* (MEMfF) on catalase activity was lower than that of vitamin E or MEMfP.

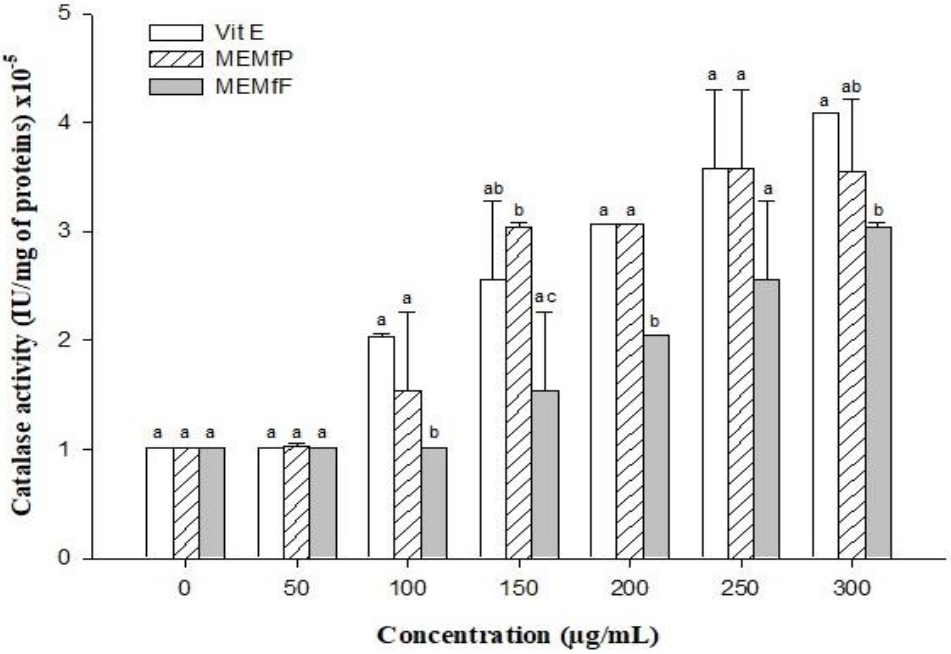

Figure 3: Catalase activity of vitamin E and M. foetida extracts. Data are mean ± SEM of 3 independent experiments; a, b, c: groups affected with the same letter do not differ significantly at p > 0.05 (Student-Newman-Keuls test). MEMfF: methanol extract of M. foetida fruit, MEMfP: methanol extract of M. foetida plant.

4. Discussion

Oxidation reactions produce free radicals or ROS that initiate multiple chain reactions with the possible ultimate outcome being damage or death of the cells In biological systems, there are two types of free radicals, namely oxygen based radicals (ROS) and nitrogen based radicals (Reactive nitrogen species, i.e., RNS). The oxygen based radicals comprise oxygen free radicals such as superoxide, hydroxyl radicals, peroxyl radicals; and non-radicals such as hypochlorous acid, hydrogen peroxide, ozone. These ROS are normally generated during metabolic process of oxygen [20]. The RNS include nitrogen based radicals and non-radicals such as nitrogen dioxide, nitric oxide radicals and peroxynitrite. The RNS are derived from nitric oxide via inducible nitric oxide synthase (iNOS), and may also occur following the action of the enzyme superoxide via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [21]. Excess free radicals in the body induces oxidative stress, a condition that has been identified as the main cause of development and progression of several diseases such as cancer, diabetes, cardiovascular diseases and neurodegenerative diseases [22,23]. It has been proven that supplementing the living system with exogenous antioxidants or stimulating the endogenous antioxidant defenses of the body enables alleviation of the oxidative damages induced by ROS. In this line plants have been shown to possess a wide variety of antioxidants capable of attenuating ROS- induced oxidative damages [6,24]. Plant derived bioactive compounds with antioxidant properties include polyphenols, flavonoids, vitamin C, etc. Favonoids comprise the most studied group of polyphenols. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. Quercetin, myricetin, catechins etc., are some most common flavonoids [24]. Polyphenols are effective ROS scavengers and metal chelators due to the presence of multiple hydroxyl groups. They therefore offer protection against development of many diseases [24-26]. Vitamin C, a soluble plant antioxidant, quickly debugs the reactive species of oxygen and nitrogen such as superoxide, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxide radicals and hypochlorous acid thereby protecting from the oxidative damage [27]. In the present study, the whole plant and fruit extracts of *M. foetida* showed different richness in antioxidants phenolic compounds, flavonoids and vitamin C with the overall high levels of antioxidants in the whole plant extract as compared to the fruit extract. The whole plant composed of all portions of the plants with the exception fruit contains the vegetable components such as leaves, stem and roots that are usually sources of antioxidants. Some studies have also shown variable antioxidant content in different part of plants [28,29].

Lipid peroxidation is a reaction in living organisms which alters the membrane permeability and causes tissue damage through free radicals. In biological environments, the most favorable substrate for peroxidation is represented by polyunsaturated fatty acids (PUFA), components of cell and subcellular membranes [26]. Free radical having deleterious effects on PUFA include superoxide anion (O_2•−), hydroperoxyl (perhydroxyl) radical (HO_2•), hydroxyl radical (•OH), nitric oxide (NO), and other species such as hydrogen peroxide (H_2O_2), and peroxynitrite (ONOO−), and metals such as iron [26,30]. The antioxidant enzymes (glutathione peroxidase, catalase, superoxide dismutase) and exogenous antioxidant compounds constitute the main defenses against free radical and excess metal ions [26]. *M. foetida* extracts showed antioxidant activity on 3 different assays used in this study. In general the whole plant extracts of *M. foetida* (MEMfP) and the reference compound, vitamin E, showed higher inhibition of lipid peroxidation, reduction of ferric ions and stimulation of catalase activity than *M. foetida* fruit (MEMfF) extract. This could be attributed to the differential richness of both extracts in antioxidants. In fact polyphenols are effective ROS scavengers and metal chelators due to the presence of multiple hydroxyl groups. The hydrogen donating ability of phenolic compounds could reduce/ inactive pro-oxidants including the reduction Fe^{3+} to Fe^{2+} [26,27]. Vitamin C serves as co-substrate to several enzymes involved in key functions of the organism. Thanks to its reversible oxidization to ascorbyl radical and then to dehydroascorbate, vitamin C can efficiently scavenge/neutralize ROS [31]. The antioxidants of the *M. foetida* may also contribute to the stimulatory activity of antioxidant enzyme such as catalase. Catalase is a tetrameric hem-containing enzyme with the potential to directly dismutate H_2O_2 into H_2O and O_2. This enzyme is indispensable for ROS detoxification during stressed conditions [32].

Other studies on *M. foetida* leaves showed that the methanol extract contained 15 ± 0.01 µM gallic acid and 4 ± 0.09 µM Catechin [10], and that the aqueous and ethanol extracts reduced ferric ions [11]. These findings sustain the antioxidants properties *M. foetida*, and the relative difference of their evaluated paramters as compared to the present study could be due to the use of whole plant extract, the extraction solvent used or the locality (region or country) of the plant. In fact the effect of factors such as extraction solvent and plant sources on biological activities, especially antioxidant properties of plant extracts have been reported in the literature [33,34].

The whole plant of *M. foetida* thanks to its high antioxidants could be considered as the appropriate source of antioxidants for this plant for further studies or any usage. However study could also assess the contribution of the fruit portion in antioxidant properties in a possible mixture of the plant extract. Notwithstanding, the noticeable antioxidant content and activity of *M. foetida* could justify the use of this plant in the treatment of many common diseases in Africa such as malaria and diabetes [8].

Summarily both whole plant and fruit methanol extracts of *M. foetida* displayed antioxidant content and activity with the whole plant extract having higher antioxidant properties. These findings support the use of *M. foetida* in the management of antioxidant linked diseases such as malaria and diabetes. Further studies will help to delineate the actual phytochemicals responsible for the antioxidant properties of *M. foetida*.

Conflict of Interest

The authors report no conflicts of interest with this study.

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