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Research Article

An investigation into the chemical composition and in vitro antioxidant activity of ethanolic extracts of *Ficus elastica* and *Mercurialis perennis leaves*

Attama Chika[1]*, Luka Lawrence[1], Yahaya Muhammad Falalu[2], Njiddah Kwaji[3], Thomas Polar[3]

[1]Department of Chemistry, Modibbo Adama University, Yola. Nigeria.

[2]Department of Petroleum Chemistry and Engineering, American University of Nigeria, Yola, Adamawa State. Nigeria.

[2]Adamawa State University, Mubi. Nigeria.

*Corresponding author's e-mail: attamachika@gmail.com

Abstract

The study explored the chemical constituents and the inhibition of free radicals potential of ethanol crude extracts from *Ficus elastica* and *Mercurialis perennis*. The Phytochemical analysis of both crude extracts shows the presence of alkaloids, terpenoids, flavonoids, tannin, proteins, reducing sugar, phytosterol, glycosides except for saponin which was present in Ficus elastica and absent in *Mercurialis perennis*. The presence of these phytochemicals are likely responsible for their curative and pharmacological relevance. Antioxidant potential of *Ficus elastica* and *Mercurialis perennis* ethanolic crude extracts were analyzed by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and Hydrogen peroxide radical scavenging methods. Essential oil of *Mercurialis perennis* compared to that of *Ficus elastica* demonstrated the best radical scavenging. The ethanolic crude extract antioxidant results obtained for the DPPH scavenging at 517 nm for the highest percentage inhibition showed that *Mercurialis perennis, Ficus elastica* and Ascorbic acid have 80.23, 79.72 and 91.24 respectively while the hydrogen peroxide percentage inhibition at 230 nm showed 90.23, 82.75 and 90.60 for *Mercurialis perennis, Ficus elastica* and Ascorbic acid respectively. On the other hand, the lowest percentage inhibition using DPPH are 55.48, 62.25 and 65.97 for *Ficus elastica, Mercurialis perennis* and Ascorbic acid respectively. While the Hydrogen peroxide percentage antioxidant activity of *Ficus elastica, Mercurialis perennis* and Ascobic acid was 60.97, 73.51 and 77.43 respectively. The plants' crude extracts showed high radical scavenging and chelating activity. The negligible reducing activity with respect to concentration values shows that these plants will serve as good and cheap source of antioxidants.

Keywords: Ficus elastic; Mercurialis perennis; phytochemical; Antioxidant.

Introduction

Resources from plants have gained wide range of applications in various industrial setups for the production of several useful products for the benefit of mankind such as perfume, food and dietary supplements, cosmetics, pharmaceutical drugs, etc. According to [1], a survey shows that 75-80% of the world's population relies over such plants as they are famous for healing several diseases and are considered as a healthy source for life. Medicinal principles are present in different parts of plants like root, stem, bark, heartwood, leaf, flower, fruits, seeds or plant exudates. Plants derived substances have obtained greater attention in the recent years to prevent and cure human diseases as they are considered to be more bio-friendly [2].

Phytochemicals are chemical compounds that occur naturally in plants. The efficiency of medicinal plants for therapeutic purposes is often based on their organic constituents such as flavonoids, tannins, alkaloids and essential oils [3]. Oxidative stress has an important role in tissue damage and leads to pathological conditions such as cancer [4]. Oxidants and free radicals are harmful for the body health when their overload cannot steadily be destroyed and consequently generate an occurrence called oxidative stress. This course of action happening due to disproportionate production of free radicals and antioxidants plays a key role in the

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formation and development of chronic diseases such as cancer, rheumatoid arthritis, cardiovascular and autoimmune disorders or even aging [5].

Ficus elastica belongs to the family *Moraceae* commonly known as the rubber fig, rubber bush, rubber tree, or Indian rubber bush. It is a widely-spread evergreen tree up to 30 m tall, having thick and dark green 7-20 cm long leaves with smooth edges and pointless tips. *Ficus elastica* Roxb and *Ficus bengalensis* Linn are anti-flammatory and analgesic [6]. According to [7], Latex used to set traps for birds, Produces gum Food for livestock Planted on boundaries. Qualitative phytochemical analysis of methanol extracts of Ficus elastica, Roxb shows the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins, saponins and terpenoids. Carbohydrates, steroids, tannins are present higher amount in the extract [8].

Some of *Ficu*s species are cultivated for their edible fruits (*Ficus sycomorus* Linn.), while others for providing shade and as ornamental plants. Many phytoconstituents including triterpenes and sterols were isolated and identified from different species of Ficus [9]. In folk medicine, Ficus plants are reported to have hypotensive and antidiabetic activities, also it is used to treat cough, chest conditions and also it is used as mild laxative, galactagogue, antirheumatic, digestive and as anthelmintic against intestinal parasites. It has been also used as anti-inflammatory in urinary tract, in sore throat, ulcerated nose, to reduce fever, to cure tuberculosis and piles. Externally, they have been used to treat postulous, eczema, to cure tinea, for leprosy, to treat cracks in the soles of the feet and dressing to boils [9].

Mercurialis is a genus of herbaceous plants belonging to the *Euphorbiaceae* family that grow in ruderal lands and grow less so in forests [10]. *Mercurialis perennis L*. is also commonly called *Mercorella bastarda* [11]. Dog's mercury, *Mercurialis perennis L. (Euphorbiaceae*), is a forest forb and a typical member of undisturbed understory communities, but it can also be found in habitats under different levels of anthropogenic disturbance. Species of the genus *Mercurialis* are known for a great variety of life histories and sexual systems, and dioecious *M. perennis* has proven to be a valuable model system for studying patterns of sex ratio variation and sexual dimorphism [12]. Dog's mercury *(Mercurialis perennis L*.) is a perennial medicinal plant, nowadays used in complementary medicine for the topical treatment of difficult healing wounds, burns, haemorrhides and against conjunctivitis [13]. Previous phytochemical studies on *M. perennis* led to the identification of piperidine alkaloids, flavoniod, glycoside, simple phenolics, terpenes, triacylglycerols, tocopherols and sterols. However, constituents from Dog's Mercury are still poorly known [29]. Arial part of *Mercurialis perennis* is used as laxative, anti-galactogogue [14].

This study provides an insight into the phytochemical constituents which are responsible for therapeutic properties of most plants and reveal the antioxidant properties of the ethanolic crude extracts extracted from these two plants. This will serve as a motivation for using the plants for the promotion of human health and this work will also serve as a baseline reference material for anyone who may want to venture into further research on this plant species. The work also provides proves to validate their use in ethno-medicine and also to improve the effectiveness of modern drugs. This large spectrum of usage of plants stirred interest on natural products researches and yet there are quite a number of unexplored plants and that is why this research tries to investigate *Mercurialis perennis*, *Ficus elastica* and their therapeutic properties, specifically the antioxidant property.

Material and methods

Materials used

Conical flasks, Beaker, Glass rod stirrer, volumetric flasks, Test tube, Wash bottles, Sample container, Measuring cylinder, 1Liter of plastic container, pipette, Spatula, Rhetort stand and Clamp, pestle and mortar, weighing balance, water bath, 5% ferric chloride, Heating mantle, Whatman filter paper, steam Distillation Apparatus, Methanol, conc. Nitric acid, , $FeCl_3$, ethanol, , Dil. Ammonia solution, Chloroform, Mayer's reagent, conc. H_2SO_4, DPPH, H_2O_2 solution, phosphate buffer, glacial acetic acid, Fehling's solution A & B, Distilled water,

Sample collection

The plant leaves were collected from the bush area of Sukur Settlement, Madagali Local Government Area of Adamawa State, Nigeria

Preparation of sample

The leaves were washed and air dried in Chemistry laboratory 2, Science Complex of the Faculty of Science, Adamawa State University, Mubi under shade at room temperature. The leaves were weighed and ground to get a coarse powder form using sterile mortar and pestle. The powder was stored in an air tight container and was used for successive analysis [15].

Plant Preparation and extraction

100 g of pulverized form each of plant leaves were weighed and extracted using ethanol solvent in air tight container for 24 h. The resultant mixture was filtered with filter paper (Whatman No. 1) under gravity. The filtrate was dried at 60^0C on a water bath to yield the leaves ethanolic extract residue [16].

Phytochemical Screening

Phytochemical screening was performed using standard procedures. By using different specific reagents, the presences of main groups of natural products were detected in ethanolic extracts of *Ficus elastica and Mercurialis perennis*.

Test for essential oils

Two drops of $FeCl_3$ were added to 90 % alcohol containing small quantity of the extracts and greenish coloration appeared which also indicated the presence of essential oils [17].

Test for alkaloids

Mayer's test: To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids [18].

Test for phenolic compounds and tannins

Ferric Chloride test: The extract (50mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compound alkaloids [18].

Detection of saponins

Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins [19].

Test for flavonoids

Dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids [20].

Detection of cardiac glycosides

Killer-killani test: To 0.5g of extract dissolve in 5ml water was added to 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This is underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer [19].

Detection of phytosterols

Salkowski's Test: Extracts was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes [19].

Test for reducing sugars (Fehling's Test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction [20].

Detection of proteins and aminoacids

Xanthoproteic Test: The extract was treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins [19].

Test for Tterpenoids (Salkowski Test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids [20].

Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform.

The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution is observed for colour changes [20].

Test for Anti-Oxidant Activity

DPPH Free radical scavenging activity

The DPPH Assay was performed by following the method of [21], used with minor modification. The hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the purple coloured methanol solution of 2, 2-diphenyl-1-picryl hydrazyl (DPPH). This Spectrophotometric assay uses the stable free radical, DPPH as a reagent. One thousand microlitres of diverse concentrations (2.5-25 µL/mL) of the extracts in ethanol were added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The DPPH radical scavenging effect was calculated as inhibition of percentage (I%) using to the following formula:

$$I\% = \frac{\text{absorbance of Blank} - \text{absorbance of sample}}{\text{absorbance of Blank}} X100$$

Where, A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A (sample) is the absorbance of the test compound. The values of inhibition were calculated for various concentrations of the extract. Tests were conceded out in triplicate.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity was performed by following the method of [22]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (2.5-25µg/mL) in methanol were added to a H_2O_2 solution (0.6mL, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. Blank solution contained the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging was calculated as:

$$H_2O_2 \text{ scavenging effect } (\%) = \frac{\text{A control} - \text{A sample}}{\text{A control}} X100$$

Where A (control) is the absorbance of the control, and A (sample) is the absorbance in the presence of the sample or standards

Results and discussion

Phytochemical Screening of Ethanol Extract of Ficus elastica and Mercurialis perennis Leaves

From the result obtained shown on Table 1, the preliminary phytochemical investigation revealed the presence of glycosides, flavonoids, tannins, alkaloids, volatile oil (essential oil) , phytosterols, reducing sugar, protein/amino acids, terpenoids and anthraquinones. Saponin was present in *Ficus elastica* and absent in *Merrcurialis perennis*. The leaves of *Ficus elastica* and *Mercurialis perennis* appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments.

The phytochemical result for *Ficus elastica* agrees with the reports [8]. From a similar study on the family of *Ficus*, [23] reported an agreement with a research on the phytochemical composition of crude ethanol extract of *Ficus sycomorus* leaves, the ethylacetate fraction contains alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones. [24] reported the presence of tannins, terpenes, alkaloids, cardiac glycosides, anthraquinone, phlobatannins, saponins, active proteins, isolated from various parts of *F. Thonningii* leaves. When compared to a another specie of the family *Ficus*, [25-27] reported that preliminary phytochemical screening of the stem bark of *Ficus platyphylla* revealed the presence of reducing sugar, saponin, alkaloid, flavonoids, terpenoids, steroids, glycosides, phlobatannins, anthraquinones, cardenolides, volatile oils and phenols flavonoids and tannins.

On the other hand, [28] reported the presence of terpenes, alkaloids, flavoniod, glycoside, simple phenolics, and terpenes in *Mercurialis perennis* which agrees with the result of this study. [29] reported that phytochemical screening of ethanol extract of *Mercurialis annua*, another specie of *Mercurialis* showed the presence of flavonoids, its aqueous-ethanol extract showed the presence of terpenoids and flavonoids while the chloroform extract showed the presence of terpenoids, flavonoids and proteins. From the report submitted by [30] and which agrees with the result obtained from this study, it was observed that methanol extract of *Micrococca mercurialis* leaf has alkaloids, flavonoids, reducing-sugars, tannins, saponins and

anthroquinones. These phytochemicals helps us to know that the *Micrococca mercurialis* possess antidiarrhoel, antihelmintic, anticancerous activities of the plant and various biological active compounds. [31] reported in their study that methanolic extract of the leaf of *Micrococca mercurialis* showed the presence of alkaloids, flavonoids, reducing sugars, gums, tannins and saponins and methanol extract of *Euphorbia thymifolia Linn* belonging to the family of *euphorbiaceae* revealed the presence of Alkaloids, carbohydrates, glycosides, saponins and flavonoids.

Ficus elastica and *Mercurialis perennis* are used by ethnomedical practitioners for treating various ailments. The pharmacodynamic basis supporting the use of *Ficus elastica* and *Mercurialis perennis* extracts in ethnomedicinal systems has been established and pharmacological studies have demonstrated the anti-inflammatory, analgesic, antimicrobial, anthelmintic, antioxidant, antiproliferative, antitrypanosomal, and antimalaria effects of the plant extracts. The remarkable therapeutic effects exhibited by these plantsare a result of the presence of an array of phytochemicals which include flavonoids, alkaloids, tannins, terpenoids and other active proteins.

Figure 1. Phytochemical Screening of *Ficus elastica* and *Mercurialis perennis*

Table 1. Phytochemical constituents of ethanolic extract of *Ficus elastica* and *Mercurialis perennis* leaves

Phytonutrients	*Ficus elastica*	*Mercurialis perennis*
Essential oil	+	+
Alkaloid	+	+
Phenol/Tannin	+	+
Saponin	+	-
Flavonoids	+	+
Glycosides	+	+
Phytosterol	+	+
Reducing sugar	+	+
Protein/amino acid	+	+
Terpenoids	+	+
Anthraquinones	+	+

Key: + Present; - Absent

DPPH Radical Scavenging Activity for Crude Extracts of Mercurialis perennis, Ficus elastica and ascorbic Acid

From Table 2, the crude extract showed good antioxidant potential when compared to standard ascorbic acid by DPPH scavenging assay method. The percentage inhibitions were found to be 62.25%, 55.48%, 65.97% at the lowest concentration (2.5 µL/mL) and 79.72%, 80.23% 91.24% at the highest concentration (10 µL/mL) for *Ficus elastica, Mercurialis perennis* and Ascorbic acid respectively. In line with the report of [23], the result of *in vitro* DPPH radical scavenging activity of the crude ethanol plant extract fraction of *Ficus Sycomorus L.* showed an increase in antioxidant activity with increase in concentration of extract of 88, 89 and 90% of inhibition at 60, 80 and 100 µg/ml respectively. Based on the result of this study, when compared

to *Ficus elastica*, *Sycomorus* is a better antioxidant source.

Table 2. Result for percentage inhibition of dpph by the plants crude extracts and ascorbic acid at 517 nm

Concentration (µL/mL)	*FE (%)*	*MP (%)*	AA*(%)*
2.5	62.25	55.48	65.97
5	71.58	61.99	75.12
7.5	75.32	72.33	81.46
10	79.72	80.23	91.24

FE=*Ficus elastica*, *MP= Mercurialis perennis*, AA= Ascorbic Acid

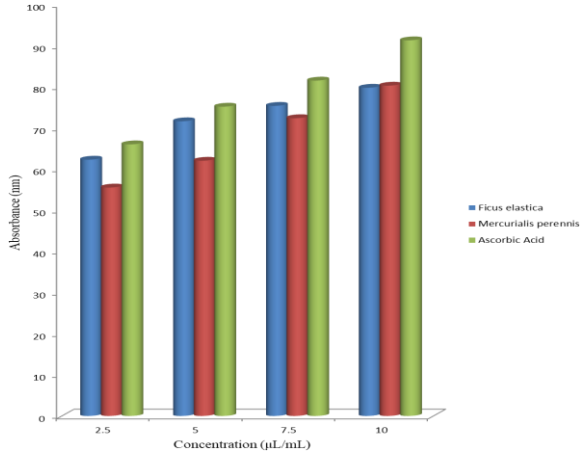

Figure 2. DPPH (%) Inhibition of plants' crude extract and Ascorbic acid

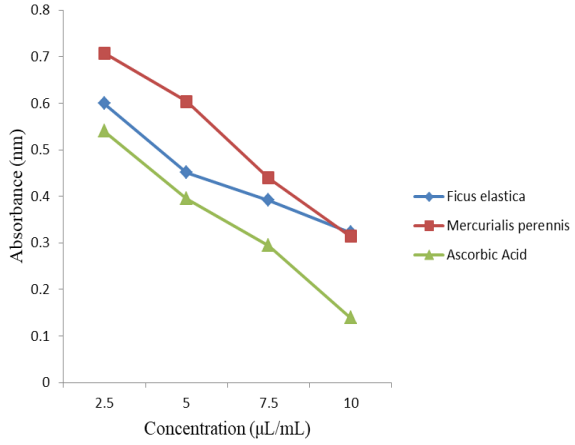

Figure 3. Graph of DPPH Absorbance against Concentration of Plants' Crude Extract and Ascorbic acid

Hydrogen Peroxide Radical Scavenging Activity for Crude Extracts of Mercurialis perennis, Ficus elastica and Ascorbic Acid

From Table 3, the plants' crude extract showed good antioxidant potential when compare to standard ascorbic acid by Hydrogen Peroxide scavenging assay method. The percentage inhibitions were found to be 60.97%, 73.51%, 77.43% at the lowest concentration (2.5µL/mL) and 82.75%, 90.23%, 90.60% at the highest concentration (10µL/mL) for *Ficus elastica*, *Mercurialis perennis* and ascorbic acid respectively. [32] reported the DPPH radical scavenging activity (percentage of inhibition) of an *euphorbiaceae*, the extract from *Jojoba pomace* with 6.71, 36.86, 57.9 and 64.56% inhibition while that of *Jatropha pomace* with 23.77, 45.33, 57.62 and 73.43% inhibition respectively against ascorbic acid with different concentrations of the extract 25, 50, 75, 100, µgmL^{-1}, respectively. This agrees with the concentration dependent results obtained from this study, but are less in antioxidant activity when compared to the *Mercurialis perennis* and *Ficus elastica*.

Table 3. Result for Percentage Inhibition of Hydrogen Peroxide by the plants' crude extracts and Ascorbic Acid at 230 nm

Concentration (µL/mL)	*FE (%)*	*MP (%)*	AA *(%)*
2.5	60.97	73.51	77.43
5	70.92	86.37	83.15
7.5	80.05	89.71	89.54
10	82.75	90.23	90.60

FE=*Ficus elastica*, *MP= Mercurialis perennis*, AA= Ascorbic Acid

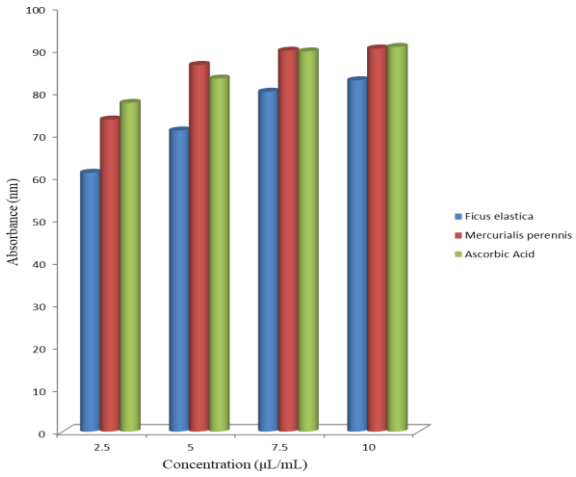

Figure 4. Graph of H_2O_2 Absorbance against Concentration of Plants' Crude Extract and Ascorbic acid

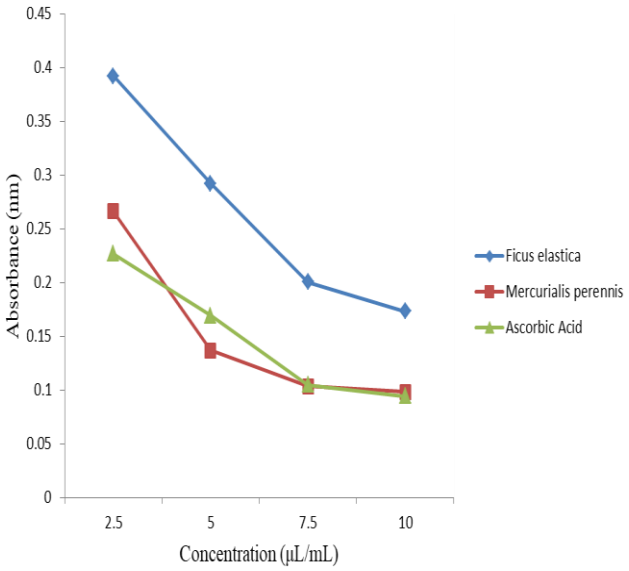

Figure 5. Graph of H_2O_2 Absorbance against Concentration of Plants' Crude Extract and Ascorbic acid

Conclusions

Phytochemical screenings showed that the ethanolic extracts of both *Mercurialis perennis* and *Ficus elastica* possess and are rich in phyto-constitution. The ethanolic extract showed the presence of alkaloids, reducing sugar, amino acids, phytosterol, terpenoids, anthraquinon, saponin, tannin, flavonoids and essential oils. The presence of the identified phytochemicals makes the plants pharmacologically active. Antioxidant activity of both crude extract and essential oil of *Mercurialis perennis* and *Ficus elastica* was studied using DPPH and hydrogen peroxide scavenging assays. Both plant components types showed antioxidant activity when compared with standard ascorbic acid. The quantitative DPPH and hydrogen peroxide assays shows that the plant essential oil and crude extracts have potent antioxidant activity which can be an excellent option for biological and chemical analysis and can be further subjected for the isolation of the therapeutically active compounds. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases.

Conflict of interest

The Authors of this work declare no conflict of interests.

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