



**STUDY OF THE PHYTOCHEMICAL PROPERTIES OF STEM BARK EXTRACTS OF
Vitex doniana (BLACK PLUM)**

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Running title: Bioactive chemical compositions of extracts of *V. doniana*

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ABSTRACT

V. doniana, a tropical plant used by traditional herbalists in combating oxidative stress-related diseases in Africa. The research is determined to evaluate the phytochemical constituents of extracts of *V. doniana*. The extracts of exactly 200 g of the weight of the sample powder in 500 mL beakers containing 400 mL of aqueous, methanol and ethyl acetate separately, was successfully performed by maceration. The Phytochemicals constituents of various extracts of the *V. doniana* was separately determined. The phytochemicals in all extracts were characterized by HPLC/PAD. The percentage yield of the extracts recorded was 5.41 %, 3.22 % and 2.20 % for methanol (MEV), ethyl acetate (EEV) and water (WEV) extracts of *V. doniana*, respectively. All the phytochemicals evaluated were detected in MEV and WEV except anthraquinones, while alkaloids and saponins were not detected in EEV. In 250 µg/mL extract, the flavonoid levels recorded in MEV, WEV, and EEV were 42.67±0.47, 42.50 ± 1.65, and 45.17±0.71 µg/mL, respectively. At 250 µg/mL of WEV, MEV, and EEV extracts, lower % free radical scavenging properties recorded were 75.63±0.04, 69.84±0.00, and 68.83±0.01 %, respectively, compared to d (97.83±0.00 %). An increase in reducing ability was recorded in 250 µg/mL of MEV (1.16±0.08), followed by WEV (1.09±0.04), and EEV (1.00±0.01) compared to standard Ascorbic acid (1.48±0.03). These could explain the medicinal potentials of extracts of *V. doniana*.

Keywords: Phytochemical, stem, extract, *Vitex doniana*

INTRODUCTION

The utilization of plants preparations in combating/fighting many human disorders started with life (Agbafor and Nuwachukwu, 2011). The unlimited significance of many medicinal plants have been reported by various researchers (Sofowora,1993, Agbafor, 2004 and Agbafor and Nuwachukwu, 2011), and they have been used in Nigeria by traditional doctor in treating or



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managing human diseases such as asthma, and hypertension (FAO, 1996, Agbafor and Nuwachukwu, 2011). The treatment of human-related abnormalities by plant products have been reported and effective (Ates *et al.*, 2003, Agbafor, 2004, Adiaratou *et al.*, 2005, and Agbafor and Nuwachukwu, 2011). The teas extracts are also reported as free radical scavengers against sunburn of skin (sunscreen) (Olubunmi *et al.*, 2017).

The bioactive chemical, nutrients, and dietary fiber contents in plants do work together as a protective agent against various diseases. These chemicals are also called metabolites with higher flavor color advantages (Johns, 1996; Craig, 1999). The sources of oxidative stress-related disorders in humans are reactive oxygen species (ROS). The processes of aerobic respiration, lipid peroxidation, and inflammation have been a source of these ROS that can cause tissue damage by oxidative stress (Valko *et al.*, 2007, Agbafor and Nuwachukwu, 2011). The accumulation of these free radicals in the physiological systems can cause oxidative stress-related tissue damages such as Inflammatory diseases, diabetes mellitus, neurodegenerative diseases through damaging the lipids, proteins and DNA molecules of biological membranes (Gulcin, 2007; Agbafor and Nuwachukwu, 2011).

V. doniana, a tropical plant called black plum in English belong to the verbaceae family (Hans-Jorgen, 1990, Agbafor and Nuwachukwu, 2011). *Vitex* species exhibited a characteristic by which an organism has the two sexes of reproductive organs (Sofowora, 1993, Agbafor and Nwachakwa, 2011). The use of the stem bark of *V. doniana* by native doctors has demonstrated higher protective ability against diarrhea, dysentery and liver problems in cows. Its high contents of phosphorous and potassium serve it to be very effective against the postnatal bleeding (Udoh and Udoh, 2005; Nadkarn, 2001). Atawodi, (2005) has demonstrated the protective ability of extracts of *V. doniana* against diabetics' mellitus, and *Trypanosome brucei* different studies. The decoction of *V. doniana* is proven to be effective against stomachache and small intestine related damages (Atawodi, 2005, Agbafor and Nuwachukwu, 2011). Regarding the medicinal significance of this plant as acclaimed by herbalists its evidence on pharmacological and



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pharmaceutical properties have not been reported. Thus, this experiment aimed at evaluating the chemical contents of extracts of *V. doniana* using HPLC for their characterizations.

MATERIALS AND METHOD

Plant samples collection

The *V. doniana* stem bark was obtained in a village near Lapai, Lapai Local Government Area of Niger State, Nigeria. The location of Lapai is Guinea Savanna, South ($8^{\circ} 49'N$ $6^{\circ}41'E$). The identification and authentication of plant specimen were performed by a plant Taxonomist of the Department of Biology, Faculty of Natural Sciences, IBB University, Lapai, Niger State of Nigeria.

Reagents

All the chemicals used were graded industrially, obtained from BDH and Sigma Chemical Companies, England, and the water used was distilled.

Preparation of extracts

The successive extraction was performed using the maceration method. The exactly 200 g of the plant sample powder was macerated in 2 L of ethyl acetate, methanol, and water, separately. The duration for the extraction was 1 week, shaken daily with the renewal of the solvents 2 times per day at room temperature (Agbafor, 2004).

Assay of chemical contents

The qualitative analysis of the plant constituents was assessed (Trease and Evans, 1999; El-Olemmy *et al.* 1994 and Harbone, 1993). The analyses were conducted to evaluate the active chemicals in extracts *V. doniana* which include; Saponins, phenols and tannins, flavonoids, glycosides, alkaloids, carbohydrates, and anthraquinones.

Characterization of phytochemical by High-performance liquid chromatography (HPLC)

The (HPLC) (Shimadzu Corporation, Kyoto Japan) was used to assess the chemical composition of the plant extract. The components of HPLC are the mobile phase that contains 0.2 % of formic acid and acetonitrile (v/v, 20:80) with mode (isocratic) that has the flow rate of 0.6



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mL/min with 10 μ l volume of 100 mg/mL of extract in water detected at UV 254 nm. The HPLC was set to have 20 % as solvent B at 40 °C for 27 min as total run time. The identification of chemicals in the extracts was performed using standards of flavonoids and phenols (Krishnaiah *et al.*, 2009).

Assay of flavonoids

This was evaluated using aluminum chloride colorimetric method, which was modified by Yakubu and Mohammed, (2016). Briefly, 10 mL of extracts of *V. doniana* was mixed with 30 mL of 5% sodium nitrite in a tube containing 2 mL of distilled water. At 4 and 5 min interval, exactly 10% aluminum chloride and 1 M NaOH at 3 and 20 mL, respectively, were mixed and the volume of the mixture in the tube was risen to 10 mL by distilled water. After thorough shaken by a vortex machine, the spectrophotometer was used to reading the absorbance at 510 nm. The expression used for the total flavonoid was mg rutin equivalents/g tofu (mg RE/g FW).

Antioxidant Activity

The ability of scavenging (DPPH) of free radical by extracts of *V. doniana* was assessed (Ursini *et al.*, 1994, Ndatsu *et al.*, 2013). Briefly, 1 mL of extracts of *V. doniana* was added to 15 mL of 20% methanol containing DPPH radicals and the absorbance of the mixtures were read at 516 nm.

Reducing Power

The extract ability to reduce FeCl₃ was determined using the method of Nwanna and Oboh (2007). Exactly 1.5 mL of *V. doniana* stem bark extracts were added to 20 mL of methanol containing 1.5 mL 100 M of sodium phosphate buffer (PH 6.6) and 100 % potassium ferricyanide each. After incubation of mixtures at 50 °C for 20 min, the exactly 1.5 mL of 10% trichloroacetic acid was added. Then, upon centrifugation of all mixtures at 650 rpm for 10 min, about 2 mL of the supernatant and water, and 1 mL 0.1 % of ferric chloride were mixed. At 700 nm the absorbance was read. A higher reducing ability of extract shows by a higher absorbance.



RESULTS

Phytochemical studied

Table 1 shows the results of bioactive components in the extracts *V. doniana*. It shows that all chemicals evaluated except anthraquinone were shown in both WEV and MEV (Table 1). Also beside, EEV does not contain alkaloids and saponins, while all other chemicals evaluated were presence (Table 1). The % yield recorded was WEV (5.41 %), MEV (3.22 %), and EEV (2.20 %) (Table 1).

Table 1: Assay of Phytochemicals

Phytochemical	Extracts of <i>V. doniana</i>		
	WEV	MEV	EEV
Alkaloids	+	+	-
Flavonoids	+	+	+
Anthraquinone	-	-	+
Phenols and Tannins	+	+	+
Saponins	+	+	-
Carbohydrate	+	+	+
Glycoside	+	+	+
Terpenoids	+	+	+
% Yield	5.41 %	3.22 %	2.20 %

(+) present (-) not present. WEV: Water extract of *V. doniana*, MEV: Methanol extract of *V. doniana* and EEV: Ethyl acetate extract of *V. doniana*.

Characterization of chemicals

Figures 1 and 2 show the results of HPLC chromatogram of EEV and MEV, respectively. The time of retention for EEV was 4.691 min and is related to caffeic acid while that of MEV was 3.169, 3.585, 4.712, 6.919 and 7.685 min and are related to Betulinic, Gallic, Caffeic, Rutin and Ferulic acids, respectively.

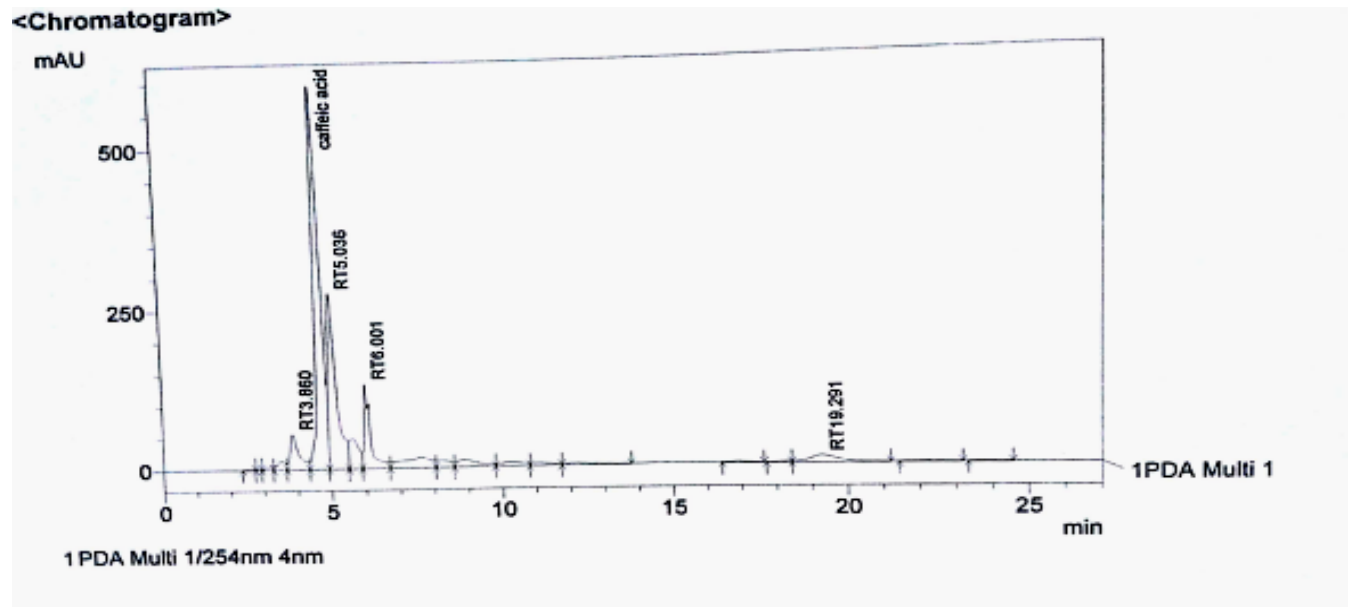


Figure 1: HPLC Spectrum for EEV

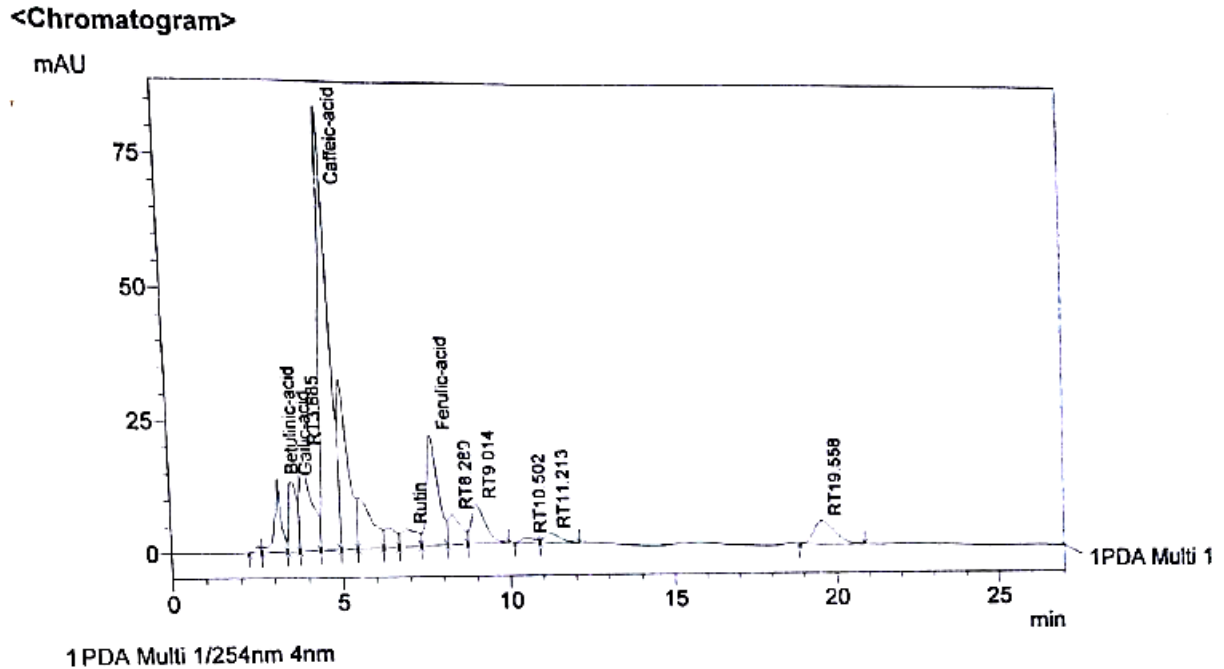


Figure 2: HPLC Spectrum for MEV

Total Flavonoid Concentration

Table 2 showed the results of the flavonoid contents. It revealed that the concentrations of flavonoids in the extracts are concentration dependent (Table 2). No significant different of flavonoids were recorded in MEV (42.67 ± 0.47 and 42.50 ± 0.24 μg of QE/g), EEV (45.17 ± 0.71 and 41.50 ± 1.41 μg of QE/g) and WEV (42.50 ± 1.65 , 40.60 ± 0.47 μg of QE/g) at 250 and 125 $\mu\text{g}/\text{mL}$, respectively, as compared to other extracts (Table 2). While at 62.5 $\mu\text{g}/\text{mL}$, no



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significant different were also recorded in MEV ($32.67 \pm 0.47 \mu\text{g/mL}$), EEV ($36.83 \pm 0.71 \mu\text{g/mL}$) and WEV ($32.00 \pm 0.47 \mu\text{g/mL}$) (Table 2).

Table 2: Total flavonoid content of extracts

Conc. ($\mu\text{g/ml}$)	TFC (μg of QE/g of Extract)		
	MEV	EEV	WEV
250	42.67 ± 0.47^a	45.17 ± 0.71^a	42.50 ± 1.65^a
125	42.50 ± 0.24^a	41.50 ± 1.41^a	40.60 ± 0.47^a
62.5	32.67 ± 0.47^b	36.83 ± 0.71^b	32.00 ± 0.47^b
31.25	23.83 ± 0.24^c	28.83 ± 0.24^c	26.17 ± 0.24^c
15.65	18.00 ± 0.94^c	19.33 ± 0.47^c	19.00 ± 1.89^c

The data are presented in mean \pm standard deviation and means with the same superscript in a column are not statistically different at $p < 0.05$ by ANOVA and Dunnett's test. WEV: Water extract of *V. doniana*, MEV: Methanol extract of *V. doniana* and EEV: Ethyl acetate extract of *V. doniana*.

The scavenging ability (DPPH)

Table 3 is showing the DPPH results in extracts. It reveals that the scavenging ability of extracts is concentration dependence (Table 3). The % DPPH recorded in ascorbic acid at all concentrations were not significantly different and ranged from 96.83 ± 0.00 - 97.83 %, also its contents were higher significantly at all concentrations compared to other extracts (Table 3). At 250 and 125 $\mu\text{g/mL}$, the % DPPH in EEV, MEV, and WEV was not significantly different and ranges from 64.23 ± 0.03 - 75.23 ± 0.04 % (Table 3). While at 62.50-15.63 $\mu\text{g/mL}$, the % DPPH recorded in EEV, MEV, and WEV was not significant difference and ranged from 22.16 ± 0.02 - 38.72 ± 0.01 (Table 3).

Table 3: Scavenging (DPPH) ability of extracts

Sample	DPPH (%)				
	250 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	62.50 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	15.63 $\mu\text{g/mL}$
ASA	97.83 ± 0.00^a	97.38 ± 0.00^a	97.63 ± 0.00^a	97.08 ± 0.02^a	96.83 ± 0.00^a
EEV	68.84 ± 0.01^b	64.23 ± 0.03^b	27.54 ± 0.02^c	22.16 ± 0.02^c	14.05 ± 0.01^d
MEV	69.84 ± 0.00^b	64.95 ± 0.03^b	22.72 ± 0.06^c	28.57 ± 0.00^c	9.62 ± 0.01^d
WEV	75.23 ± 0.04^b	69.26 ± 0.01^b	34.23 ± 0.02^c	38.72 ± 0.01^c	23.11 ± 0.01^c



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The data are presented in mean \pm standard deviation and means with the same superscript in a column are not statistically different at $p < 0.05$ by ANOVA and Dunnett's test. ASA: Ascorbic acid, WEV: Water extract of *V. doniana*, MEV: Methanol extract of *V. doniana* and EEV: Ethyl acetate extract of *V. doniana*.

Reducing power

Table 4 shows the extracts reducing ability results. It revealed that the reducing capacity of all extracts is concentration dependence (Table 4). At 250-62.5 $\mu\text{g/mL}$, the reducing ability of ascorbic acid and other extracts were not significant difference and ranged from 0.75 ± 0.01 - 1.10 ± 0.03 (Table 4).

Table 4: The reducing ability of extracts of the *V. doniana*

Sample	Absorbance				
	250 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$	15.63 $\mu\text{g/mL}$
ASA	1.10 ± 0.03^a	1.08 ± 0.06^a	0.99 ± 0.00^a	0.60 ± 0.00^b	0.62 ± 0.02^b
MEV	1.16 ± 0.08^a	0.97 ± 0.01^a	0.72 ± 0.02^a	0.52 ± 0.01^b	0.30 ± 0.01^c
EEV	1.20 ± 0.01^a	0.84 ± 0.03^a	0.75 ± 0.01^a	0.40 ± 0.00^b	0.21 ± 0.00^b
WEV	1.19 ± 0.04^a	0.90 ± 0.00^a	0.75 ± 0.02^a	0.42 ± 0.00^b	0.25 ± 0.01^c

The data are presented in mean \pm standard deviation and means with the same superscript in a column are not statistically different at $p < 0.05$ by ANOVA and Dunnett's test. ASA: Ascorbic acid, WEV: Water extract of *V. doniana*, MEV: Methanol extract of *V. doniana* and EEV: Ethyl acetate extract of *V. doniana*.

DISCUSSION

The medicinal properties/effects of plant extracts in treating/managing many oxidative stress-related cell damages are due to their phytochemical compositions (Chukwuka *et al.*, 2011, Agbafor and Nuwachukwu, 2011). These bioactive substances (alkaloids, flavonoids, phenols, tannins, saponins minerals, and other biochemical contents) were reported many times for their medicinal significance toward the oxidative stress-related tissue injuries (Olaleye, 2007; Varadarajan, 2008; Chukwuka *et al.*, 2011; Agbafor and Nuwachukwu, 2011). The *V. doniana* preparations are commonly used by traditional doctors in fighting a different kind of abnormalities. They are usually found growing in African continents (Ruffo *et al.*, 2002; Agbafor



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and Nuwachukwu, 2011). African herbalists used different parts of *V. doniana* preparations in treating various abnormalities such as anaemia, gonorrhoea, dysentery, jaundice and liver disorder in humans (Babalola, 1993 and James *et al.*, 2010). The curing ability to extract preparations (*V. doniana*) against many ailments by the herbalists signify its phytochemical contents (Agbafor and Nuwachukwu, 2011). All these findings are supported by the reports of Emmanuel *et al.* (1996), Ali, *et al.* (2017) and Agbafor and Nuwachukwu, (2011) that the *V. doniana* leaf extracts contained some valuable bioactive compounds. The flavonoids, saponins and tannins contents of many medicinal plants could justify their preventive ability against allergic and cancer formation (Chukwuka *et al.*, 2011, Agbafor and Nuwachukwu, 2011). The ability of these biological compounds in combating animal disorders (diabetes, microbial diseases, atherosclerosis, cancer and inflammatory oxidative stress-related ailments have been reported (Chukwuka *et al.*, 2011; James *et al.*, 2010; Agbafor and Nuwachukwu, 2011). Shukla *et al.*, (2015) and Agbafor and Nuwachukwu, (2011) have reported that the neutralizing ability of a plant preparation against free radicals depends on its phytochemical compositions. Subsequently, the content of extract of flavonoids is indication of its ability to fight the activity of ROS. The ROS reducing ability of flavonoids are due to its OH group, and it acts against oxidative stress-related human disorders (Agbafor and Nwachukwa, 2011). The ability of *V. doniana* stem bark extract to neutralize the effect of free radical through DPPH justify its antioxidant potential. These findings are supported by that of Norshazila *et al.* (2010), Agbafor and Nuwachukwu (2011 and Jose *et al.* (2013) that free radical scavenging by DPPH is the common way of evaluating the scavenging ability of ROS chain breakage mechanisms. The scavenging potential of plant extracts is associated with the presence of their bioactive chemicals contents (Frankel, 1995; Kahkonen *et al.*, 1999, Agbafor and Nuwachukwu, 2011). It has been established that all the medicinal plants have scavenging potentials to prevent the tissue damages done by reactive oxygen species (Pattanayak *et al.* 2012). The chain of reactions of free radical species termination occurs by the presence of antioxidant substances. These substances exhibited a high neutralizing ability to donate electrons to attain their stability (Pin-Deer, 1998). A significant increase in reducing power demonstrated by extracts of *V. doniana* bark justifies its



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ability to neutralize the free radical activity. The reduction of free radicals exhibited by leave extracts of *V. doniana* in different solvent was reported *in vitro* studies (Agbafor and Nuwachukwu, 2011, James *et al.*, 2014). A significant decrease of liver injuries exhibited in rats by leave and stem aqueous extracts of *V. doniana* haven been reported (James *et al.*, 2010; Agbafor and Nuwachukwu, 2011).

CONCLUSION

This is concluded that the concentrations of bark extracts of *V. doniana* demonstrated higher scavenging ability toward oxidative-stress related tissue damages. This signifies extracts as a protective agent against oxidative stress, which could be supported by its phytochemical components as shown in the HPLC analysis. Thus, these findings are in line with the usage of the plant extracts in managing/combating the numerous oxidative stress-related human disorders.

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