PharmacologyOnLine December 30, 2014

Archives • 2014 • vol.3 •153-162

STUDY OF ANTIOXIDANT POTENTIAL OF *CADABA FRUTICOSA* BY USING VARIOUS *IN VITRO* MODELS

Murugesan Amudha*, Shanmugam Rani

Department of Pharmacy, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.

*amudhapharma3@gmail.com

Abstract

To investigate the antioxidant potential, total flavonoid and phenolic content in a variety of extracts of aerial parts of *Cadaba fruticosa* (L) Druce. The samples such as ethyl acetate and ethanolic extracts were tested using six in vitro models such as DPPH, Nitric oxide radical, Iron chelating, Hydroxyl radical, Superoxide radical scavenging activity and Total antioxidant activity to evaluate the *in vitro* antioxidant potential of *C. fruticosa* by spectrophotometrically. Total flavonoid and phenolic content in sample were estimated using aluminium chloride colorimetric and Folin-Ciocalteu method. The results were analyzed statistically by the regression method. IC_{50} (Half maximal inhibitory concentration) of the ethanolic extract was found to be 577µg/mL (DPPH), 583µg/mL ($NO^{\bullet}$), 545µg/mL (Iron chelating), 596µg/mL ($OH^{\bullet}$), 568µg/mL ($O_2{-}{\bullet}$) and 556µg/mL (TAC). Furthermore, the TFC and TPC of the ethanolic extract were found to be 2.54mg gallic acid equivalent per gram of extract and 1.92mg quercetin equivalent per gram of extract respectively. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The results of the present comprehensive analysis demonstrated that *C. fruticosa* possess potent anti oxidant activity, high flavonoid and phenolic content. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. These results clearly indicated that *C. fruticosa* is effective against free radical mediated diseases as a natural antioxidant.

Keywords: Antioxidant, *C. fruticosa*, Chelating, Flavonoid, *In vitro* models, Phenols, Scavenging

Introduction

Several decades of dietary research findings suggested that consuming greater amount of antioxidant rich foods might help to protect against diseases (1). Antioxidants are substances that detain or obstruct the oxidation of cellular oxidizable substrates. They recruit their effect by scavenging reactive oxygen species (ROS), and blocking the generation of ROS (2). ROS are free radicals concerned in many human diseases. Superoxide anion radical (O_2–•), the hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2) are the most common kind of ROS (3). In current decades, focus on plant research has expanded all over the globe. Gathered evidences revealed immense potential of medicinal plants applied in various traditional systems, for their biological actions and antioxidant principles (4).

Cadaba fruticosa (L) Druce (Capparidaceae) which is known as Indian Cadaba, native to India, Egypt, Ethiopia, Kenya, Saudi Arabia. This plant possesses hepatoprotective, cytotoxic and antidiabetic, anticancer, antifungal, antiprotozoal, a ntibacterial and Wound healing activities (5). Total antioxidant potential of plant extracts cannot be assessed by using one single method, due to the complex constitution of phytochemicals as well as of oxidative processes. Hence the present study was designed to estimate the antioxidant activity of ethyl acetate and ethanolic extracts of aerial parts of *Cadaba fruticosa* (L) Druce through a number of *in vitro* models like DPPH, Nitric oxide radical, Iron chelating, Hydroxyl radical, Superoxide radical scavenging activity and Total antioxidant activity. Further an attempt has also been made to find the relationship between flavonoid, phenolic content and antioxidant activity of this plant.

Methods

Collection and Preparation of Plant

The aerial parts of plant were collected from the natural habitats of Viruthunagar District of Tamilnadu, India on Sep 2013. The plant was authenticated by Botanist Dr. V. Chelladurai, Research officer-Botany (Retd.), Central council for research in Ayurveda and Siddha, Government of India and the herbarium of voucher specimen number P2401 has been deposited at the herbarium in Department of Botany, Presidency College, Chennai (India). The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The aerial parts of plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Plant sample extraction

500 g of powdered sample was extracted with Pet. ether for 7h, then ethyl acetate for 12h and then ethanol for 48h using soxhlet apparatus. The extracts were gathered and concentrated under reduced pressure in a rotary evaporator. All extracts were kept in desiccators until use. The % yield of this extraction was shown in Fig 1.

Evaluation of antioxidant activities by in vitro models

From the above three extract ethyl acetate and ethanolic extract were employed for the determination of antioxidant activity by using different in vitro models. All the experiments were performed with various concentrations ranging from 125 to 1000µg (125, 250, 500 and 1000 µg/mL) in triplicates.

DPPH radical-scavenging assay

The effect of extracts on DPPH radical was assayed using the Mensor method (6). A methanolic solution of 0.5mL of DPPH (0.4mM) was added to 1 mL of the various concentrations of two extracts and allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. Rutin used as standard. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactiviy(\%) = \frac{A_{518}Control - A_{518}Sample}{A_{518}Control} \times 100$$

Where,

A_{518} control is the absorbance of DPPH radical+ methanol;

A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Nitric oxide radical scavenging activity

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic conditions, NO• reacts with oxygen to produce nitrate and nitrite. The quantities of which can be determined using Griess reagent. According to method (7) 2 mL of 10

mM sodium nitroprusside was dissolved in 0.5 mL of phosphate buffer saline (pH 7.4) and mixed with 0.5 mL of various concentrations of different extracts.

The mixture was then incubated at 25^0 C. After 15 min of incubation, 0.5 mL of the incubated solution is withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of Naphthylethylenediamine dichloride (0.1% W/V)]. The mixture was then incubated at room temperature for 30 min and its absorbance was measured at 546 nm. Ascorbic acid was used as control. The amount of nitric oxide radical inhibition was calculated following this equation.

$$\% \text{ inhibition of } NO^{\bullet} = [A_0-A_1]/A_0 \text{ X } 100$$

Where

A_0 is the absorbance before reaction,

A_1 is the absorbance after reaction has taken place with Griess reagent.

Iron chelating activity

The Benzie and strain method (8) was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 mL of 0.05% O-Phenanthroline in methanol, 2 mL ferric chloride (200µM) and 2 mL of various concentrations of different extracts were incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator.

$$\% \text{ inhibition of Fe radical} = [A_0-A_1]/A_0 \text{ X } 100$$

Where

A_0 is the control absorbance,

A_1 is the test absorbance

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao method (9). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 mL deoxydribose (2.8mM),0.1 mL EDTA (0.1 mM), 0.1 mL H_2O_2 (1mM), 0.1 mL ascorbate (0.1mM), 0.1 mL KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations of two extracts in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. 1 mL of TBA (1%) and 1 mL of trichloroacetic acid (2.8%) are added and incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm, against a blank.

$$\% \text{ inhibition of } OH^{\bullet} \text{ radical} = [A0-A1]/A0 \text{ X } 100$$

Where

A0 is the absorbance of control,

A1 is the absorbance of test.

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne (10). The assay mixture contained sample with 0.1mL of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 mL of EDTA (0.1M EDTA), 0.05 mL riboflavin (0.12 mM) and 2.55 mL of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was incubated at 25^0 C for 5 min and the absorbance was measured at 560 nm against the blank.

$$\% \text{ inhibition of } O_2^{-\bullet} = [A_0-A_1]/A_0 \text{ X } 100$$

Where

A_0 is the absorbance of control,

A_1 is the absorbance of test

Total antioxidant activity

The antioxidant activity of the extracts was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (11). An aliquot of 0.4 mL of various concentrations of two extracts were mixed in a vial with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^0 C for 90 min. After cooling the samples to room temperature, the absorbance of the mixture was read at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

Total flavonoids

The total flavonoid content was determined by

Alumi-num chloride method (4). The reaction mixture (3.0 mL) that comprised of 1.0 mL of extract (1:10 dilution), 0.5 mL of aluminum chloride (1.2%) and 0.5 mL of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent (mg/g).

Total phenols

The total phenol content was determined by Folin-Cio-calteu reagent method (12). 0.5 mL of extract (1:5 dilution) and 0.1 mL of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min. 2.5 mL saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance was mea-sured at 760 nm. The total phenol content was expressed in terms of Gallic acid equivalent (mg/g).

Results

Total antioxidant potential of the plant using various concentrations in the range 125- 1000 µg/ mL of the ethyl acetate and ethanolic extract of *C. fruticosa* were tested for free radical scavenging property by means of different *in vitro m*odels.

The DPPH scavenging capacity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 75.23% and 69.46% inhibition at 1000 µg/ mL whereas for rutin (standard) was found to be 86.23% at 1000 µg/mL and that was depicted in Fig 1. It was revealed that free radicals were scavenged by extracts in a concentration- dependent manner. The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 577µg/mL and 624µg/mL which was less than the standard rutin ($IC_{50,}$ 508µg/mL).

The NO• scavenging capacity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 72.45% and 67.56% inhibition at 1000 µg/ mL whereas for ascorbic acid (standard) was found to be 79.43% at 1000 µg/mL and that was illustrated in Fig 2. The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 583µg/mL and 635µg/mL which was less than the standard ascorbic acid ($IC_{50,}$ 527µg/mL).

The iron chelating capacity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 78.54% and 79.23% inhibition at 1000 µg/ mL whereas for EDTA (standard) was found to be 89.78% at 1000 µg/mL and that was presented in Fig 3. The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 545µg/mL and 531µg/mL which was less than the standard ascorbic acid which shows IC_{50} 470µg/mL.

The OH• scavenging capacity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 68.45% and 62.56% inhibition at 1000 µg/ mL whereas for ascorbic acid was found to be 82.54% at 1000 µg/mL and that was demonstrated in Fig 4. The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 596µg/mL and 649µg/mL which was less than the standard ascorbic acid ($IC_{50,}$ 510µg/mL). The O_2–• scavenging capacity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 72.54% and 61.56%

inhibition at 1000 µg/ mL whereas for ascorbic acid (standard) was found to be 84.34% at 1000 µg/mL and that was presented in Fig 5. It was revealed that free radicals were scavenged by extracts in a concentration- dependent manner The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 568µg/mL and 667µg/mL which was less than the standard ascorbic acid ($IC_{50,}$ 506µg/mL).

Total antioxidant activity of the ethanolic and ethyl acetate extract of *C. fruticosa* showed 73.98% and 60.68% inhibition at 1000 µg/ mL whereas for ascorbic acid (standard) was found to be 84.95% at 1000 µg/mL and that was presented in Fig 6. The IC_{50} value of ethanolic and ethyl acetate extract of *C. fruticosa* were found to be 556µg/mL and 690µg/mL which was less than the standard ascorbic acid ($IC_{50,}$ 499µg/mL). The total amount of phenols and flavonoids content of ethanolic and ethyl acetate extract of *C. fruticosa* were depicted in Table 1.

Discussion

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. Normal pro--oxidant molecules including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals in aerobic metabolism. Physiological concentrations of free radicals are needed to mediate normal progression of fertilization, and embryo development. Even if, above physiologi-cal levels of free radicals can result in oxidative stress which leads to sperm or ovum damage, deformity, endometriosis, miscarriage and infertility (13). In traditional societies nutrition and health care are powerfully interrelated and many plants have been consumed both as food and for medicinal purposes. The utilization of non-cultivated plants plays a central role in the diet, but very few

ethnopharmacological and phytochemical studies have accorded in detail with the potential health benefits of such plants (14). In the traditional medicine practice, the boiled leaves are eaten as an anthelmintic; decoction with other ingredients is employed in the treatment of amenorrhea, dysmenorrheal and uterine obstruction. It was reported that the leaves and stem bark possesses L-stachydrine and L-3-hydroxy stachydrine.Presence of quercetin, isoorientin, hydroxybenzoic acid, syringic acid, vanillic acid and 2-hydroxy- 4-methoxy benzoic acid. The stembark contains an alkaloid cadabicine, and dry pods contain cadabalone (15).

Hence the aerial parts of *C. fruticosa* were used for the *in vitro* antioxidant study. In this research, the potential sources of the anti-oxidants, total phenols and flavonoids in the plant *C. fruticosa* were studied. Anti oxidant activity should not be concluded based on a single test model. In order to confirm the antioxidant potentials of the *C. fruticosa* extract, the present study employed different *in vitro* models, such as DPPH, $NO^{\bullet}$, $OH^{\bullet}$, $O_2{-}^{\bullet}$ scavenging, iron chelating and total antioxidant activity. DPPH antioxidant assay is based on the capability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical have an odd electron, which is accountable for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron provided by an antioxidant molecule, the DPPH is decolorized, that can be quantitatively measured from the changes in absorbance.

The antioxidant properties on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Hydroxyl radical is the most reactive of the reactive oxygen species (ROS), and it has the shortest half-life compared with other ROS. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipids, and The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe2+ salts as an important catalytic component (16). Superoxide anion shows a vital role in plant tissues and is concerned in the formation of other cell-damaging free radicals. Superoxide anion scavenging activity can be correlated with high content of flavonoids. Study suggested that the flavonoids may be involved in the dismutation of super-oxide anion radical. Hydrogen peroxide even if a weak oxidizing agent is vital because of its ability to penetrate biological membranes, once inside the cell it can probably react with Fe2+ and Cu2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects (17). Plant phenolics and flavonoids are extensively dispersed in plant tissues as well as contribute a vital function in free radical scavenging and anti oxidant activity. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases (18). Flavonoids like quercetin and kaempferol have been shown to exhibit the action through effects on permeability of membrane, and by hindrance of membrane-bound enzymes such as the phospholipase and ATPase (17). The antioxidant activity of phenolic compounds is mostly due to their redox characteristics, which can play an vital role in quenching singlet and triplet oxygen, neutralizing and absorbing free radicals or decomposing peroxides (19). Total phenolics and flavonoids which contain hydroxyl functional groups, in ethanolic extract were highest when compared to ethyl acetate extract. Since phenolics and flavonoids are accountable for the anti-oxidant activity, and high amount present in the extract specifies good antioxidant activity. Female patients who suffer from hypogonadotropic hypogonadism (HH) connected with amenorrhea, anovulation, and infertility which may be attributed to the iron effect on the pituitary gland as well as on the female reproductive system. Treatment with combination of antioxidants and iron chelators could probably neutralize the deleterious effects of ROS (20) and maybe reverse endocrine problems, improving reproductive capability and fertility potential

Conclusion

From the report it was revealed that the potential of the extracts to different scavenging properties in different *in vitro* models, denoting that they may be valuable therapeutic agents for managing free radical-related pathological damage. From the above results and discussion it can be concluded that the ethanol extract of *C. fruticosa* acquires the potent antioxidant substances which may be responsible for its activity as well as rationalize the basis of using this plant's extract as folkloric remedies. Even though the scavenging activity of the extracts was appreciably lower than those of standard antioxidant but it was an evident that the extract did prove the proton-donating ability and could serve as free radical inhibitors probably as primary antioxidants.

This study recommended that the ethanolic extract of *C. fruticosa* might be helpful in preventing or slowing the progress of various types of ailments. Advance studies are needed to explicate the exact mechanistic pathway and the isolation of various active compounds responsible for the anti oxidant as well as fertility potentials of C. fruticosa.

Acknowledgment

The author M. Amudha was grateful to the University Grant Commission, New Delhi India for providing UGC-BSR fellowship.

References

1. Antioxidants and Health: An Introduction. National Center for Complementary and Alternative Medicine. U.S. Department of Health and Human Services 2012; Available at www.nlm.nih.gov/medlineplus/antioxidants.html.
2. Michael S, Kalirajan A, Padmalatha C, Ranjith Singh AJA. *In vitro* antioxidant evaluation and total phenolics of methanolic leaf extracts of *Nyctanthes arbor-tristis* L. Chin J Nat Med 2013; 11(5): 484–87.
3. Agarwal A, Allamaneni SSR. Role of free radicals in female reproductive diseases and assisted reproduction. Reprod Biomed Online. 2004; 9 (3): 338-47.
4. Garg D, Shaikh A, Muley A, Marar T. *In vitro* antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosa-sinensis* stem and leaves. Free Radicals and Antioxidants 2012; 2 (3): 41-6.
5. Telrandhe UB, Uplanchiwar. Phyto-Pharmacological Perspective of *Cadaba farinosa* forsk. American Journal of Phytomedicine and Clinical Therapeutics 2013; 1 (1): 011-022.
6. Mensor LL, Meneze FS, Leitao GG, Reis AS et al., Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method.*Phytother.Res*.2001; 15: 127-30.
7. Marcocci I, Marguire JJ, Droy-lefaiz MT, Packer L. The nitric oxide scavenging properties of Ginko biloba extract. Biochem. Biophys. Res. Commun. 1994; 201: 748- 55
8. Benzie IEF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*.1996; 239: 70-76.
9. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin, Int.J.Pharm.1990; 58: 237-240.
10. Winterbourne CC, Hawkins RE, Brain M and Carrel RW. The estimation of red cell superoxide dismutase activity. J. Lab.chem.Med.1975; 85: 337-341.
11. Prieto P, Pineda M, Aguilar M . Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Anal. Biochem, 1999; 269: 337-341.
12. McDonald S, Prenzler P, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry. 2001; 73:73–84
13. Rong-zhen Z, Dao-wei Z. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. Journal of integrative agriculture 2013; 12 (10): 1826-38
14. Sahaa MR, Hasana MR, Aktera R, Hossaina MM et al.,. In vitro free radical scavenging activity of methanol extract of the leaves of Mimusops elengi linn. Bangl. J. Vet. Med. 2008; 6 (2): 197–202.
15. Khare CP. Indian Medicinal Plants- An illustrated medical dictionary. 2007; Springer.p 107.
16. Deepalakshmi K, Mirunalini S, Krishnaveni M, Arulmozhi V. In vitro and in vivo antioxidant potentials of an ethanolic extract of Ganoderma lucidum in rat mammary carcinogenesis. Chinese J Nat Med. 2013; 11 (6): 621−627.
17. Hausteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharm. 1983; 32:1141.
18. Sahoo S, Ghosh G, Das D, Nayak S. Phytochemical investigation and in vitro antioxidant activity of an indigenous medicinal plant Alpinia nigra B.L. Burtt. Asian Pac J Trop Biomed 2013; 3(11): 871-876.
19. Banerjee SK, Bonde CG. Total phenolic content and antioxidant activity of extracts of Bridelia retusa spreng bark: Impact of dielectric constant and geographical location. J Med Plants Res 2011; 5 (5): 817- 22.
20. Rachmilewitz EA, Weizer-Stern O, Adamsky K et al., "Role of iron in inducing oxidative stress in thalassemia: can it be prevented by inhibition of absorption and by antioxidants?" Annals of the New York Academy of Sciences 2005; 1054, pp. 118–123.

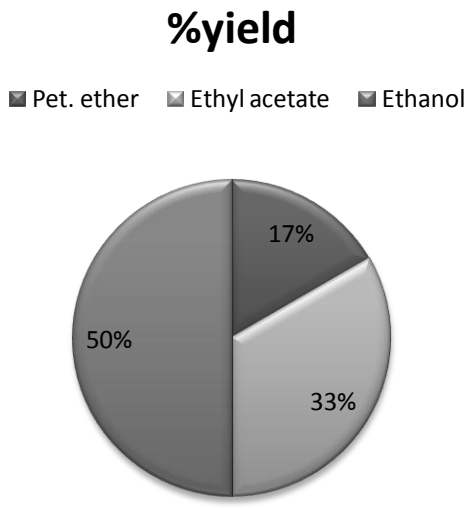

Figure 1. % Yield of three extracts of aerial parts of *C. fruticosa*

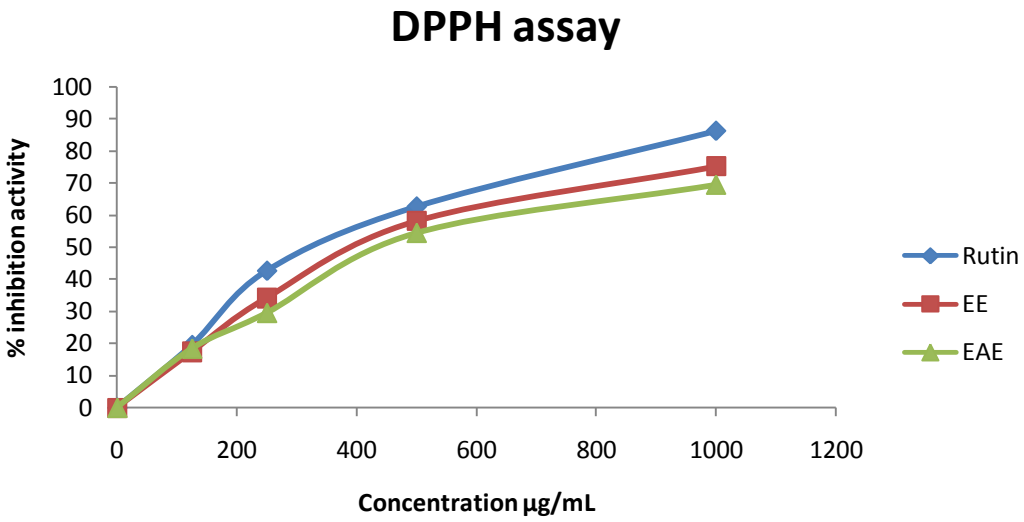

Figure 2. DPPH free radical-scavenging activities of *C. fruticosa* extract in comparison with rutin. n = 3,*x* ± *s*

.

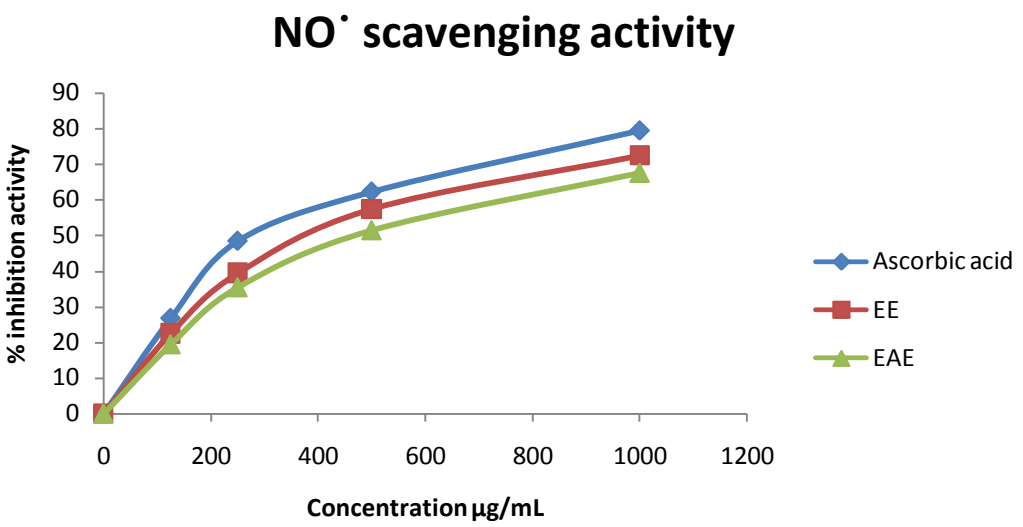

Figure 3. NO•-scavenging activities of *C. fruticosa* extract in comparison with ascorbic acid. $n = 3, x \pm s$

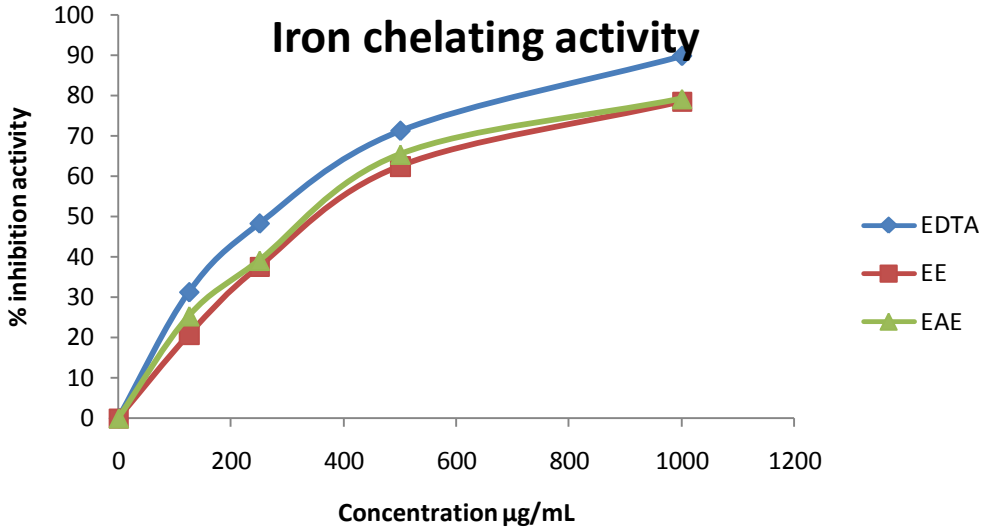

Figure 4. Iron chealating activities of *C. fruticosa* extract in comparison with EDTA. $n = 3, x \pm$.

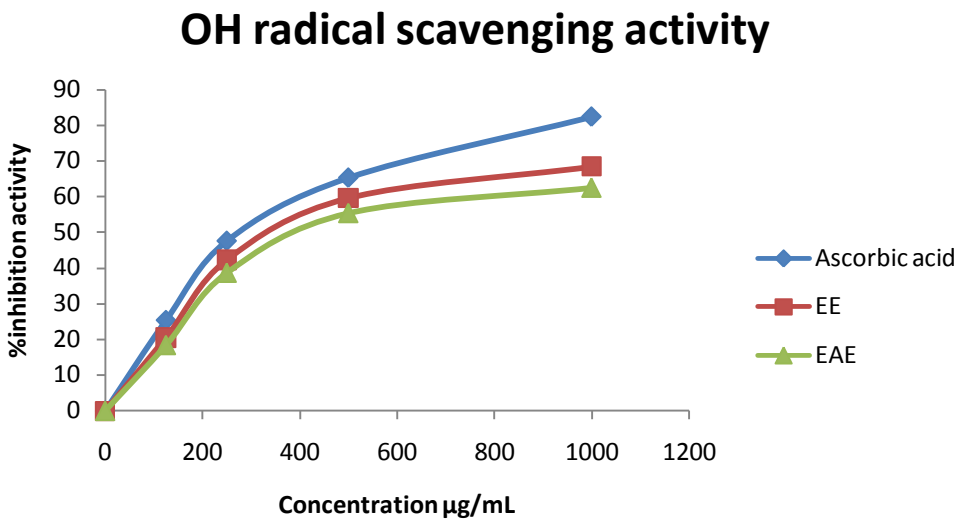

Figure 5. OH• -scavenging activities of *C. fruticosa* extract in comparison with ascorbic acid. $n = 3, x \pm s$

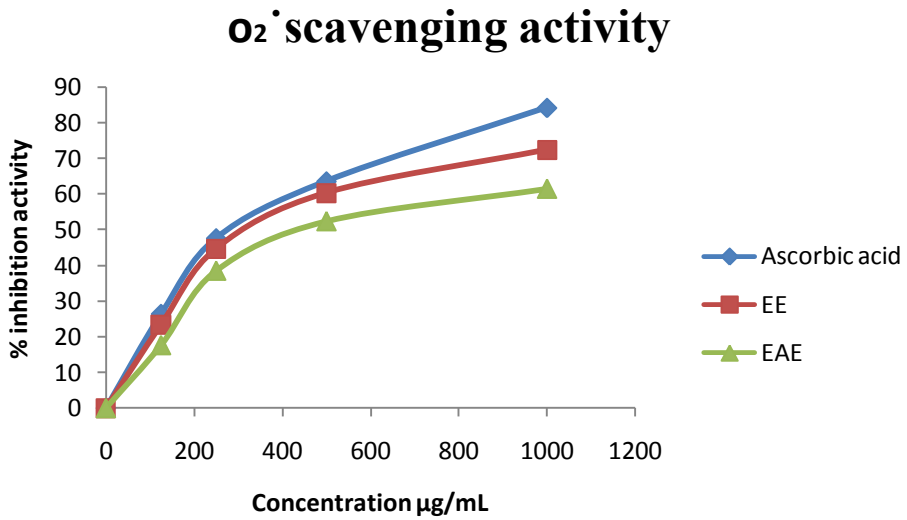

Figure 6. O_2•-scavenging activities of *C. fruticosa* extract in comparison with ascorbic acid. $n = 3, x \pm s$

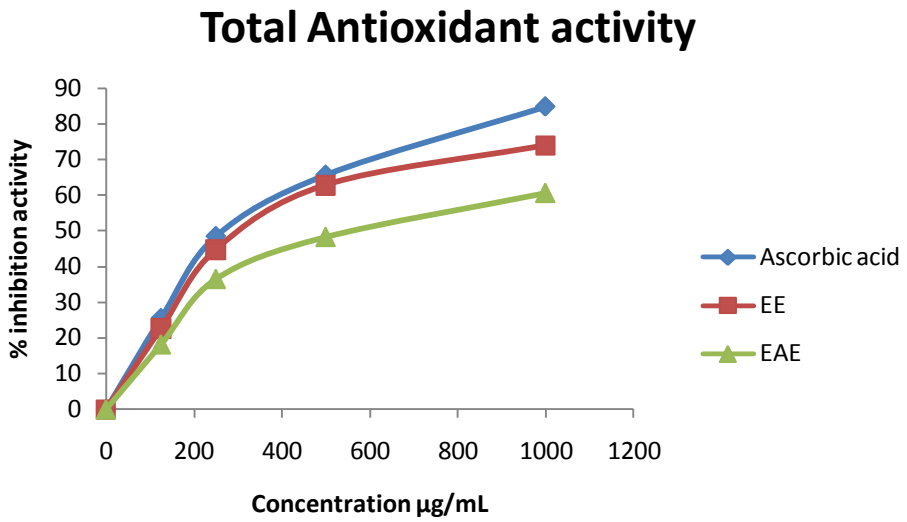

Figure 7. Total antioxidant activities of *C. fruticosa* extract in comparison with ascorbic acid. $n = 3, x \pm s$

Table 1 The total amount of phenols and flavonoids content of ethanolic and ethyl acetate extract of *C. fruticosa*

S.NO.	CONTENT	*C. FRUTICOSA*	
		STANDARD EQUIVALENT IN ETHANOLIC EXTRACT (MG/G) *	STANDARD EQUIVALENT IN ETHYL ACETATE EXTRACT (MG/G) *
1	TOTAL PHENOLS	1.92 ± 0.15	1.23 ± 0.08
2	TOTAL FLAVONOIDS	2.54 ± 0.45	1.68 ± 0.83

(* The results obtained were expressed as Mean ± S.D. of triplicates)