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FREE-RADICAL SCAVENGING ACTIVITIES OF FLOWER AND FRUIT EXTRACTS *CADABA FRUTICOSA* (L.) DRUCE

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ABSTRACT

Objective: In the present study, antioxidant activities flower and fruit of the pet-ether, chloroform, acetone and methanolic extracts from *Cadaba fruticosa* (L.). Flower and fruit were investigated by employing established *in-vitro* studies. *C. fruticosa* is one of the folk herbal used in various traditional medicaments. Many people of rural and urban areas depend upon treatment, which has been developed through prolonged traditional experience. **Method**: The ability of the plant extract to act as hydrogen/electrons donor or scavenger of radicals were determined by *in-vitro* antioxidant assays using 2,2-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\cdot}$) scavenging, reducing power assay, superoxide radical (O_2^{*-}) scavenging activity, phosphomolybdenum assay, FRAP and metal chelating activity ,were performed to know the antioxidant potency of the plant extract of flower and fruit of *C. fruticosa*. **Result**: Results are evaluate higher in flower and fruit of *C. fruticosa* recorded antioxidant as well as to phytochemical quantitative of total phenol and flavonoid present in the plant extracts of *C. fruticosa*. The plant *C. fruticosa* methanolic extract of flower showed greater IC_{50} for DPPH assay (11.23µg/ml) and compare to other extract, higher Reducing power activity flower in methanol (1.296 ascorbic acid /100g extract), better fruit in phosphomolybdenum reduction (104.6mg/g extract) and higher superoxide radical scavenging activity in fruit extract (92.06%). However, the better metal chelating ability was shown by the water extracts of flower (9.11 Ascorbic acid /100g) compared to other solvent extracts. **Conclusion**: The result indicates the antioxidant and total phenol activity potential of *C. fruticosa*.

KEYWORDS: *Cadaba fruticosa*, DPPH assay antioxidant activity and total phenol activity.

1. INTRODUCTION

Medicinal plants are therefore being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing (Peschel *et al.*, 2006). Drug discovery is an on-going requirement to find safe, effective, and affordable cures for an expanding spectrum of human ailments. Plants develop a valuable source of an extensive type of therapeutic molecules and consequently hold a large outlook for new medicines. Natural antioxidants are required to prevent and/or cure the disorders caused by free radicals (Valko *et al.*, 2009). Reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals (Saeed *et al*., 2012).

Free radicals have been implicated in the development of several disorders, including cancer, neurodegeneration and inflammation (Ferguson, 2010), giving rise to studies of antioxidants for the prevention and treatment of diseases. Oxidation interfered by free radical effects is additionally chargeable for the rancidity of perishable food rich in unsaturated fatty acids and the natural antioxidants are suggested as a superior alternative for the synthetic ones such as BHA or BHT (Li *et al.*, 2008). Therefore, there is increasing interest day by day in the substances revealing antioxidant properties, which are supplied to humans and animals as food components or as specific preventative pharmaceuticals (Sasikurkcu *et al.*, 2009).

2. MATERIAL AND METHODS

2.1. Plant material

The flower and fruit parts of *C. fruticosa* were collected from Maruthamalai tail of Western Ghats, during December 2017. The collected plant material was identified and authenticated by Botanical survey of India, Southern Circle, Coimbatore

(NO.BSI/SRC/5/23/2017/Tech./3493) and the voucher specimen has been deposited in Bharathiar University Herbarium, Department of Botany, and Coimbatore. The collected fresh plant for leaves, stem, and root were cleaned thoroughly with running tape water to remove dust and shade dried for a week at room temperature. The powders were in airtight container.

2.2. Plant extracts preparation

The powder of plant material was extracted as one of the extraction method of Soxhlet extractor based extracted continuously with petroleum ether, chloroform, acetone and methanol. Each time before extracting with the following dissolvable, the thimble was dried in hot air oven underneath 40 ºC. The distinctive dissolvable concentrates were thought by rotating vacuum evaporator and after that air-dried.

2.3. Qualitative phytochemical analysis

The phytochemical screening (Sadasivam, Manickam 2008) of the plant *C. fruticosa* flower and fruit were done qualitatively to determine the presence of following phytochemicals qualitative analysis.

2.3.1 Carbohydrates test

2 ml of plant extricate include few drops of an alcoholic arrangement of α-naphthol at that point, the blend is shaken well and 1 ml of concentrated sulphuric corrosive is included gradually at the edges of the test tube. A violet ring shows the nearness of sugars.

2.3.2. Proteins test

Two drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) are added to 2 mL of extract. A purple colour indicates the presence of amino acids.

2.3.3. Alkaloids test

To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 ml) was added; an orange-red precipitate shows the presence of alkaloids.

2.3.4. Flavonoids test

A mixture containing a piece of magnesium ribbon (magnesium turning) and conc. Hydrochloric acid is added to plant extract. It gives a red colour which indicates the presence of flavonoids.

2.3.5. Tannins test

The extract (2ml) was mixed with 2ml of distilled water and some drops of ferric chloride (FeCl3) solution were added. Development of fresh green precipitate obtained the presence of tannins.

2.3.6. Phenolic compounds

The plant extract 500µl is dissolved in 5ml of distilled water and 2 ml of 1% solution of gelatin containing 10 g sodium chloride is added to it, then absorbing white precipitate indicates, it as the presence of phenolic compounds.

2.3.7. Steroid and terpenoids

The extract (about 100 mg) was separately shaken with chloroform (2 ml) followed by the addition of concentrated H_2SO_4 (2 ml) along the side of the test tube, a reddish-brown colouration of the interface indicates the presence of Steroid and terpenoid (Ayoola *et al.,* 2008).

2.3.8. Saponins test

The 50mg extract is mixed with distilled water and made up to 20ml. The mixture is shaken in a graduated cylinder for 15 min and the foam appeared of two cm layer as a result of the presence of saponins.

2.4. Quantification of total phenolics, tannins and flavonoids

2.4.1. Quantification of total phenolics and tannin

The total phenol content was determined according to the method described by Makkar, (2003). 100 µL aliquots for plants extracts (5mg/ ml) were taken in the test tubes and made up to the volume of 1mL with distilled water. Then 500µl of Folin - Ciocalteu reagent (1:1 with distilled water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vertexing the mixture, the test tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the blank. The mixture without plant extract was taken as blank. The examination of the reaction was conducted in triplicate and the results were shown standard as Gallic acid equivalents.

Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) Makkar. (2003). 75 mg of PVPP was weighed into a 2 ml Eppendorf tube and to this 900 µL distilled water and then 750 µL of the sample extracts were added. The content was vortexed and kept in the test tube at 4 ºC for 4hrs. Then the sample was centrifuged at 4000 x g for 10 minutes at room temperature and the supernatant was collected. The supernatant has only simple phenolics other than the tannins (the tannins would have been precipitated along with the PVPP. The phenolic substance of the supernatant was measured and communicated as the substance of non-tannin phenolic. From the results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) - Non tannin phenolics (%)

2.4.2. Quantification of total flavonoids

The flavonoid content of all the extracts were quantified as it act as a major antioxidants in plants reducing oxidative stress. Estimation as per described by (Zhishen *et al*., 1999). Initially 150 µl of all the plant extracts were taken in different test tubes. To each extracts 2 ml of distilled water was added. Then 150 µL of $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation 150 µl of $AlCl_3$ (10%) was added to all the test tubes. The test tubes were incubated for 6 minutes at room temperature. Then 2 ml of NaOH was added to all the test tubes which

were made up to 5 ml using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour was developed due to the presence of flavonoid was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

2.5. *In vitro* antioxidant studies

2.5.1. DPPH Radical Scavenging Activity (shimade *et al*., 1992)

The antioxidant activity of the extracts was determined in terms of hydrogen donating ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$), according to the method of Blois, 1958). Plants extracts at various concentrations (20 - 100 µl) was added to 5 ml. of 0.1 m methanolic solution of $DPPH^{\cdot}$ and allowed to stand for 20 min at 27 ºC. The absorbance of the sample was measured at 517 nm. Methanol served as blank and solution without extract served as control. The mixture of methanol, DPPH and standard (ascorbic acid) served as positive control. More significantly the IC_{50} of the extracts were also calculated.

2.5.2. Assay of superoxide radical (O_2^{*-}) scavenging activity

The assay was established on the capacity of the plant extract to inhibit formazan creation by scavenging superoxide radicals generated in riboflavin- light-NBT system (Beauchamp and Fridovich, 1971). Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 2.33µg riboflavin and 12 mM EDTA, and 11.55 g NBT. Reaction was started by illuminating the reaction mixture with of sample extracts (100 µl) for 90 seconds. Reaction mixture with extract kept in dark served as negative control while the mixture without extract was taken as blank. Immediately after illumination, the absorbance was measure at 590 nm. The activity was compared to ascorbic acid. The percentage inhibition of superoxide anion formation was calculated using the following formula:

% of Inhibition in superoxide radical (O_2^{*-}) scavenging activity = (Control OD – Sample OD / Control OD) × 100

2.5.3. Reducing power assay

The reducing power of different extracts of *C. fruticosa* was determined by the method reported by (Oyaizu, 1986). 100 µL of extract was taken in 2.5 mL of 0.2 M phosphate buffer (pH 6.6) was added. To this, 2.5 ml of 1% Potassium ferricyanide solution was added and the mixture was incubated at 50ºC for 20 minutes. After the incubation, 2.5 ml of 10 % TCA was added. The content was centrifuged at 3000 rpm for 10 minutes. The upper layer of the supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride. The absorbance of the reaction mixture was measured spectrophotometer at 700 nm.

% of Reducing power = (Control OD – Sample OD / Control OD) × 100

2.5.4. Phosphomolybdenum assay

The antioxidant capacity of the extracts has been determine with the phosphomolybdenum reduction assay according to (Prieto *et al*., 1999). The assay was based on the reduction of the extract and subsequent formation of a complex (Green colour). 0.5 ml of extract combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was incubated at 95 ºC for 90 minutes. The absorbance was taken at 695nm using spectrophotometer. The results were calculated in ascorbic acid equivalents.

Percentage of Phosphomolybdenum = (Control OD – Sample OD / Control OD) × 100

2.5.5. Metal chelating activity

Iron II chelating activity was measured by the inhibition of the formation of Iron-(II)-ferrozine complex after preincubation of the sample. The Fe^{+} was monitored by measuring the formation of ferrous iron –ferrozine complex against methanol blank at 562nm.The chelating of ferrous ions by various extracts in plant was estimated by the method of (Dinis *et al.*, 1994). The chelating of ferrous ions by various extracts of *C. fruticosa was* estimated. Initially, about 100µl the extract samples were added to 50µl of 2 mM $Fecl_2$ solution. Then the reaction was initiated by the addition of 200µl of 5mM ferrozine and the test tubes were vortexed well and left standing at room temperature for 10 minutes. The reaction mixture containing deionized water in place of sample was considered as the negative control absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). EDTA was against the standard metal chelating agent and the results were expressed as mg EDTA equivalents/g extract chelate the ferrous ion was calculated by,

Percentage chelation = [1-(ABS sample/ABS control)]×100

Ec_{50} value (mg extract / ml) is the effective concentration at which ferrous ions were chelated 50% by the extract.

2.5.6. FRAP -Ferric Reducing Antioxidant power

The antioxidant capacities of phenolic extracts of samples were estimated according to the procedure described by (Pulido *et al.* 2000). FRAP reagent (2.7 ml), prepared freshly and incubated at 37^{o}C, was mixed with 270 µL of distilled water and 50 µl of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 ^{o}C for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml of 20 mM/L TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM/L HCl plus 2.5 ml of 20 mM/L $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 mM/L acetate buffer (pH 3.6) described by Siddhuraju and Becker, (2003)(15). After the incubation, the absorbance readings were taken immediately at 593 nm. Results were calculated in ferrous sulphate equivalents.

2.6. Statistical Analysis

The results of scavenger activity and total phenolic and total flavonoids contents were performed from the averages of all samples (n=3) reading Mean ± SD used Excel 2003. Analysis of antioxidant variance using one-way ANOVA was performed to test the significance of differences between means at the 0.05 level of significance using the statistical analysis software, SPSS (IBM-SPSS statistic version 20).

3. RESULTS

3.1. Qualitative phytochemical screening

The qualitative of phytochemical screening was evaluated for flower and fruit of *C. fruticosa,* and their appreciable appearance determined the major primary and secondary metabolics results are shown in table 1. The results revealed that the primary metabolites such as carbohydrates, protein, are present in appreciable amount in acetone and methanolic extract fruit and minimum in flower. The secondary metabolites such as phenolic, saponin and tannins were found to be variously distribution in flower and fruit *C. fruticosa*. Alkaloids were not found in extract. The phenol was found to be higher in all extract. The flavonoids are minimum present in acetone and methanolic extract.

Table 1: Qualitative phytochemicals screening test.

Phytochemical test	Pet ether		Chloroform		Acetone		Methanol	
	CFFl	CFFr	CFFl	CFFr	CFFl	CFFr	CFFl	CFFr
Carbohydrates test	--	--	--	--	+	++	+	++
Protein test	--	+	--	+	+	++	+	++
Alkaloid test	--	--	--	--	--	--	--	--
Flavonoid test	-	+	-	+	+	+	+	+
Tannin test	+	+	+	+	+	++	++	++
Phenolic compound	++	++	++	++	++	++	++	++
Steroid and terpenoid	+	+	+	++	++	++	++	++
Saponin test	+	+	+	+	--	++	++	++

+ Presence of chemical compound, (-) Absence of chemical compound (+) (++) Based on the intensity of characteristic colour. **CFFl** *C. fruticosa* flowers; **CFFr-** *C. fruticosa* fruits

3.2. Quantification of total phenolics, tannin and flavonoid

The results of total phenolics and tannin contents are showed in Table 2. Methanol extracts of *C. fruticosa* flower revealed highest phenolic (32.41±0.08 GAE/100g) and maximum in methanolic extract in fruit (32.15±0.24GAE /100g extract), when estimation of tannin methanol extracts of *C. fruticosa* flower revealed highest amount shows (6.51±0.65 GAE/100g) and lowest in methanolic extract in fruit (5.93g ± 3.01 GAE /100g extract). The results of flavonoid content are presented in Table 2. In this estimation of the methanol extract of *C. fruticosa* flower revealed maximum amount of flavonoid content (18.20g ± 0.94/100g) followed by methanol extract of fruit (15.78 ± 3.59 g/100g).

Table: 2. Total phenolics, tannins and flavonoid content of flower and fruit extract of *C. fruticosa.*

Plant material	Extract	Total phenol (GAE mg/100g)	Tannin (GAE mg/100g)	Flavonoid (RE mg/100g)
C.F. Flower	Pet ether	10.99 ± 0.82^{h}	3.59 ± 0.55^{d}	7.74 ± 0.04^{i}
	Chloroform	18.49 ± 2.94^{f}	1.27 ± 0.01^{i}	16.78 ± 1.36^{c}
	Acetone	32.11 ± 0.11^{d}	2.71 ± 1.27^{f}	15.06 ± 2.22^{e}
	Methanol	**32.41 ± 0.08^{b}**	**6.51 ± 0.65^{b}**	**18.20 ± 0.94^{b}**
C.F. Fruit	Pet ether	6.33 ± 0.30^{i}	2.06 ± 0.31^{g}	10.44 ± 0.63^{g}
	Chloroform	15.25 ± 6.93^{g}	1.52 ± 0.26^{h}	8.03 ± 0.12^{h}
	Acetone	19.88 ± 1.79^{e}	3.40 ± 0.04^{e}	12.98 ± 0.80^{f}
	Methanol	**32.15 ± 0.24^{c}**	**5.93 ± 3.01^{c}**	**15.78 ± 3.59^{d}**
Standard		54.12 ± 0.17^{a}	17.48 ± 2.11^{a}	22.11 ± 0.45^{a}

Value are mean of triplicate determine (n=3) mean ± standard deviation. GAE- Gallic acid, RU-Rutin, DIG-Diosgenin. The letters a>b>c>d>e>f>g>h>i, represents where significant statistical difference at $p<0.05$ among extract.

3.4 *In vitro* antioxidant studies

3.4.1. Radical scavenging activity using DPPH· method

The free radical-scavenging activities in different extracts of flower and fruit *of C. fruticosa*, such as standard vitamin C, was determined by the DPPH radical

scavenging assay and the results are shown in Figure 1. The reduction in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical produced by hydrogen donation. Flowers extract methanol shows the minimum (11.23µg/ml) inhibitory activity. In fruit methanol shows (12.67µg/ml) the minimum inhibitory activity. The DPPH radical has been generally used to test the capacity of mixes as free radical foragers or hydrogen contributors to assess the cancer prevention agent action of the plant concentrates and nourishments (Soares *et al.*, 1997).

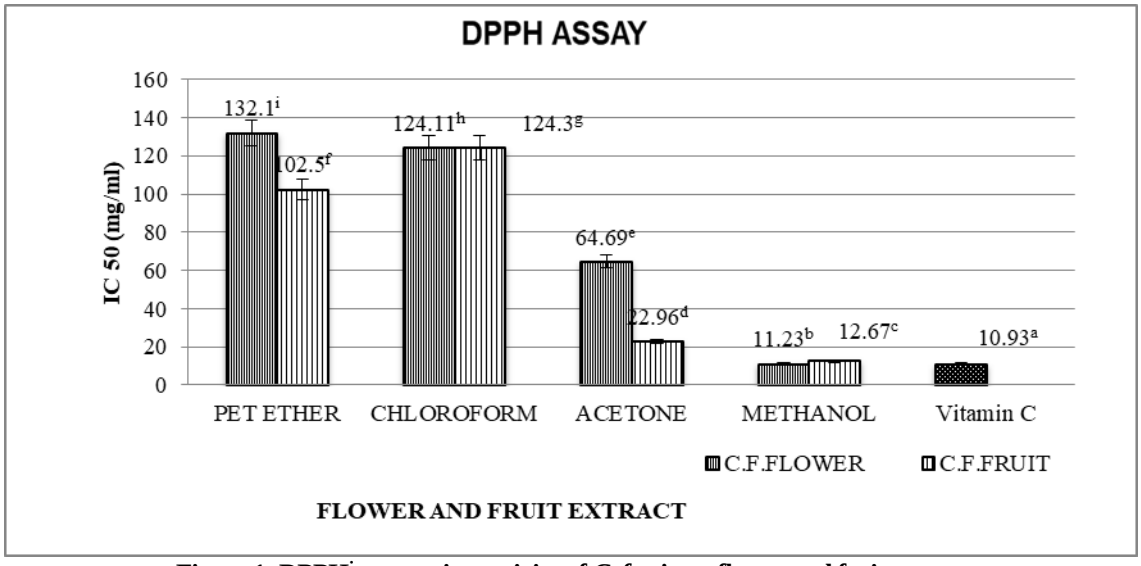

Figure 1: DPPH˙ scavenging activity of *C. fruticosa* flower and fruit extract

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at $p<0.001$ among extract. IC_{50}- Inhibitory concentration of stem extracts

3.4.2. Phosphomolybdenum assay

Phosphomolybdenum assay is successfully used to determine the ability of extracts to reduce Mo (VI) to Mo (V) and subsequent formation of green phosphate /Mo(V) complex at an acid pH. The total antioxidant capacity of different solvent extracts of flower and fruit of *C. fruticosa* shown in figure 2. *C. fruticosa* fruit showed higher activity (104.6mg/g extract) in acetone compared to the other extracts. Methanol extract of flower shows (97.19mg/g extract) in Phosphomolybdenum reduction.

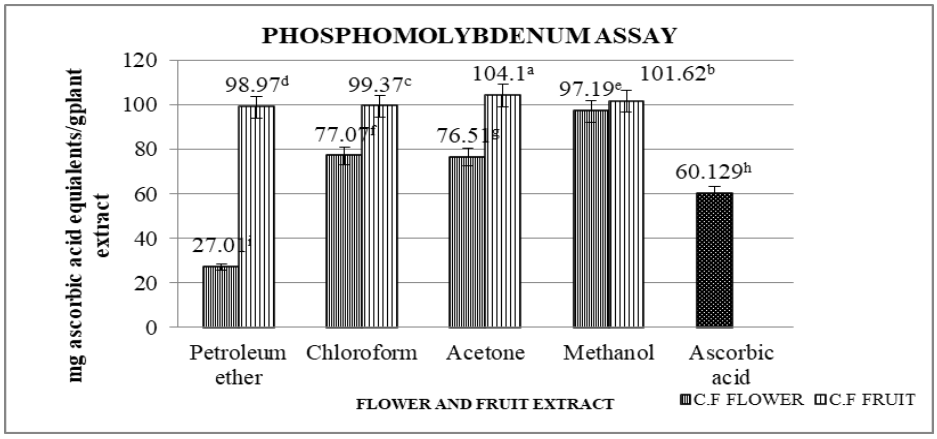

Figure 2: Phosphomolybdenum activity of *C. fruticosa* flower and fruit extract.

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at $p<0.001$ among extract.

3.4.3. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power assay analysis to examine the antioxidant effect of any substance in the reaction substance as reduction ability. Antioxidant potential of flower and fruit extracts of *C. fruticosa* estimated from their ability to reduce TPTZ- Fe (III)

complex to TPTZ-Fe (II) and the results are given in Figure 3. *C. fruticosa* extract of flower and fruit methanol extract revealed higher activity and the values were (2248.92 MmolFe(II)E/mg; 2939.38 MmolFe (II)E/mg) extract.

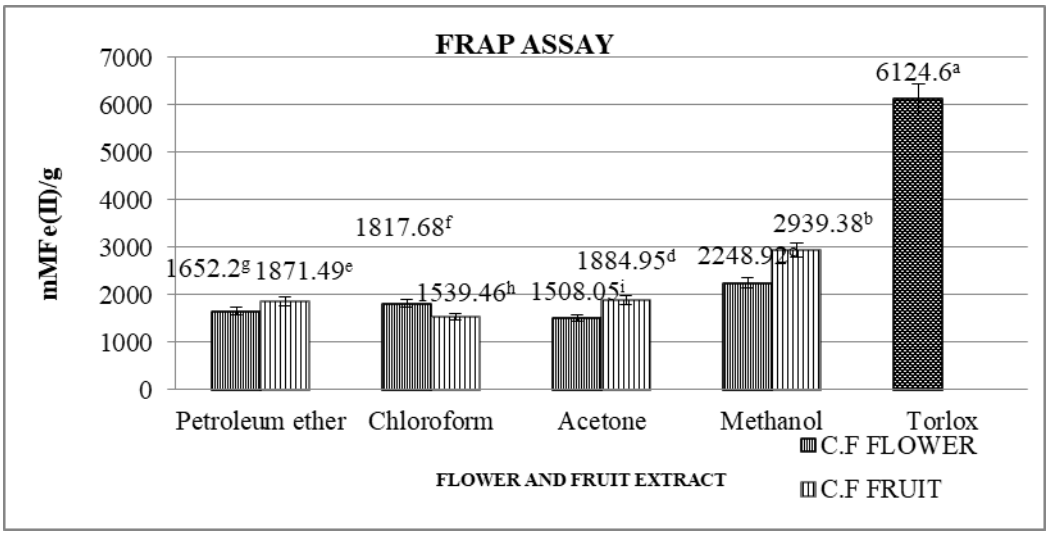

Figure 3: FRAP assay of *C. fruticosa* flower and fruit extract.

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at p<0.001 among extract.

3.4.4. Reducing power assay

The presence of antioxidants causes the diminished of Fe_3+/ferricyanide complex to the ferrous form. The reducing power of and flower and fruit extracts of *C. fruticosa* was calculated and the results were presented in the Figure 4 and 5. The *C. fruticosa* methanol extract minimum in flower (1.296 ascorbic acid /100g) and fruit (1.125 ascorbic acid /100g) compare to standard the values.

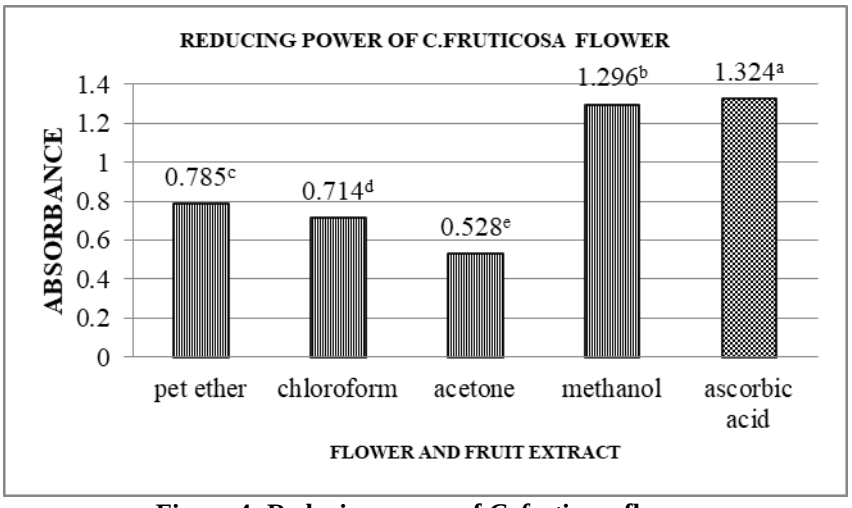

Figure 4: Reducing power of *C. fruticosa* flower

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at p<0.001 among extract.

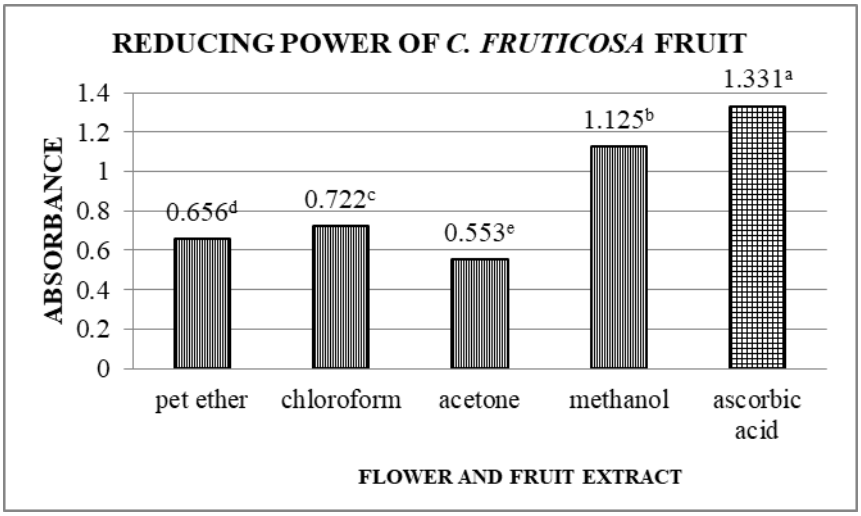

Figure 5: **Reducing power of *C. fruticosa* fruit**

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at p<0.001 among extract.

3.4.5. Metal chelating activity

Ferrozine can quantitatively chelate with Fe2+ and frame a red hued complex. This response is restricted within the sight of other chelating specialists and results in an abatement of the red shade of the ferrozine-Fe^{2+} complex. Iron is an essential mineral for normal physiology, which it may cellular injury in increasing level. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress (Hippeli and Elstner, 1999). An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. Along these lines oxidation response is captured and no free radicals are delivered. The Fe^{+} chelating capacity was absorbed of *C. fruticosa* flower and fruit extracts shown in Figure 7. In *C. fruticosa* maximum chelation's was observed for the methanol extract of flower (9.11 g ascorbic acid/100g) and the least in fruit methanol extract (3.4g ascorbic acid /100g).

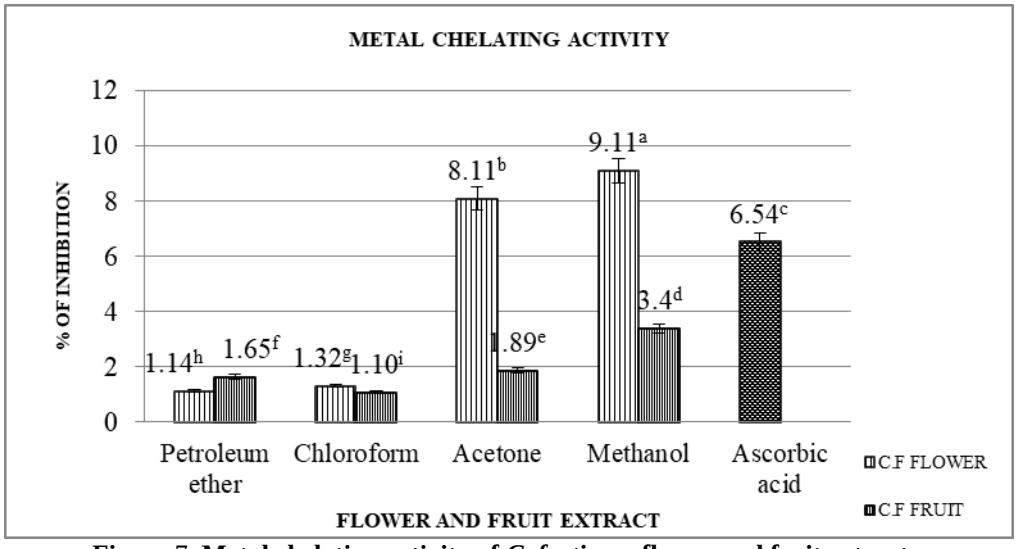

Figure 7: **Metal chelating activity of *C. fruticosa* flower and fruit extracts.**

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at p<0.001 among extract.

3.4.6. Superoxide radical (O_2^{*-}) scavenging activity

The superoxide anion radical scavenging activities of *C. fruticosa* flower and fruit extracts are shown in Figure 6. The extracts were found to be an efficient scavenger of superoxide radical generated in riboflavin- NBT- light system *in vitro*. The methanol extract of *C. fruticosa* fruit extract showed higher superoxide radical scavenging

activity fruit in (92.06%) compared to other solvent extracts. The methanol extract of *C. fruticosa* flower showed superoxide radical scavenging activity (81.57%). The scavenging activity against chemically generated superoxide radicals of the crude extracts and flavonoids was measured by means of spectrophotometric measurement of the product on reduction of nitro blue tetrazolium (NBT) (Robak & Gryglewski, 1988).

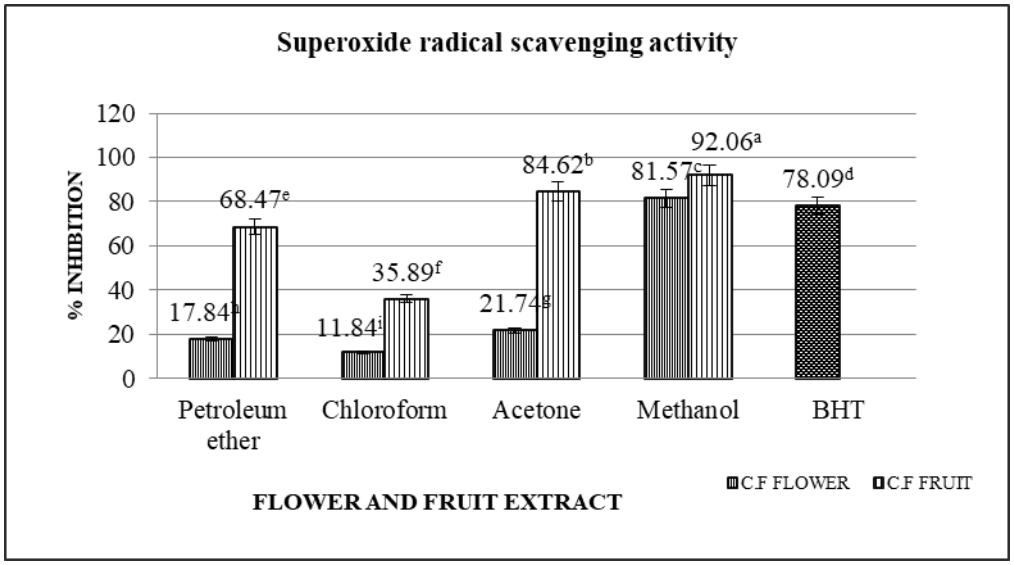

Figure 6: Superoxide scavenging activity of *C. fruticosa* flower and fruit extracts.

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g, the letter represents where significant statistical difference at p<0.001 among extract.

3.4.7. Antioxidant activity by the ABTS$^{\bullet+}$ assay

Antioxidant activity of plant extracts was assessed spectrophotometrically by [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] ABTS cautioned colourization assay and the absorbance was taken at 734 nm (Re *et al.*, 1999). The complete of antioxidant activity determined as the concentration of Trolox becoming equivalent antioxidant activity showed as µl/g extracts. The Fe+ chelating capacity of different the solvent extracts of *C. fruticosa* flower and fruit extracts shown in Figure 7. In *C. fruticosa* maximum chelation's was observed for the methanol extract of flower and fruit (467.59 Torlox/100g; 391.2 Torlox/100g.) extract.

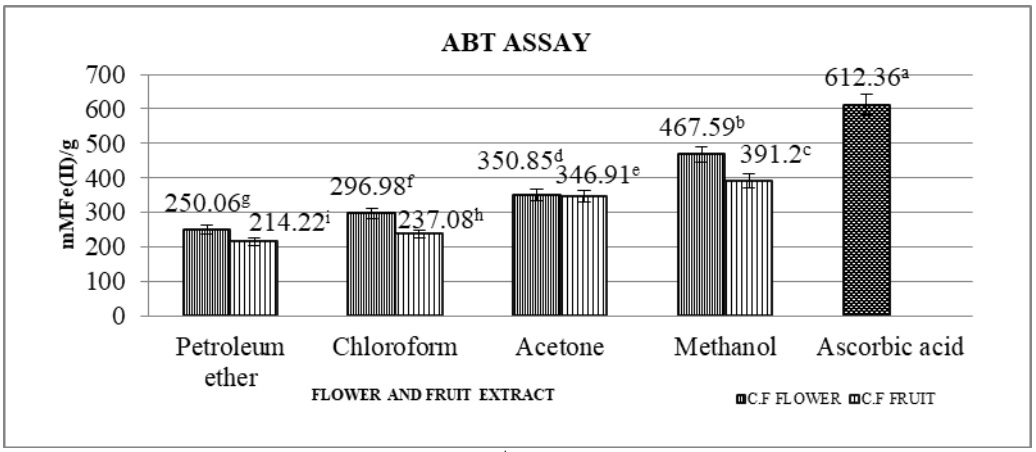

Figure 7: Antioxidant activity by the ABTS$^{\bullet+}$ assay of *C. fruticosa* flower and fruit extracts.

Value are concentration independent analysis (n=3) mean ± standard deviation. a>b>c>d>e>f>g>h>i the letter represents where significant statistical difference at p<0.001 among extract.

DISCUSSION

Phenolic compound have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids (Rice-Evans *et al.*, 1996). Flavonoid and phenols have vital scavenging role in oxidation, inflammation and cancer (Dzoyem *et al.*, 2015). Tannins are the polyphenolic compounds obtained from plants tremendous activity against diarrhoea, haemorrhage, virus and haemorrhoids, bacteria, fungi and parasites and also impact anticancer and cytotoxic activity (Cai *et al.*, 2016). Scavenging activity of *C.fruticosa* flower and

fruit extract for free radicals was estimated by DPPH assay. It is very sensitive and short time assay for checking the antioxidant potential of the plant extracts and compounds.

Antioxidants are an important role in the prevention of diseases of the human body by virtue of scavenging free radicals. Public threaten disease like cancer, immune system decline to ageing, are prevented by antioxidants such as vitamins, carotenoids, phenolic compounds. (Aksoy *et al.,* 2013). DPPH is one of a stable nitrogen centred dark violet coloured powder which changes from violet to yellow colour upon reduction (Chanda *et al.,* 2009). The principal behind the Iron chelating assay was to decolorize the iron-ferrozine complex by the scavenger's ability or plant extract ability. The coloured complex if formed by the reaction of Fe (II) with ferrozine. The complex of iron-ferrozine was obstructed by the scavenging constituents that chelates with Fe (II), thus reducing the colour intensity of the solution (Wu *et al.,* 2015). Phosphomolybdenum assay also important in vitro antioxidant activity to access the total antioxidant capacity of the plant extracts. The principal follows the conversion of Mo (VI) to Mo (V) by extract or the compound which possess antioxidant potential resulting in green phosphate Mo (V). The electron/hydrogen donating pattern of antioxidant depends upon its structure and series of redox reactions occurring in the activity (Gupta *et al.,* 2016). Reducing power of *C. fruticose* flower and fruit extract was assessed by using the potassium ferricyanide reduction method. An antioxidant compound in the test sample cause conversion of iron (Fe^{+3}) to ferrous (Fe^{+2}) by donating hydrogen and the yellow colour of the reaction mixture changes to green. The intense green colour in the assay shows the strong antioxidant capacity of the sample which has reducing powder (Wang *et al.,* 2016).

CONCLUSION

The plant *C. fruticosa* is a value of natural medicine and antioxidants activity source, which seemed to provide potential nutraceuticals for human health. The current research determined the phytochemical screening studies and antioxidants activity for importance medicinal properties with providing a cost-effective and reliable source of medicine for the welfare of humanity.

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REFERENCES

1. Peschel W, Sanchez-Rabaneda F, Dieckmann W, Plescher A, Gartzia I, *et al.* An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem, 2006; 97: 137-150.
2. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 2007; 39: 44–84.
3. Saeed N, Khan MR, Shabbir M. Antioxidant activity total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L.BMC Complement. Altern Med, 2012; 12: 221.
4. FergusonChronic LR. Inflammation and mutagenesis Mutat. Res Fund. Mol, 2010; 690: 3-11.
5. Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT – Food Science and Technology, 2008; 41: 385-390.
6. Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G, Mete E. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus castus* L. Fruits from Turkey Food and Chemical Toxicology, 2009; 47(10): 2479-2483.
7. Sadasivam, Manickam. author. Biochemical Methods, 2nd edn. Tamilnadu: New age international (P) limited, publishers, 2008; 8-56.
8. Makkar HPS. Quantification of tannin in tree and shrub foliage: A laboratory manual. Dondrecht. The Netherlands: Kluwer academic publishers, 2003.
9. Zhishen J, Mengecheng T, an Jianming, W. The determination of flavonoids contents on mulberry and their scavenging effects on superoxide radical. Food chemistry, 1999; 64: 555-559.
10. Blios MS. Antioxidants determination by the use of a stable free radical. Nature, 1958; 4617: 1199-1200.
11. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assay applicable to acrylaminde gels. Analytical Biochemistry 1971; 44: 276-277.
12. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr, 1986; 44: 307–315.
13. 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.
14. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys, 1994; 315: 161-9.
15. Pulido R, Bravo L, and Sauro-Calixto F. Antioxident activity of dietary polyphenols as determine by a modified ferric reducing/antioxidant power assay. Journal of Agricultural Food chemistry, 2000; 48: 3396-3402.
16. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygis*. Free Radic Res 1997; 26: 469–478.
17. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol, 1988; 36: 317–322.

18. Hippeli S, Elstner EF, Transition metal ion-catalyzed oxygen activation during pathogenic processes. FEBS Lett., 1999; 443: 1–7.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 1999; 26: 1231–1237.
20. Rice-Evans C, Miller NJ, (1996) Antioxidant activities of flavonoids as bioactive components of food, Biochem. Soc. Trans, 1996; 24: 790-795.
21. Dzoyem JP, Eloff JN. Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. J Ethnopharmacol, 2015; 160: 194–201.
22. Cai Y, Luo Q Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 2004; 74(17): 2157-2184.
23. Aksoy L, Kolay E, Agilonu Y, Aslan Z, Kargioglu M. Free radical scavenging activity, total phenolic content, total antioxidant status and total oxidant status of endemic *Thermopsis turcica*. Saudi J. Biol. Sci., 2013; 20: 235-239.
24. Chanda S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. Afr J Microbiol, 2009; 3: 981–996.
25. Wu L, Du Y, Lok J, Lo E H, Xing C. Lipocalin-2 enhances angiogenesis in rat brain endothelial cells via reactive oxygen species and iron-dependent mechanisms. J Neurochem, 2015; 132: 622–628.
26. Gupta K , Maurya S , Agarwal S , Kushwaha A , Kumar R , Antioxidant assessment of extracts obtained through hot extraction process. Cell Mol Biol., 2016; 62: 129.
27. Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of in-vitro antioxidant activity of polysaccharides. Oxid Med Cell Longev., 2016: Article ID 5692852,13.