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Estimation of total phenolic content and Studies on antioxidant activity of different extracts of *Cadaba farinosa* Forsk by hydroxy radical and iron chelating techniques

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Abstract

Cadaba farinosa (family Capparidaceae) is generally known as “Indian cadaba” in the traditional ayurvedic system. The current study, aerial parts of different concentrates (Pet.ether, ethyl acetate and methanol) of *Cadaba farinosa* was evaluated for its *in-vitro* antioxidant potential by hydroxy radical taking ascorbate as a standard. The iron-chelating activity is taking

Ethylenediamine tetraacetate as standard and estimation of total phenol content as equivalent to mg/g of Gallic acid. The methanolic concentrates of *Cadaba farinose* & ascorbic acid exhibited antioxidant potential possessing IC_{50} 205 μ g/ml & 65 μ g/ml (Hydroxy radical), methanolic concentrates of *Cadaba farinose* & Ethylenediamine tetraacetate exhibited antioxidant potential possessing IC_{50} 240 μ g/ml & 70 μ g/ml (iron-chelating activity). The methanolic and EA concentrates of *Cadaba farinose* contain total phenolic content 9.86 ± 0.62 and 3.98 ± 0.54 , respectively. The IC_{50} value was originated that methanolic concentrates of *Cadaba farinose* more efficient in hydroxy radical, iron chelating activity compared EA & PE concentrates. The methanolic extract of *Cadaba farinose* having more free radical activity due to the presence of phenolic content as a bioactive compound. This result indicates that aerial parts of methanolic concentrate *Cadaba farinose* could serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.

Keywords

Cadaba farinosa, hydroxy radicals, iron chelating, total phenolic content

Introduction

Free radicals induce lipid peroxidation and provoke damage in the cell membrane. Incorporation of antioxidants in fats & oils or foods can prevent the deterioration of lipids in foods. Some of these compounds which retard lipid peroxidation are synthetic antioxidants, whereas others occur as natural dietary constituents (Duh, 1999). The human body is in a constant battle to keep from free radicals. The first line of defence is the preventive antioxidants which quench the free radicals generated in the body. An early-stage Augmentation of antioxidant status should either prevent or greatly curtail tissue injury (Lobo, Patil, Phatak, & Chandra, 2010). Both enzymatic and non-enzymatic reactions generate free radicals. The primary source of free radicals in enzymatic reactions includes those involved in phagocytosis, respiratory chain, in prostaglandin synthesis, and cytochrome p⁴⁵⁰ system. Non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionising radiations result in free radical production (Liu, Stem, & Roberts, 1999). Both enzymatic and non-enzymatic antioxidant mechanisms

handle the elimination and neutralisation of ROS. Due to the adverse effects of synthetic antioxidants on human health, restrictions have been imposed on their usage (Bajpai, Sharma, Kang, Baek, & Antioxidant, 2014). Natural antioxidants have significantly been intensified as synthetic antioxidants exert a carcinogenic effect.

Cadaba farinosa (family Cappariaceae) is generally known as “Indian cadaba” in the traditional ayurvedic system. Quercetin, isoorientin, hydroxybenzoic acid, syringic acid, vanillic acid and 2-hydroxy-4-methoxy benzoic acid were isolated from *Cadaba farinosa* (Khare, 2007). *Cadaba farinosa* was used for different diseases like anthelmintic, antisyphilitic, aperients, stimulant, antiscorbutic, antiphlogistic (Ambasta & Anonymous, 1986). *Cadaba farinosa* was used rheumatic pain (Ambasta et al., 1986). The flower buds are stimulant, antiscorbutic, purgative, antiphlogistic and anthelmintic, especially for round worm (Nadkarni, 1954). *Cadaba farinosa* was used as hepatoprotective activity (Umesh et al., 2010). *C. farinosa* was used for the treatment of wound healing (Habib, Ghulam, & Abdul, 2004) and anticancer (Graham, Quinn, Fabricant, & Farnsworth, 2000). Still, no literature is available on the antioxidant activity of aerial parts *Cadaba farinosa*. Thus, the present study to assess antioxidant activities of aerial parts *Cadaba farinosa* by invitro techniques like Hydroxyl radical assay, Iron chelating activity and determination of total phenolic compound.

Table 1 Activity of PE extract of *Cadaba farinosa* on Hydroxyl radical method

S.no	Extract (µg/ml)	% inhibition (±SEM) *	
		PE extract	Ascorbate
1	100	24.28± 0.012	57.34±0.024
2	200	30.18 ± 0.020	63.12±0.028
3	400	41.29 ± 0.032	71.40±0.033
4	800	47.25 ± 0.024	84.37±0.018
		IC50 = 910 µg/ml	IC50 = 65 µg/ml

Table 2 Activity of EA extract of *Cadaba farinosa* on Hydroxyl radical method

S.no	Extract (µg/ml)	% of inhibition (±SEM)*	
		(EA extract)	Ascorbate
1	100	30.28±0.024	57.34±0.024
2	200	37.48±0.016	63.12±0.028
3	400	48.08±0.028	71.40±0.033
4	800	55.45±0.034	84.37±0.018
		IC50 = 458 µg/ml	IC50 = 65µg/ml

Table 3 Activity of Methanolic extract Cadaba farinosa on Hydroxyl radical method

S.no	Extract ($\mu\text{g/ml}$)	% inhibition ($\pm\text{SEM}$)*	
		Methanolic extract	Ascorbate
1	100	35.12 \pm 0.012	57.34 \pm 0.024
2	200	50.10 \pm 0.042	63.12 \pm 0.028
3	400	59.18 \pm 0.038	71.40 \pm 0.033
4	800	68.84 \pm 0.024	84.37 \pm 0.018
		IC50 = 205 $\mu\text{g/ml}$	IC50 = 65 $\mu\text{g/ml}$

Table 4 Iron-binding potential of Cadaba farinosa PE extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition ($\pm\text{SEM}$)*	
		PE extract	Ethylenediamine tetraacetate
1	100	24.18 \pm 0.052	55.18 \pm 0.023
2	200	33.46 \pm 0.018	62.45 \pm 0.034
3	400	42.45 \pm 0.032	69.22 \pm 0.047
4	800	50.16 \pm 0.045	75.38 \pm 0.028
		IC50 = 810 $\mu\text{g/ml}$	IC50 = 70 $\mu\text{g/ml}$

Table 5 Iron-binding potential of Cadaba farinosa of EA extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition ($\pm\text{SEM}$)*	
		EA extract	Ethylenediamine tetraacetate
1	100	26.86 \pm 0.028	55.18 \pm 0.023
2	200	38.34 \pm 0.010	62.45 \pm 0.034
3	400	56.28 \pm 0.042	69.22 \pm 0.047
4	800	63.59 \pm 0.028	75.38 \pm 0.028
		IC50 = 508 $\mu\text{g/ml}$	IC50 = 70 $\mu\text{g/ml}$

Table 6 Iron-binding potential of Cadaba farinosa Methanolic extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition($\pm\text{SEM}$)*	
		Methanol extract	EthyleneDiamine tetra acetate
1	100	36.82 \pm 0.024	55.18 \pm 0.023
2	200	48.34 \pm 0.036	62.45 \pm 0.034
3	400	56.65 \pm 0.082	69.22 \pm 0.047
4	800	64.22 \pm 0.053	75.38 \pm 0.028
		IC50 = 240 $\mu\text{g/ml}$	IC50 = 70 $\mu\text{g/ml}$

Table 7 The total Phenolic content of various extracts of aerial parts of Cadaba farinosa

S.No	Extracts	Total phenol content (mg/g of Gallic acid) (±SEM)*
1	Petroleum ether extract of <i>Cadaba farinosa</i>	0.69 ± 0.24
2	Ethyl acetate extract of <i>Cadaba farinosa</i>	3.98 ± 0.54
3	Methanolic extract of <i>Cadaba farinosa</i>	9.86 ± 0.62

Methodology

Gathering & Identification of Plant

The aerial parts *Cadaba farinosa* (family Cappariaceae) were gathered from Senkottai, Tirunelveli District of Tamilnadu, India. Plant identification was made from Botanical investigation of India, Palayamkottai. The *Cadaba farinosa* were desiccated under shadowy, segregate, crushed through a grinder. (Alagumanivasagam.G, Muthu.A, & Manavalan.R, 2012).

Preparation of Concentrates

The pulverised materials were packed in a muslin cloth and extracted with pet.ether, ethyl acetate and methanol as solvents respectively according to the increasing order of polarity (selvin & Muthu, 2010) through hot constant percolation method in Soxhlet equipment (Borse, Muthu, Thangatripathi, & Borse, 2012) for twenty-four hours. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophiliser till dry powder was acquired (SatheeshKumar, Muthu, & Manavalan, 2010).

Assessment of Antioxidant potential through invitro methods:

The variety of concentrates of *Cadaba farinosa* were used for assessment of antioxidant activity by (Kunchandy & Rao, 1990) method was adopted for Hydroxyl radical assay, (Benzie & Strain, 1996) method was utilised to determine the Iron chelating activity and determination of total phenolic compound were estimated by the methods of (Malik & Singh, 1980).

Results and Discussion

Hydroxyl radical scavenging activity

Hydroxyl radical is the most ROS and causes severe injury to the adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by

generating the hydroxyl radicals using ascorbic acid–iron EDTA. The hydroxyl radicals were produced by the oxidation reaction with the DMSO to give in HCHO, which provides a suitable method to identify hydroxyl radicals by treatment with Nash reagent (Pavithra & Vadivukkarasi, 2015). Hydroxyl radical activity was expressed in terms of % inhibition of generated free radicals respectively for various concentrations. Hydroxyl radical potential of PE extract of *Cadaba farinosa* appears in Table 1. The more Hydroxyl radical potential of PE extract and standard at 800 µg/ml was recorded at 47.25% and 84.37%. IC₅₀ of PE extract and standard was marked as 910µg/millilitre and 65µg/ millilitre correspondingly.

Hydroxyl radical potential of EA extract of *Cadaba farinosa* appeared in Table 2. The more Hydroxyl radical scavenging potential of EA extract and standard 800 µg/ml was recorded 55.45% and 84.37% correspondingly. EA extract and Quercetin IC₅₀ was recorded as 458µg/ml and 65µg/ml correspondingly.

Hydroxyl radical scavenging potential of methanolic extract of *Cadaba farinosa* appeared in Table 3. Hydroxyl radical scavenging potential was more in methanolic extract and quercetin (standard) at 800µg/ml was recorded 68.84% and 84.37%. Methanolic extract and standard IC₅₀ was recorded as 205µg/ml and 65µg/ml correspondingly.

IC₅₀ values and Hydroxyl radical potential revealed that methanol extract of *Cadaba farinosa* is a better activity in scavenging superoxide radical when compared EA and PE extracts. The methanolic extract of *Cadaba farinosa* exhibited higher ability in scavenging Hydroxyl radical when compared to the standard quercetin.

Iron chelating potential

The iron-chelating potential of all the extract was measured by Fe-ferrozine complex formation. Ferrozine-Fe complex is producing red coloured, which absorbs at 562nm (Yamaguchi, Ariga, Yoshimara, & Naxazawa, 2000). The iron complex potential of PE extract *Cadaba farinosa* and Ethylenediamine tetraacetate appears in Table 4. The iron-chelating potential was expressed in terms of % inhibition of generated free radicals, respectively concerning various concentrations. The more iron-binding potential of PE extract and Ethylenediamine tetraacetate 800 µg/ml were recorded, 50.16% and 75.38%.

The IC₅₀ of PE extract of *Cadaba farinosa* and Ethylenediamine tetraacetate were found as 810µg/ml and 70µg/ml correspondingly.

The iron complex potential of EA extract of *Cadaba farinosa* and Ethylenediamine tetraacetate was presented in [Table 5](#). The more iron-binding capacity of EA extract and Ethylenediamine tetraacetate 800 µg/ml was recorded at 63.59% and 75.38%. The IC₅₀ value of ethyl acetate extract of *Cadaba farinosa* and Ethylenediamine tetraacetate was found to be 508µg/ml and 70µg/ml correspondingly.

The iron complex potential of methanolic extract of *Cadaba farinosa* and Ethylenediamine tetraacetate is shown in [Table 6](#). The more iron-binding potential of methanolic extract and Ethylenediamine tetraacetate 800 µg/ml were recorded, 64.22% and 75.38%. The IC₅₀ value of methanol extract of *Cadaba farinosa* and Ethylenediamine tetraacetate was recorded as 240µg/ml and 70µg/ml correspondingly.

IC₅₀ values and iron-binding potential revealed that methanol extract of *Cadaba farinosa* is a massive activity in iron-chelating potential when compared ethyl acetate and petroleum ether extract. But when compared to all the three extracts, the methanol extract of the *Cadaba farinosa* showed a better result.

Total phenol

Phenolic compounds are famous as powerful chain-breaking antioxidants. The phenolic compounds might contribute directly to antioxidative action. The total amount of phenolic content of various extract of an aerial plant of *Cadaba farinosa* was present in [Table 7](#).

Based on the result, the methanolic extract of *Cadaba farinosa* was found higher content of phenolic components than that of petroleum ether and ethyl acetate extract of *Cadaba farinosa*. Phenols are essential to plant constituents because of their scavenging ability due to their hydroxyl groups ([Hatano, Edamatsu, & Mori, 1989](#)).

Conclusions

Among the three various extracts, methanolic extract of *Cadaba farinosa* exhibited higher potency of antioxidant activity due to the presence of total

phenolic compounds. Phenolic compounds are known to act as an antioxidant. These results indicate that aerial parts of methanolic extract *Cadaba farinosa* could serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.

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None

Conflict of interest statement

There are no conflicts of interest.

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