



Evaluation of in vitro antioxidant activity of different extracts of entire plant of *Ipomoea pestigridis* Linn

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ABSTRACT

Ipomoea pestigridis (Linn) (family Convolvulaceae) is commonly known as "Tiger Foot Morning Glory" in English and locally known as 'Pulichuvadi' or 'Pulichuvadu' in Malayalam. The current study, aerial parts of different concentrates (Pet. ether, ethyl acetate, and methanol) of *I.pestigridis*, was evaluated for its *in-vitro* antioxidant potential by nitric oxide activity, total antioxidant activity, iron chelating activity taking ascorbate & Ethylenediamine tetraacetate as the standard correspondingly. An IC₅₀ value was originated that EA concentrates of *I.pestigridis* more efficient in nitric oxide activity, total antioxidant activity, Iron chelating capacity compared methanolic & PE concentrates. The ethyl acetate concentrates of *I.pestigridis* & ascorbic acid exhibited antioxidant potential possessing IC₅₀ 226 μg/ml & 66 μg/ml (Nitric oxide). 185 μg/ml & 60 μg/ml (total antioxidant), 287 μg/ml & 65 μg/ml (iron-chelating Activity) respectively. The difference in the scavenging potential of the extracts can be due to variation in the percentage of bioactive compounds present in different solvents. *In vitro* antioxidant studies obviously show EA concentrates of *I.pestigridis* have better antioxidant activity. These results indicate that aerial parts of methanolic concentrate *I.pestigridis* could serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.



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INTRODUCTION

Free radicals might be defined as one or more unpaired electrons present in the molecules as it's outmost orbital and are able of autonomous existence. It is regularly represented as hydrogen rad-

ical (H[•]), which contains one proton and one electron (unpaired) is the simplest free radical. A free radical reaction might be finished by a reaction among two free radicals or by neutralization by substances such as the antioxidants (Fang *et al.*, 2002). Free radicals may be generated both *in vivo* and *in vitro* through one of the subsequent mechanisms. Loss of a single electron from a regular molecule. Addition of an electron to regular molecule (Halliwell and Gutteridge, 1999). Ethnomedical literature contains a huge amount of herbs that may be used for the various diseases, in which ROS and free radical participate vital responsibility. A huge numerical herbs are used for strong antioxidant activity (Badami *et al.*, 2003). Current reports revealed that there is a converse connection among the intake of antioxidant-rich foods and the occurrence of human diseases (Halliwell and Gutteridge, 1999).

Table 1: Nitric oxide scavenging activity of *I. pestigridis* PE Extract

S.no	Extract ($\mu\text{g/ml}$)	% of activity($\pm\text{SEM}$)*	
		PE extract	Ascorbic acid
1	125	34.65 \pm 0.025	48.54 \pm 0.046
2	250	40.34 \pm 0.042	57.31 \pm 0.034
3	500	45.34 \pm 0.052	68.24 \pm 0.045
4	1000	55.02 \pm 0.021	76.34 \pm 0.012
		IC ₅₀ = 750 $\mu\text{g/ml}$	IC ₅₀ = 135 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Ipomoea pestigridis (Linn) (family Convolvulaceae) is commonly known as "Tiger Foot Morning Glory" in English and locally known as 'Pulichuvadi' or 'Pulichuvadu' in Malayalam (Sahu and Gupta, 2014; Pawar and Patil, 2004). *I.pestigridis* was used for the treatment of wound healing (Austin, 1975; Amor-Prats and Harborne, 1993). *I.pestigridis* was used for different diseases like headaches, swellings, poisonous stings, snake bites. *I.pestigridis* was used for analgesic, antimicrobial, thrombocytic, cytotoxic activity (Pratap et al., 2011). Still, no literature are available on the antioxidant activity of the entire plant of *I.pestigridis*. Thus, the present study to assess the antioxidant activities of the entire plant of *I.pestigridis*.

MATERIALS AND METHODS

Collection and Identification of Plant

The entire plant of *I.pestigridis* (family Convolvulaceae) were gathered from kalakad, Tirunelveli District of Tamilnadu India. Plant identification was made from Botanical investigation of India, Palayamkottai. The *I.pestigridis* were desiccated under shadowy, segregated, crushed through a grinder (Satheeshkumar et al., 2011).

Preparation of Concentrates

The pulverized materials were progressively concentrated with PE (40-60°C) through hot constant percolation method in Soxhlet equipment (Harborne, 1984) for twenty-four hours. At that moment, the marc was subjected to EA (76-78°C) for 24 hrs & then marc was subjected to methanol for 24 hrs. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired. (AlagumaniVasagam et al., 2010).

Assessment of Antioxidant potential through invitro methods

The variety of concentrates of *I.pestigridis* were used for assessment of antioxidant activity by Garrat (1964) method was adopted for NO radical assay & Prieto

et al. (1999) method described for total antioxidant activity, and Benzie and Strain (1996) method was adopted to determine the Iron chelating activity.

RESULTS AND DISCUSSION

Nitric oxide scavenging activity

Nitric oxide scavenging activity of PE concentrates of *I. pestigridis* appeared in Table 1. The PE concentrates of *I. pestigridis* exhibit a more Nitric oxide scavenging activity of 55.02% at 1000 $\mu\text{g/ml}$ & ascorbate was recorded 76.34% at 1000 $\mu\text{g/ml}$. The IC₅₀ of the PE concentrates of *I. pestigridis* & ascorbic acid were recorded 750 $\mu\text{g/ml}$ & 135 $\mu\text{g/ml}$ correspondingly.

Nitric oxide scavenging activity of the EA concentrates of *I. pestigridis* appeared in Table 2. The EA concentrates of *I. pestigridis* exhibit a more Nitric oxide scavenging activity of 69.34% at 1000 $\mu\text{g/ml}$ & ascorbic acid was recorded at 76.34% at 1000 $\mu\text{g/ml}$. The IC₅₀ of the EA concentrates of *I. pestigridis* & ascorbic acid were recorded 198 $\mu\text{g/ml}$ & 135 $\mu\text{g/ml}$ correspondingly.

Nitric oxide scavenging activity of methanol concentrates of *I. pestigridis* appeared in Table 3. The methanol concentrates of *I. pestigridis* exhibit a more Nitric oxide scavenging activity of 66.22% at 1000 $\mu\text{g/ml}$ & ascorbic acid was recorded at 76.34% at 1000 $\mu\text{g/ml}$. The IC₅₀ of methanol concentrates of *I. pestigridis* & ascorbic acid were recorded 435 $\mu\text{g/ml}$ & 135 $\mu\text{g/ml}$ correspondingly.

IC₅₀ values & Nitric oxide scavenging potential revealed that ethyl acetate concentrates of *I. pestigridis* is better activity in scavenging Nitric oxide scavenging activity when compared methanol & PE extracts.

Phosphomolybdic acid method

Total antioxidant activity of PE concentrates of *I. pestigridis* appeared in Table 4. The PE concentrates of *I. pestigridis* exhibit a more total antioxidant activity of 39.55% at 300 $\mu\text{g/ml}$ & ascorbic acid was

Table 2: Nitric oxide scavenging activity of *I. pestigridis* EA Extract

S.no	Extract ($\mu\text{g/ml}$)	% of activity($\pm\text{SEM}$)*	
		EA extract	Ascorbic acid
1	125	43.54 \pm 0.022	48.54 \pm 0.046
2	250	55.32 \pm 0.043	57.31 \pm 0.034
3	500	64.56 \pm 0.065	68.24 \pm 0.045
4	1000	69.34 \pm 0.054	76.34 \pm 0.024
		IC ₅₀ = 198 $\mu\text{g/ml}$	IC ₅₀ = 135 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 3: Nitricoxide scavenging activity of *I. pestigridis* methanol Extract

S.no	Extract($\mu\text{g/ml}$)	% of activity($\pm\text{SEM}$)*	
		Methanolic extract	Ascorbate
1	125	32.34 \pm 0.025	48.54 \pm 0.046
2	250	44.84 \pm 0.054	57.31 \pm 0.034
3	500	52.38 \pm 0.062	68.24 \pm 0.045
4	1000	66.22 \pm 0.038	76.34 \pm 0.024
		IC ₅₀ = 435 mg/ml	IC ₅₀ = 135 mg/ml

*Every value was articulated as mean \pm SEM for 3 experimentation

Table 4: Total antioxidant activity of *I. pestigridis* PE Extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition($\pm\text{SEM}$)*	
		PE extract	Ascorbate
1	50	12.23 \pm 0.043	50.76 \pm 0.024
2	100	21.32 \pm 0.012	61.68 \pm 0.035
3	200	29.22 \pm 0.076	74.64 \pm 0.048
4	300	39.55 \pm 0.046	98.12 \pm 0.021
		IC ₅₀ = 460 $\mu\text{g/ml}$	IC ₅₀ = 57 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 5: Total antioxidant activity of *I. pestigridis* EA Extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition($\pm\text{SEM}$)*	
		EA extract	Ascorbate
1	50	33.45 \pm 0.043	50.76 \pm 0.024
2	100	40.65 \pm 0.054	61.68 \pm 0.035
3	200	50.85 \pm 0.062	74.64 \pm 0.048
4	300	59.46 \pm 0.014	98.12 \pm 0.021
		IC ₅₀ = 190 $\mu\text{g/ml}$	IC ₅₀ = 57 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 6: Total antioxidant activity of *I. pestigridis* methanol Extract

S.no	Extract ($\mu\text{g/ml}$)	% inhibition($\pm\text{SEM}$)*	
		Methanolic extract	Ascorbate
1	50	17.82 \pm 0.035	50.76 \pm 0.024
2	100	26.54 \pm 0.042	61.68 \pm 0.035
3	200	37.22 \pm 0.033	74.64 \pm 0.048
4	300	44.32 \pm 0.012	98.12 \pm 0.021
		IC ₅₀ = 340 $\mu\text{g/ml}$	IC ₅₀ = 57 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 7: Iron-binding potential of *I.pestigridis* PE concentrates

S.no	Extract ($\mu\text{g/ml}$)	% inhibition ($\pm\text{SEM}$)*	
		PE extract	Ethylenediamine tetraacetate
1	125	29.56 \pm 0.018	57.52 \pm 0.014
2	250	37.48 \pm 0.038	64.76 \pm 0.022
3	500	45.23 \pm 0.042	82.12 \pm 0.045
4	1000	55.48 \pm 0.023	96.34 \pm 0.034
		IC ₅₀ = 760 $\mu\text{g/ml}$	IC ₅₀ = 65 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 8: Iron-binding potential of *I.pestigridis* of EA concentrates

S.no	Extract ($\mu\text{g/ml}$)	% inhibition ($\pm\text{SEM}$)*	
		EA extract	Ethylenediamine tetraacetate
1	125	38.12 \pm 0.010	57.52 \pm 0.014
2	250	53.92 \pm 0.024	64.76 \pm 0.022
3	500	61.43 \pm 0.036	82.12 \pm 0.045
4	1000	69.87 \pm 0.065	96.34 \pm 0.034
		IC ₅₀ = 217 $\mu\text{g/ml}$	IC ₅₀ = 65 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

Table 9: Iron-binding potential of *I.pestigridis* Methanolic concentrates

S.no	Extract ($\mu\text{g/ml}$)	% inhibition($\pm\text{SEM}$)*	
		Methanol extract	EthyleneDiamine tetraacetate
1	125	30.84 \pm 0.045	57.52 \pm 0.014
2	250	43.22 \pm 0.063	64.76 \pm 0.022
3	500	55.84 \pm 0.072	82.12 \pm 0.045
4	1000	67.32 \pm 0.028	96.34 \pm 0.034
		IC ₅₀ = 385 $\mu\text{g/ml}$	IC ₅₀ = 65 $\mu\text{g/ml}$

* Every value was articulated as mean \pm SEM for 3 experimentation

recorded at 98.12% at 300 $\mu\text{g/ml}$. The IC_{50} of the PE concentrates of *I. pestigridis* & ascorbic acid were recorded 460 $\mu\text{g/ml}$ & 57 $\mu\text{g/ml}$ correspondingly.

The total antioxidant activity of the EA concentrates of *I. pestigridis* appeared in Table 5. The EA concentrates of *I. pestigridis* exhibit a more total antioxidant activity of 59.46% at 300 $\mu\text{g/ml}$ & ascorbic acid was recorded at 98.12% at 300 $\mu\text{g/ml}$. The IC_{50} of the EA concentrates of *I. pestigridis* & ascorbic acid were recorded 190 $\mu\text{g/ml}$ & 57 $\mu\text{g/ml}$ correspondingly.

The total antioxidant activity of methanol concentrates of *I. pestigridis* appeared in Table 6. The methanol concentrates of *I. pestigridis* exhibit a more total antioxidant activity of 44.32% in both table (Table 6) and text. The IC_{50} of the methanol concentrates of *I. pestigridis* & ascorbic acid were recorded 460 $\mu\text{g/ml}$ & 57 $\mu\text{g/ml}$ correspondingly.

IC_{50} values & total antioxidant potential revealed that ethyl acetate concentrates of *I. pestigridis* is better activity in scavenging total antioxidant potential when compared methanol & PE extracts

Iron chelating potential

The iron complex potential of PE concentrates *I. pestigridis* & Ethylenediamine tetraacetate were appeared in Table 7. The more iron-binding potential of PE concentrates & Ethylenediamine tetraacetate 1000 $\mu\text{g/ml}$ were recorded, 55.48% & 96.34%. The IC_{50} of PE concentrates of *I. pestigridis* & Ethylenediamine tetraacetate were found as 760 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

The iron complex potential of EA concentrates of *I. pestigridis* & Ethylenediamine tetraacetate were presented in Table 8. The more iron-binding capacity of EA concentrates & Ethylenediamine tetraacetate 1000 $\mu\text{g/ml}$ was recorded at 69.87% & 96.34%. The IC_{50} value of ethyl acetate concentrates of *I. pestigridis* & Ethylenediamine tetraacetate were found 217 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

The iron complex potential of methanolic concentrates of *I. pestigridis* & Ethylenediamine tetraacetate were presented in Table 9. The more iron-binding potential of methanolic concentrates & Ethylenediamine tetraacetate 1000 $\mu\text{g/ml}$ were recorded, 67.32% & 96.34%. The IC_{50} value of methanol concentrates of *I. pestigridis* & Ethylenediamine tetraacetate was recorded as 385 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

IC_{50} values & iron-binding potential revealed that ethyl acetate concentrates of *I. pestigridis* is a huge activity in iron-chelating potential when compared methanol & petroleum ether concentrates. But when compared to all the three concentrates,

the ethyl acetate concentrates of the *I. pestigridis* showed the better result.

Assessment of antioxidant activity, so many *in vitro* methods have been used a variety of concentrates of *I. pestigridis*. The results of antioxidant activity by NO radical activity, total antioxidant activity & iron-chelating potential were expressed in terms of % inhibition of generated free radicals, respectively, with respect to various concentrations. NO is produced from L-arginine through vascular endothelial cells, phagocytes, and certain cells of the brain. NO is a free radical since its unpaired electron and displays significant reactivity with confident types of proteins and other free radicals. The toxicity of Nitric oxide becomes a side effect when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion (ONOO^-) (Nagmoti *et al.*, 2012). NO produced from sodium nitroprusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylenediamine, producing pink coloured, which absorbs at 546 nm (Balakrishnan *et al.*, 2009). Among the three different plant concentrates tested, interestingly, in the NO radical activity of the EA extract of *I. pestigridis* exhibited more NO radical potential comparable with that of ascorbic acid.

The iron-chelating potential of all the concentrates was measured by Fe-ferrozine complex formation. Ferrozine-Fe complex is producing red coloured, which absorbs at 562nm (Yamaguchi *et al.*, 2000). It was revealed that Ethylenediamine tetraacetate, which forms σ bond with iron, are efficient as secondary antioxidants, for the basis that they decrease the redox potential, thereby stabilizing the oxidized form of the iron ion (Duh *et al.*, 1999). The iron-chelating potential of ethyl acetate extract of *I. pestigridis* exhibited a higher ability in scavenging compared to standard Ethylenediamine tetraacetate.

CONCLUSIONS

The current trends, antioxidative activity of the herbs having more interest due to their possible use as natural additives to substitute synthetic ones. Among the three various extract ethyl acetate extracts of *I. pestigridis* exhibited higher potency of antioxidant activity. These results indicate that ethyl acetate extract of *I. pestigridis* might serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.

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