Original Article

Evaluation of *In-vitro* Antioxidant Potential of Whole Plant of Various Extracts of *Trianthema decandra*.

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ABSTRACT

The present investigation was to examine the *in-vitro* antioxidant potential of various extracts of whole plant of *Trianthema decandra* by different *in-vitro* methods. The antioxidant activity was determined by DPPH activity, superoxide radical scavenging and iron chelating activity. An IC_{50} value was found that methanolic extract of *Trianthema decandra* is more effective in free radical scavenging activity than that of other two extracts. So, the *in-vitro* studies clearly showed that the methanolic extract of *Trianthema decandra* has a significant antioxidant activity. These *in-vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *In-vitro* antioxidant, DPPH activity, Superoxide radical activity, Iron chelating activity.

INTRODUCTION

Oxygen-derived free radicals such as super oxide anion and hydroxyl radical are cytotoxic and promote tissue injuries. Antioxidants act as a major defense against radical-mediated toxicity by protecting against the damages caused by free radicals[1]. The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress[2]. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[3].

Trianthema decandra (Aizoaceae) is a prostrate, glabrous, succulent, annual herb found almost throughout India. It is

commonly known as gadabani in Hindi and vellai sharunai in Tamil[4]. *Trianthema decandra* has been used in various parts of Asia, Africa, Australia and South America for curing various diseases. In African countries the plant has been popular use for skin diseases, wound healing, fever and tooth aches[5]. The juice of leaves is used to treat the black quarter[6]. The bitter roots are used for curing bacterial infections and it is also given in combination with ginger as a cathartic[7]. The leaves contain huge amount of vitamin C which is used in the treatment of oedema[8]. A decoction of the herb is used as a vermifuge and is useful in rheumatitis. It is also an antidote to alcoholic poison[9]. However, no data are available in the literature on the antioxidant activity of whole plant of *Trianthema decandra*. Therefore we undertook the present investigation to examine the antioxidant activities of various extract of whole plant of *Trianthema decandra* through different *in vitro* models.

MATERIALS AND METHODS

Collection and Identification of Plant materials

The whole plant of *Trianthema decandra*, were collected from Senkottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India (Voucher Number 6471 dated 10-09-1998). Palayamkottai. The plant material, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[10] for 24 hrs then mark was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by *in vitro* Techniques

DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[11]. A methanolic solution of 0.5 ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows.

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

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

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method[12]. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[12]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5mM NBT) solution, 0.2ml of EDTA (0.1M EDTA), 0.05ml riboflavin (0.12mM) and 2.55ml 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 illuminated for 30 min

and the absorbance at 560nm was measured against the control samples. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity

The method of Benzie and strain (1996)[13] 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 2ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[14]. They are also involved in autoimmune disorders like rheumatoid arthritis etc.

DPPH scavenging activity

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Trianthema decandra* depicted in Table 1. The IC_{50} values of the petroleum ether extract of *Trianthema decandra* and Rutin were found to be 1350µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Trianthema decandra* depicted in Table 2. The IC_{50} values of the ethyl acetate extract of *Trianthema decandra* and Rutin were found to be 1150µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of methanolic extract of *Trianthema decandra* depicted in Table 3. The IC_{50} values of the methanolic extract of *Trianthema decandra* and Rutin was found to be 420µg/ml and 480µg/ml respectively.

From the result indicated the IC_{50} values of methanolic extract of *Trianthema decandra* exhibits significant antioxidant activity when compared to that standard Rutin. IC_{50} value of plant extract and Rutin was recorded as 420µg/ml and 480µg/ml respectively. But other two extracts showed lower activity when compared to that of methanolic extract of *Trianthema decandra* and standard Rutin.

Superoxide anion scavenging activity

Percentage scavenging of superoxide anion activity of petroleum ether extract of *Trianthema decandra* was depicted in table 4. The IC_{50} value of plant extract and Quercetin was recorded as 1080µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion activity of ethyl acetate extract of *Trianthema decandra* was depicted in table 5. The IC_{50} value of plant extract and Quercetin was recorded as 380µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion activity of methanolic extract of *Trianthema decandra* was depicted in table 6. The IC_{50} value of plant extract and Quercetin was recorded as 180µg/ml and 60µg/ml respectively.

Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that methanolic extract of *Trianthema decandra* is more effective in scavenging superoxide radical than that of Quercetin (standard).

Iron chelating activity

Iron binding capacity of the petroleum ether extract of *Trianthema decandra* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000µg/ml) were examined and the values were presented in table 7. The IC_{50} value of plant extract and EDTA was recorded as 1170µg/ml and 65µg/ml respectively.

Iron binding capacity of the ethyl acetate extract of *Trianthema decandra* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000µg/ml) were examined and the values were presented in table 8. The IC_{50} value of plant extract and EDTA was recorded as 450µg/ml and 65µg/ml respectively.

Iron binding capacity of the methanolic extract of *Trianthema decandra* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000µg/ml) were examined and the values were presented in table 9. The IC_{50} value of plant extract and EDTA was recorded as 70µg/ml and 65µg/ml respectively.

From the result indicated the IC_{50} value of methanolic extract was found more effective than that of EDTA as a standard. Based on the above results indicated, the methanolic extract of *Trianthema decandra* exhibited significant antioxidant activity was comparable to that of petroleum ether & ethyl acetate extracts of *Trianthema decandra*.

CONCLUSION

The present study was clearly indicated the methanolic extract of *Trianthema decandra* showed strong antioxidant activity when compared with other extracts. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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Table 1. Effect of Petroleum ether extract of *Trianthema decandra* on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	17.97 ± 0.070	18.85 ± 0.076
2	250	20.53 ± 0.010	22.08 ± 0.054
3	500	23.56 ± 0.015	52.21 ± 0.022
4	1000	35.62 ± 0.091	69.83 ± 0.014
		IC_{50} = 1350µg/ml	**IC_{50} = 480µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 2**.** Effect of Ethyl acetate extract of *Trianthema decandra* on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	14.04 ± 0.081	18.85 ± 0.076
2	250	24.93 ± 0.067	22.08 ± 0.054
3	500	39.27 ± 0.042	52.21 ± 0.022
4	1000	45.98 ± 0.039	69.83 ± 0.014
		IC_{50} = 1150µg/ml	**IC_{50} = 480µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 3**.** Effect of Methanolic extract of *Trianthema decandra* on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	21.51 ± 0.012	18.85 ± 0.076
2	250	24.31 ± 0.049	22.08 ± 0.054
3	500	62.63 ± 0.036	52.21 ± 0.022
4	1000	69.60 ± 0.024	69.83 ± 0.014
		IC_{50} = 90µg/ml	**IC_{50} = 480µg/ml**

*All values are expressed as mean ± SEM for three determinations

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Table 4. Effect of Petroleum ether extract of *Trianthema decandra* on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	18.06 ±0 .015	1
2	250	26.32 ± 0.049	2
3	500	37.62 ± 0.030	3
4	1000	62.80 ± 0.027	4
		IC_{50} = 1080µg/ml	**IC_{50} = 60µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 5. Effect of Ethyl acetate extract of *Trianthema decandra* on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	13.48 ± 0.051	1
2	250	15.54 ± 0.029	2
3	500	55.43 ± 0.031	3
4	1000	63.98 ± 0.019	4
		IC_{50} = 380µg/ml	**IC_{50} = 60µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 6. Effect of Methanolic extract of *Trianthema decandra* on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	40.29 ± 0.015	1
2	250	61.93 ± 0.029	2
3	500	68.47 ± 0.032	3
4	1000	85.38 ± 0.028	4
		IC_{50} = 180µg/ml	**IC_{50} = 60µg/ml**

*All values are expressed as mean ± SEM for three determinations

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Table 7. Effect of Pet. ether extract of *Trianthema decandra* on Iron-chelating method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	Standard (EDTA)
1	125	18.20 ± 0.020	1
2	250	28.80 ± 0.037	2
3	500	33.35 ± 0.029	3
4	1000	47.35 ± 0.022	4
		IC_{50} = 1170µg/ml	**IC_{50} = 65µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 8. Effect of Ethyl acetate extract of *Trianthema decandra* on Iron-chelating method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (EDTA)
1	125	14.93 ± 0.016	1
2	250	24.54 ± 0.011	2
3	500	81.41 ± 0.029	3
4	1000	87.87 ± 0.021	4
		IC_{50} =450µg/ml	**IC_{50} = 65µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 9. Effect of Methanolic extract of *Trianthema decandra* on Iron-chelating method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	53.65 ± 0.044	1
2	250	63.35 ± 0.029	2
3	500	75.39 ± 0.036	3
4	1000	81.18 ± 0.013	4
		IC_{50} = 70µg/ml	**IC_{50} = 65µg/ml**

*All values are expressed as mean ± SEM for three determinations