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Free Radical Scavenging Activity OF Various Extracts from Root of *Clerodendrum Phlomidis* (Linn.) Evaluated by three *in vitro* methods

M Sathish*, CB Tharani, V Niraimathi

College of Pharmacy, Dept of Pharmaceutical Chemistry, Madras Medical College, Chennai-600 003, India.

ABSTRACT

The free radical scavenging activity of root of *Clerodendrum phlomidis* extracts, obtained by sequential extraction with various polarities of solvents (Petroleum ether, chloroform, ethyl acetate and ethanol) was evaluated by three different *in vitro* methods: DPPH radical scavenging, superoxide anion radical scavenging and total antioxidant activity. The ethanolic extract showed best free radical scavenging activity than that of other three extracts. The super oxide radical scavenging activity of ethanolic extract (IC_{50} = 60 µg/ml) was better than that of standard Quercetin (IC_{50} = 130 µg/ml). The good total antioxidant activity was observed in ethanolic extract of *Clerodendrum phlomidis* than that of other extracts. It showed good reducing capacity assessment. The high antioxidant capacity observed for ethanolic extract of *Clerodendrum phlomidis* suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

Keywords: *Clerodendrum phlomidis, Invitro* antioxidant, DPPH, Super oxide anion radical activity, total antioxidant activity.

Corresponding author

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INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias [1]. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing .However; there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis [2]. There is growing interest toward natural antioxidants from herbal sources [3-5]. Epidemiological and in vitro studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological system [6-8].

Clerodendrum phlomidis, Linn. f. suppl. is belonging to the family *Verbanaceae,* which is mentioned under the common name of Arni and/or Agnimantha in Ayurveda [9]. Their roots are important ingredient of Ayurvedic preparations like Dashmoolakwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaaktel, Narayan tel etc., valued for the treatment of variety of aliments [10]. *C. phlomidis* roots are valued as tonic, diuretic, febrifuge, anti-diabetic, anti-inflammatory, antidiahhoreal and antitussive [11-13]. Phytochemical studies include presence of b- sitosterol and g- sitosterol, ceryl alcohol, clerodin, clerosterol, clerodendrin-A [14] and flavanoids, pectolinarigenin, hispidulin, apigenin luteolin [15].

Literature survey revealed that there is a lack of enough scientific reports regarding antioxidant activity of the various extracts from roots of *Clerodendrum phlomidis.* Hence the objective of the present investigation was to evaluate the free radical scavenging activity of various extracts from root of *Clerodendrum phlomidis* with three *in vitro* antioxidant methods.

MATERIAL AND METHODS

Plant materials

The roots of *Clerodendrum phlomidis*, were collected from Chennai, Tamil Nadu, India. The plant material was identified by Dr.Sasikala Ethirajulu, Research officer, CCRAS, Govt.of India, Chennai. The roots of *Clerodendrum phlomidis* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The dried powder of the root was extracted sequentially by hot continuous percolation method using Soxhlet apparatus [16], using different polarities of solvents like petroleum ether, chloroform, ethyl acetate and ethanol. The dried root powder was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 hrs. Then the mark was subjected to

chloroform for 24 hrs, and the mark was subjected to ethyl acetate for 24 hrs and then mark was subjected to ethanol 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 methods

DPPH radical scavenging activity [17]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001) [17]. A methanolic solution of 0.5ml 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 518 nm 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 anion radical scavenging activity [18]

Superoxide radical (O_2^-) was generated from the photoreduction 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 et al (1975) [18]. 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 illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate 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.

Total antioxidant activity (Phosphomolybdic acid method) [19]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [19]. An aliquot of 0.4 ml of sample solution was combined 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 95^0C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

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RESULTS AND DISCUSSION

Inhibition of DPPH radical

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. The reduction capability of DPPH radicals was determined by the decreases in its absorbance at 517 nm, which is induced by antioxidants.

Table 1: DPPH radical scavenging activity of petroleum ether extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	12.67 ± 0.13	28.39 ± 0.25
2	250	13.30 ± 0.32	48.98 ± 0.46
3	500	21.04 ± 0.67	65.18 ± 0.13
4	1000	48.23 ± 0.29	70.65 ± 0.20
		IC_{50} = 1090µg/ml	**IC_{50} = 270µg/ml**

*Data presented as the mean ± SEM for three measurements.

Table 1 was depicts the percentage of DPPH radical scavenging activity of petroleum ether extract extracts of root of *Clerodendrum phlomidis.* The IC_{50} values of the petroleum ether extract of *Clerodendrum phlomidis* and Rutin were found to be 1090µg/ml and 270µg/ml respectively.

Table 2: DPPH radical scavenging activity of chloroform extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Chloroform extract)	Standard (Rutin)
1	125	13.70 ± 0.15	28.39 ± 0.25
2	250	19.63 ± 0.09	48.98 ± 0.46
3	500	26.32 ± 0.07	65.18 ± 0.13
4	1000	49.38 ± 0.21	70.65 ± 0.20
		IC_{50} = 1010µg/ml	**IC_{50} = 270µg/ml**

*Data presented as the mean ± SEM for three measurements

The percentage of DPPH radical scavenging activity of chloroform extract of *Clerodendrum phlomidis* depicted in Table 2. The IC_{50} values of the chloroform extract of *Clerodendrum phlomidis* and Rutin were found to be 1010µg/ml and 270µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Clerodendrum phlomidis* depicted in Table 3. The IC_{50} values of the ethyl acetate extract of *Clerodendrum phlomidis* and Rutin were found to be 1010µg/ml and 270µg/ml respectively.

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The percentage of DPPH radical scavenging activity of ethanolic extract of *Clerodendrum phlomidis* depicted in Table 4. The IC_{50} values of the ethanolic extract of *Clerodendrum phlomidis* and Rutin was found to be 250µg/ml and 270µg/ml respectively.

Table 3: DPPH radical scavenging activity of ethyl acetate extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	28.72 ± 0.83	28.39 ± 0.25
2	250	38.74 ± 0.42	48.98 ± 0.46
3	500	48.73 ± 0.43	65.18 ± 0.13
4	1000	61.44 ± 0.66	70.65 ± 0.20
		IC_{50} =520 µg/ml	**IC_{50} = 270µg/ml**

*Data presented as the mean ± SEM for three measurements

Table 4: DPPH radical scavenging activity of Ethanolic extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Rutin)
1	125	36.43 ± 0.25	28.39 ± 0.25
2	250	50.44 ± 0.54	48.98 ± 0.46
3	500	58.69 ± 0.48	65.18 ± 0.13
4	1000	65.58 ± 0.44	70.65 ± 0.20
		IC_{50} = 250µg/ml	**IC_{50} = 270µg/ml**

*Data presented as the mean ± SEM for three measurements

Based on the result clearly indicated, that the ethanolic extract of *Clerodendrum phlomidis* exhibits significant antioxidant activity when compared with other three extracts. In comparison of all the extracts with standard Rutin, the ethanolic extract of *Clerodendrum phlomidis* was found more radical scavenging activity than that of standard. An IC_{50} value of ethanolic extract of *Clerodendrum phlomidis* and Rutin was recorded as 250µg/ml and 270µg/ml respectively.

Inhibition of Superoxide anion radical scavenging activity

Table 5: Superoxide anion radical scavenging activity of Petroleum ether extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	Standard (Quercetin)
1	125	13.26 ± 0.48	49.44 ± 0.48
2	250	25.17 ± 0.42	61.88 ± 0.49
3	500	32.06 ± 0.52	78.39 ± 0.18
4	1000	39.14 ± 0.43	89.28 ± 0.09
		IC_{50} = 1270 µg/ml	**IC_{50} = 130 µg/ml**

*Data presented as the mean ± SEM for three measurements

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Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical [21]. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavonoids [22].

The percentage of superoxide anion scavenging activity of petroleum ether extract of *Clerodendrum phlomidis* was presented in Table 5. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 39.14% and 89.28% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 1270µg/ml and 130µg/ml respectively.

Table 6: Superoxide anion radical scavenging activity of Chloroform extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Chloroform extract)	Standard (Quercetin)
1	125	21.87 ± 0.33	49.44 ± 0.48
2	250	33.84 ± 0.58	61.88 ± 0.49
3	500	40.67 ± 0.22	78.39 ± 0.18
4	1000	49.42 ± 0.15	89.28 ± 0.09
		IC_{50} = 1010 µg/ml	**IC_{50} = 130 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of superoxide anion scavenging activity of Chloroform extract of *Clerodendrum phlomidis* was presented in Table 6. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 49.42% and 89.28% respectively. The IC_{50} value of plant and Quercetin was recorded as 1010µg/ml and 130µg/ml respectively.

Table 7: Superoxide anion radical scavenging activity of ethyl acetate extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	22.07 ± 0.32	49.44 ± 0.48
2	250	38.43 ± 0.19	61.88 ± 0.49
3	500	55.47 ± 0.13	78.39 ± 0.18
4	1000	67.39 ± 0.09	89.28 ± 0.09
		IC_{50} = 400 µg/ml	**IC_{50} = 130 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of superoxide anion scavenging activity of ethyl acetate extract of *Clerodendrum phlomidis* was presented in Table 7. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 67.39% and 89.28% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 400µg/ml and 130µg/ml respectively.

The percentage of superoxide anion scavenging activity of ethanolic extract of *Clerodendrum phlomidis* was presented in Table 8. Maximum scavenging activity of plant

extract and Quercetin at 1000 µg/ml was found to be 84.41% and 89.28% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 60µg/ml and 130µg/ml respectively.

Table 8: Superoxide anion radical scavenging activity of ethanolic extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Quercetin)
1	125	64.41 ± 0.50	49.44 ± 0.48
2	250	68.22 ± 0.11	61.88 ± 0.49
3	500	81.10 ± 0.31	78.39 ± 0.18
4	1000	84.41 ± 0.03	89.28 ± 0.09
		IC_{50} =60 µg/ml	**IC_{50} = 130 µg/ml**

*Data presented as the mean ± SEM for three measurements.

Based on the result, the ethanolic extract of *Clerodendrum phlomidis* was found more superoxide anion radical scavenging activity than that of other three extracts. When compared all the four extracts with standard Quercetin, the ethanolic extract of *Clerodendrum phlomidis* were showed significant radical scavenging activity. An IC_{50} value of ethanolic extract of *Clerodendrum phlomidis* and Quercetin was recorded as 60µg/ml and 130µg/ml respectively.

Determination of Total antioxidant activity (Phosphomolybdic acid method)

Table 9: Total antioxidant activity of Petroleum ether extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.26±0.09	26.87 ± 0.08
2	250	24.39±0.19	30.30 ± 0.05
3	500	33.42±0.27	60.64 ± 0.02
4	1000	39.41±0.14	55.23 ± 0.01
		IC_{50} = 1270µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of total antioxidant activity of petroleum ether extract of *Clerodendrum phlomidis* depicted in Table 9. The petroleum ether extract of *Clerodendrum phlomidis* exhibited a maximum total antioxidant activity of 39.41% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50}values of the petroleum ether extract of *Clerodendrum phlomidis* and ascorbate were found to be 1270µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of chloroform extract of *Clerodendrum phlomidis* presented in Table 10 The chloroform extract of *Clerodendrum phlomidis* exhibited a maximum total antioxidant activity of 45.49% at 1000 µg/ml whereas for ascorbate (standard)

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was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the chloroform extract of *Clerodendrum phlomidis* and ascorbate were found to be 1120µg/ml and 410µg/ml respectively.

Table 10: Total antioxidant activity of chloroform extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Chloroform extract)	Standard (Ascorbate)
1	125	13.36±0.16	26.87 ± 0.08
2	250	17.71±0.50	30.30 ± 0.05
3	500	40.12±0.43	60.64 ± 0.02
4	1000	45.49±0.16	55.23 ± 0.01
		IC_{50} = 1120µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

Table 11: Total antioxidant activity of ethyl acetate extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	19.20±0.02	26.87 ± 0.08
2	250	27.23±0.21	30.30 ± 0.05
3	500	49.79±0.20	60.64 ± 0.02
4	1000	56.65±0.05	55.23 ± 0.01
		IC_{50} = 550µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of total antioxidant activity of ethyl acetate extract of *Clerodendrum phlomidis* depicted in Table 11. The ethyl acetate extract of *Clerodendrum phlomidis* exhibited a maximum total antioxidant activity of 56.65% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50}values of the ethyl acetate extract of *Clerodendrum phlomidis* and ascorbate were found to be 550µg/ml and 410µg/ml respectively.

Table 12: Total antioxidant activity of ethanolic extract of *Clerodendrum phlomidis*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	39.20±0.02	26.87 ± 0.08
2	250	49.73±0.29	30.30 ± 0.05
3	500	61.29±0.70	60.64 ± 0.02
4	1000	69.65±0.05	55.23 ± 0.01
		IC_{50} = 260µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of total antioxidant activity of ethanolic extract of *Clerodendrum phlomidis* presented in Table 12. The ethanolic extract of *Clerodendrum phlomidis* exhibited a maximum total antioxidant activity of 69.65% at 1000 µg/ml whereas for ascorbate (standard)

was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the ethanolic extract of *Clerodendrum phlomidis* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

Based on the result clearly indicated the ethanolic extract of *Clerodendrum phlomidis* was found to be more effective than that of other extracts. But when compare all the extracts with standard the ethanolic extract of *Clerodendrum phlomidis* was found strong total antioxidant activity. The IC_{50} values of the ethanolic extract of *Clerodendrum phlomidis* and Ascorbate were found to be 260µg/ml and 410µg/ml respectively.

CONCLUSION

The results of the present study was clearly indicated that the ethanolic extract of *Clerodendrum phlomidis* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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