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Research Article

IN-VITRO ANTIOXIDATIVE ACTIVITY OF PHENOLIC AND FLAVONOID COMPOUNDS EXTRACTED FROM ROOT OF *CLERODENDRUM PHLOMIDIS* (LINN.)

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ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoids content, and to evaluate in-vitro antioxidant activity of various extracts from root of *Clerodendrum phlomidis*. The ethanolic extract of *Clerodendrum phlomidis* was showed significant free radical scavenging activity than that of other three extracts. The greater amount of phenols and flavonoids were found in ethanolic extract of *Clerodendrum phlomidis* than that of other extracts. The radical scavenging activity was found to be concentration dependent manner. Further studies on isolation of constituents from the extract and their biological activities are under investigation.

Keywords: *Clerodendrum phlomidis,* Antioxidant activities, Phenolics, Flavonoids, Radical scavenging.

INTRODUCTION

Free radicals produced from oxygen to form reactive oxygen species such as the singlet oxygen, superoxide, peroxyl, hydroxyl and peroxynitrite radicals, are constantly produced within living cells for specific metabolic purposes[1]. Living cells have complex mechanisms that act as antioxidant systems to counteract the damaging effects of reactive species. 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].

Natural compounds, which are present in herbal products, vegetables, fruits and grains, possess the ability to reduce oxidative damage by acting as antioxidants[6]. 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 systems[7-9].

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[10]. Their roots are important ingredient of Ayurvedic preparations like Dashmoolakwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaaktel etc, valued for the treatment of variety of aliments[11]. *C. phlomidis* roots are valued as tonic, diuretic, febrifuge, anti-diabetic, anti-inflammatory, anti diarrhoeal and antitussive[12-14].Phytochemical studies include presence of b- sitosterol and g- sitosterol, ceryl alcohol, clerodin, clerosterol, clerodendrin-A[15] and flavanoids, pectolinarigenin, hispidulin, apigenin luteolin[16].The present investigation was undertaken to determine the *in -vitro* antioxidative activity of phenolic and flavonoid compounds extracted from root of *Clerodendrum phlomidis* (Linn.).

MATERIAL AND METHODS

Collection and identification of the 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 root 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 roots was extracted sequentially by hot continuous percolation method using Soxhlet apparatus[17], 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 marc was subjected to chloroform for 24 hrs, and the marc was subjected to ethyl acetate for 24 hrs and then marc 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

Determination of Hydroxyl radical scavenging activity[18]

This was assayed as described by Elizabeth and Rao (1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbate–EDTA–H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37ºC. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Determination of Nitric oxide radical scavenging activity[19]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964).The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25ºC for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

FRAP assay[20]

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3$.$6H_2O$. The temperature of the solution was raised to 37ºC before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored

product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Iron chelating activity [20]

The method of Benzie and strain (1996) 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 2 ml 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 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Estimation of total phenol[21]

0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Estimation of total flavonoids[22]

0.5 ml of extract and 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[23]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[24].

Hydroxyl radical scavenging activity

The percentage of Hydroxyl radical scavenging activity of various extracts of *Clerodendrum phlomidis* was presented in Table 1. The IC_{50} values of petroleum ether, chloroform, ethyl acetate and ethanolic extract of *Clerodendrum phlomidis* were found to be 1470µg/ml, 1350 µg/ml, 1080 µg/ml and 110µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml.

Table 1: Hydroxyl radical scavenging activity of various extracts of *Clerodendrum phlomidis*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	11.18±0.03	20.15±0.04	24.01±0.44	32.37±0.13	1470
Chloroform extract	19.65±0.11	30.82±0.30	33.49±0.18	41.31±0.28	1350
Ethyl acetate extract	18.73±0.13	35.92±0.40	46.30±0.12	48.81±0.43	1080
Ethanolic extract	51.81±0.19	59.60±0.06	67.33±0.07	80.18±0.11	110
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

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

Data presented in the above table revealed that the ethanolic extract of *Clerodendrum phlomidis* was showed a significant antioxidant activity when compared with standard ascorbate. Similar result was not found in other extracts.

Nitric oxide radical scavenging activity

Table 2 was shows the scavenging of nitric oxide radical by various extracts of *Clerodendrum phlomidis* and ascorbate. The IC_{50} values of various extracts (petroleum ether, chloroform, ethyl acetate and ethanolic) of *Clerodendrum phlomidis* were found to be 1410µg/ml, 1365 µg/ml, 550 µg/ml and 135µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml.

Based on the below results clearly in table 2, indicated that the ethanolic extract of *Clerodendrum phlomidis* were found more effective nitric oxide scavenging activity than that of other extracts. But when compare to the all the extracts with ascorbate (standard), the ethanolic extract of the *Clerodendrum phlomidis* was showed the strong nitric oxide radical scavenging activity than that of standard.

Table 2: Nitric oxide radical scavenging activity of various extracts of *Clerodendrum phlomidis*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	16.27±0.08	20.72±0.32	28.70±0.16	33.17±0.02	1410
Chloroform extract	27.42±0.09	31.03±0.11	33.67±0.05	38.52±0.03	1365
Ethyl acetate extract	35.32±0.06	40.46±0.21	45.51±0.14	53.69±0.01	550
Ethanolic extract	48.36±0.16	56.07±0.14	62.10±0.11	69.54±0.12	135
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

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

Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components[25].

Iron binding capacity of the various extracts of *Clerodendrum phlomidis* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were summarized in Table 3.The IC_{50} values of various extracts (petroleum ether, chloroform, ethyl acetate and ethanolic) of *Clerodendrum phlomidis* were found to be 1395µg/ml, 1360 µg/ml, 475 µg/ml and 140µg/ml respectively. Whereas, the IC_{50} value of standard EDTA was observed 65µg/ml.

Based on the findings in table 3, clearly indicated that, the ethanolic extract of *Clerodendrum phlomidis* were showed more effective metal chelating activity than that of other extracts. The results indicted the plant extract possess iron binding capacity which might be due to the presence of polyphenols that averts the cell from free radical damage by reducing of transition metal ions[26].

FRAP Assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 4 was depicted the FRAP values of various extracts of *Clerodendrum phlomidis* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The IC_{50} values of various extracts (petroleum ether, chloroform, ethyl acetate and ethanolic) of *Clerodendrum phlomidis* were found to be 1410µg/ml, 1355 µg/ml, 590 µg/ml and 355µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml. The ethanolic extract of *Clerodendrum phlomidis* was showed significant antioxidant activity than that of other extracts.

Table 3: Iron-chelating activity of various extracts of *Clerodendrum phlomidis*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	14.24±0.17	20.95±0.35	27.62±0.03	36.32±0.06	1395
Chloroform extract	19.38±0.06	27.63±0.03	33.49±0.12	39.76±0.27	1360
Ethyl acetate extract	28.81±0.23	35.34±0.15	51.46±0.11	53.42±0.15	475
Ethanolic extract	45.13±0.31	62.52±0.60	75.57±0.13	83.96±0.17	140
Standard	58.68±0.01	65.87±0.02	83.83±0.01	97.90± .02	65

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

Table 4: FRAP Assay of various extracts of *Clerodendrum phlomidis*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	15.45±0.11	20.10±0.04	21.38±0.09	33.15±0.32	1410
Chloroform extract	19.35±0.05	23.49±0.03	33.18±0.03	41.60±0.36	1355
Ethyl acetate extract	24.15±0.06	38.02±0.02	46.28±0.03	52.39±0.15	590
Ethanolic extract	28.08±0.02	39.27±0.09	57.73±0.29	63.28±0.08	355
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

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

Total phenol

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[27]. The phenolic compounds may contribute directly to antioxidative action[28]. The total phenolic content of various extract of root of *Clerodendrum phlomidis* was presented in Table 5. Ethanolic extract of *Clerodendrum phlomidis* was found higher content of phenolic components than that extracts.

Table 5: The total Phenolic content of various extracts of root of *Clerodendrum phlomidis*

S. No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1.	Petroleum ether extract of *Clerodendrum phlomidis*	1.09 ± 0.01
2.	Chloroform extract of *Clerodendrum phlomidis*	2.64 ± 0.09
3.	Ethyl acetate extract of *Clerodendrum phlomidis*	3.61±0.01
4.	Ethanolic extract of *Clerodendrum phlomidis*	6.60 ± 0.07

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

Total flavonoids

The total amount of flavonoids content of various extract of root of *Clerodendrum phlomidis* was summarized in Table 6. Flavonoids present in food of plant origin are also potential antioxidants[29, 30]. The higher content of flavonoids was found in ethanolic extract of *Clerodendrum phlomidis* than that of other extracts.

Table 6: The total flavonoids content of various extracts of root of *Clerodendrum phlomidis*

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1.	Petroleum ether extract of *Clerodendrum phlomidis*	0.49 ± 0.03
2.	Chloroform extract of *Clerodendrum phlomidis*	0.81 ± 0.04
3.	Ethyl acetate extract of *Clerodendrum phlomidis*	1.10 ± 0.04
4.	Ethanolic extract of *Clerodendrum phlomidis*	2.39 ± 0.02

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

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. However, the ethanolic extract of *Clerodendrum phlomidis* was found high content of flavonoids and phenolic compounds. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

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