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Antioxidant potential of various extracts from whole plant of *Ionidium suffruticosum* (Ging)

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ABSTRACT

In the present study was to evaluate the free radical scavenging potential of various extracts from whole plant of *Ionidium suffruticosum* with the help of three *in-vitro* antioxidant models were carried out for total antioxidant activity (phosphomolybdic acid method), ferric-reducing antioxidant potential (FRAP) assay and total flavonoids method. Methanolic extract of *Ionidium suffruticosum* was found to be extremely effective in free radical scavenging activity than that of other two extracts in total antioxidant activity and FRAP methods were tested. High amount of flavonoids were found in methanolic extract of *Ionidium suffruticosum*. The results obtained in the present study indicate that the methanolic extract of whole plant of *Ionidium suffruticosum* are a potential source of natural antioxidant.

Keywords: Whole plant of *Ionidium suffruticosum*, Total antioxidant activity, FRAP assay, total flavonoid.

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INTRODUCTION

Antioxidants, free radical scavengers prevent pathological conditions of human body namely ischemia, anemia, asthma, arthritis, inflammation, neurodegenaration, and aging process [1]. Many plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties [2] and it has been established as one of the mechanisms of their action. Some of the non-nutritive antioxidants of plants are phenolic compounds, flavonoids, coumarins, benzyl isothiocyanate [3] etc.

Ionidium suffruticosum (Ging.) (Syn: *Hybanthus enneaspermus*) it belongs to the family Violaceae [4] known as Lakshmisheshta, Padmavati, Padmacharini or Purusharathna in Sanskrit, is an important plant in the Indian system of medicine. It is a small suffrutescent perennial herb found in the regions of former Madras Presidency in India, Ceylon, tropical Asia, Africa, and Australia. It grows 15-30 cm in height with many diffuse or ascending branches and is pubescent in nature [5].Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhea, leucorrhoea, dysuria, and sterility [6]. The plant is also attributed to its antimicrobial and antiplasmodial action [7, 8]. Various phytoconstituents viz. dipeptide alkaloids, aurantiamide acetate, isoarborinol, and β- sitosterol have been isolated from different parts of this plant [6, 9, 10, 11]. Moreover, the plant is reported, in ancient ayurvedic literature, to cure conditions of “kapha” and “pitta”, urinary calculi, strangury, painful dysentery, vomiting, burning sensation, wandering of the mind, urethral discharges, blood troubles, asthma, epilepsy, cough, and to give tone to the breasts [5].

So far no any reports are published on antioxidant activity of this plant. Therefore, the aim of the present investigation was to evaluate the antioxidant potential of various extract from whole plant of *Ionidium suffruticosum* through various *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Ionidium suffruticosum* (Ging), were collected form Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Ionidium suffruticosum* (Ging), 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 [12] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc 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

Total antioxidant activity (Phosphomolybdic acid method) [13]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex. 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.

FRAP assay [14]

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 37^0 C 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.

Total flavonoids [15]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 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

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reaction and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free

radicals [17]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc.

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Ionidium suffruticosum* depicted in Table 1. The petroleum ether extract of *Ionidium suffruticosum* exhibited a maximum total antioxidant activity of 49.34% 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 *Ionidium suffruticosum* and ascorbate were found to be 1020µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.31±0.16	26.87 ± 0.08
2	250	24.67±0.19	30.30 ± 0.05
3	500	32.39±0.13	60.64 ± 0.02
4	1000	49.34±0.09	55.23 ± 0.01
		IC_{50} = 1020µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of ethyl acetate extract of *Ionidium suffruticosum* depicted in Table 2. The ethyl acetate extract of *Ionidium suffruticosum* exhibited a maximum total antioxidant activity of 60.57% 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 *Ionidium suffruticosum* and ascorbate were found to be 390µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	28.30±0.19	26.87 ± 0.08
2	250	45.61±0.16	30.30 ± 0.05
3	500	53.43±0.20	60.64 ± 0.02
4	1000	60.57±0.13	55.23 ± 0.01
		IC_{50} = 390µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of methanolic extract of *Ionidium suffruticosum* presented in Table 3. The methanolic extract of *Ionidium suffruticosum* exhibited a maximum total antioxidant activity of 73.40% at 1000 µg/ml whereas for ascorbate (standard)

was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Ionidium suffruticosum* and ascorbate were found to be 190µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	41.65±0.18	26.87 ± 0.08
2	250	58.41±0.13	30.30 ± 0.05
3	500	69.33±0.12	60.64 ± 0.02
4	1000	73.40±0.25	55.23 ± 0.01
		IC_{50} = 190µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the result clearly indicated the ethyl acetate and methanolic extract of *Ionidium suffruticosum* was found to be more effective than that of petroleum ether extract. But when compare all the extracts with standard the methanolic extract of *Ionidium suffruticosum* was found strong antioxidant activity than that of other two extracts. The IC_{50} values of the methanolic extract of *Ionidium suffruticosum* and Ascorbate were found to be 190µg/ml and 410µg/ml respectively.

FRAP assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The FRAP assay showed greater variability between various extracts. Table 4 was illustrated the FRAP values of petroleum ether extract of *Ionidium suffruticosum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The maximum reducing ability at 1000µg/ml for petroleum ether extract of *Ionidium suffruticosum* and ascorbate were found to be 48.33% and 98.07% respectively. The IC_{50} values of petroleum ether extract of *Ionidium suffruticosum* and ascorbate were recorded as 1050µg/ml and 50µg/ml respectively.

Table 4: FRAP assay of Petroleum ether extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	13.42±0.32	72.04 ± 0.01
2	250	35.33±0.19	82.05 ± 0.03
3	500	41.71±0.21	86.04 ± 0.02
4	1000	48.33±0.03	98.07 ± 0.04
		IC_{50} = 1050µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the ethyl acetate extract of *Ionidium suffruticosum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were illustrated in Table 5. The maximum reducing ability at 1000µg/ml for ethyl acetate extract of

Ionidium suffruticosum and ascorbate were found to be 57.42% and 98.07% respectively. The IC_{50} values of ethyl acetate extract of *Ionidium suffruticosum* and ascorbate were recorded as 780µg/ml and 50µg/ml respectively.

Table 5: FRAP assay of Ethyl acetate extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethylacetate extract)	Standard (Ascorbate)
1	125	16.35±0.21	72.04 ± 0.01
2	250	33.55±0.24	82.05 ± 0.03
3	500	42.66±0.14	86.04 ± 0.02
4	1000	57.42±0.24	98.07 ± 0.04
		IC_{50} = 780µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the methanolic extract of *Ionidium suffruticosum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were illustrated in Table 6. The maximum reducing ability at 1000µg/ml for methanolic extract of *Ionidium suffruticosum* and ascorbate were found to be 60.38% and 98.07% respectively. The IC_{50} values of methanolic extract of *Ionidium suffruticosum* and ascorbate were recorded as 440µg/ml and 50µg/ml respectively.

Table 6: FRAP assay of Methanolic extract of *Ionidium suffruticosum*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	25.52±0.24	72.04 ± 0.01
2	250	38.21±0.29	82.05 ± 0.03
3	500	53.33±0.23	86.04 ± 0.02
4	1000	60.38±0.05	98.07 ± 0.04
		IC_{50} = 440 µg/ml	**IC_{50} = 50 µg/ml**

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

Based on the above results indicated, the methanolic extract of *Ionidium suffruticosum* was found to most effective than that of other two extracts. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Ionidium suffruticosum* showed the moderate result.

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants [17, 18]. The total amount of flavonoids content of various extract of whole plant of *Ionidium suffruticosum* were summarized in Table 7.

Table 7: The total flavonoids content of various extracts of whole plant of *Ionidium suffruticosum*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Ionidium suffruticosum*	1.48 ± 0.043
2	Ethyl acetate extract of *Ionidium suffruticosum*	2.24 ± 0.052
3	Methanolic extract of *Ionidium suffruticosum*	5.47 ± 0.060

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

Based on the above result clearly illustrated the higher content of flavonoids was found in methanolic extract of *Ionidium suffruticosum* than that of other two extracts.

CONCLUSION

The present investigation revealed that the methanolic extract of whole plant of *Ionidium suffruticosum* possess with significant antioxidant activity when compared to that of other two extracts. In addition, the methanolic extract of *Ionidium suffruticosum* was found to contain a noticeable amount of flavonoids, which play a major role in controlling antioxidants. Therefore, further works should be performed on the isolation and identification of the antioxidant components in methanolic extract of whole plant of *Ionidium suffruticosum*.

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