WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 541-552. Research Article ISSN 2277– 7105

IN *VITRO* ANTIOXIDANT EFFECT OF *IONIDIUM SUFFRUTICOSUM* (GING).

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Article Received on
09 May 2015,

Revised on 31 May 2015,
Accepted on 21 June 2015

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ABSTRACT

The present investigation has been carried out to evaluate the phytochemical constituents and *in vitro* antioxidant effect of whole plant of *Ionidium suffruticosum* (Ging). Phytochemicals were analyzed qualitatively and the result confirmed the presence of alkaloids, steroids, saponins, flavonoids, tannins etc., and the quantitative analysis was also performed for their carbohydrate, protein and phenol content. The *in vitro* antioxidant properties were evaluated using DPPH radical scavenging assay, ABTS scavenging activity, reducing power assay, nitric oxide scavenging activity. *Ionidium suffruticosum* (Ging) was found to be extremely effective in all above models than standard ascorbic acid. IC_{50} values were 27µg, 400µg, 50µg and 260µg for DPPH assay, ABTS scavenging activity, reducing power assay, nitric oxide scavenging activity respectively. The results obtained from the study indicate that the ethanol extract of whole plant of *Ionidium suffruticosum* (Ging) was a potential source of natural antioxidant.

KEYWORDS: Antioxidant activities, Ethanol extract, *Ionidium suffruticosum* (Ging), Phytochemicals.

INTRODUCTION

Free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants.[1]

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS.[2, 3] Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, changes in gene expression and induce abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and function as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radicals scavengers may be necessary.[4, 5]

The most important oxygen-containing free radicals in many disease states are hydroxyl radical (OH), superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), oxygen singlet, hypochlorite, nitric oxide radical (NO), and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids.[6]

Plants with medicinal properties "The gift of mother nature to mankind" are in use for centuries in the traditional system of medicine like Ayurvedha, Unani, Siddha etc., in India and other countries for the treatment of diseases. They are considered to be effective and non toxic. Medicinal plants contain physiologically active principles that over the bears have been exploited in traditional medicine for the treatment and various ailments [7] as they contain antioxidant properties. The medicinal herbs consist vent indispensable components of the traditional medicine practiced worldwide due to low costs, easy access and ancestral experience.[8] Keeping this view, the present investigation has been carried out to evaluate the physicochemical parameters, phytochemicals (qualitative and quantitative methods), and *in vitro* antioxidant activity by various models in the whole plant of *ionidium suffruticosum* (Ging).

The whole plant of *ionidium suffruticosum* (Ging) belongs to the family of Violaceae. The plant contains alkaloids, steroids, flavonoids, saponins, tannins. Various phyconstituents viz. leucine, isoleucine, tryptophan and phenylalanine, dipeptide alkaloids, aurantiamide acetate, isoarborinol, and β-sitosterol have been isolated from different parts of this plant. [9, 10] The aqueous extract of *ionidium suffruticosum* was found to be high content of flavonoids and phenolic compounds. This herb is considered to be extremely beneficial to men, used as a

diuretic, demulcent and tonic. [10] The root is diuretic and is used in urinary infections and bowel complaints of children. Decoction of leaves and tender stalks is demulcent. The plant is also attributed to its antimicrobial and antiplasmodial action. [11]

MATERIALS AND METHODS

Plant collection

Plant source selected for the present study was *Ionidium suffruticosum.* Selected whole plant was collected from in and around Mannargudi, identified with the help of Flora of Presidency of Madras, [12] the whole plant of *Ionidium suffruticosum* (Ging) were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Plant extract preparation

20g of plant powder (*Ionidium suffruticosum*) were soaked in 100ml of ethanol for 48hrs. Then filtered and boiled at 57ºC until it becomes precipitated. The precipitate is used for the determination of moisture content, extractive values [13], Fluorescence analysis [14] and phyto chemical analysis. [15] The *in vitro* antioxidant assays were also performed by various models such as DPPH radical scavenging assay. [16] ABTS radical Scavenging Activity, [17] reducing power assay, [18] and nitric oxide scavenging activity. [19]

RESULTS AND DISCUSSION

In the present study, the ethanol extract of *Ionidium suffruticosum* (Ging.) were tested for physicochemical properties such as purity, extractive values, solubility, phytochemical constituents, and also *in vitro* antioxidant activity. In antioxidant activity, various methods like total antioxidant capacity, ABTS scavenging assay, nitric oxide scavenging assay and reducing power assay were performed. The results were presented in the form of tables and figures and discussed.

Test for purity and Identify

The purity of *Ionidium suffruticosum* was tested and the results showed that the 2.24% of foreign matters and loss on drying was 8.41% (Table 1).

Table 1: Test for purity and identity

S.No	Parameter	Percentage (%)
1.	Foreign matters	2.24
2.	Loss on drying	8.41

Physiochemical analysis

Table-2 shows the extractive value of *Ionidium suffruticosum* in hexane (2.73%), chloroform (2.76%), and ethyl acetate (1.33%). Results indicate that the high extractive value was found in chloroform extract. The solubility test was also performed in water (7.83%) and ethanol (17.04%). The alcohol solubility was found to be higher than the water solubility.

Table 2: Physicochemical analysis

S.No	Parameters	Concentration (%)
	Successive Extractive values	
1.	i) Hexane	2.73
	ii)Chloroform	2.76
	iii)Ethyl acetate	1.33
	Solubility	
2.	i)Ethanol	17.04
	ii)Water	7.83

Fluorescence analysis

Table-3 represents the fluorescence analysis of *Ionidium suffruticosum*. In day light, the plant powder exhibited various shades of green fluorescence and various shades of green, orange, pink fluorescence under UV light. The pink and orange fluorescence indicated the presence of alkaloids and terpenoids.

Table 3: Fluorescence analysis

S.No	Treatment	Day Light			UV Light		
		0 Hr	24 Hrs	48 Hrs	0 Hr	24 Hrs	48 Hrs
1.	Drug powder	Green	Green	Green	Green	Green	Green
2.	Drug powder+ Aqueous 1N NaOH	Light green	Light green	Light green	Light green	Light green	Light green
3.	Drug powder+ Alcoholic 1N NaOH	Green	Light green	Light green	Green	Yellowish green	Yellowish green
4.	Drug powder+1N HCl	Green	Pale green	Pale green	Green	Green	Green
5.	Drug powder+50% H_2SO_4	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green
6.	Drug powder+ Hexane	Green	Light green	Light green	Green	Light green	Light green
7.	Drug powder+$CHCl_3$	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green
8.	Drug powder+ Ethyl acetate	Green	Dark green	Dark green	Green	Light green	Pink
9.	Drug powder+Acetone	Green	Dark green	Dark green	Green	Light yellow	Pink
10.	Drug powder+ Benzene	Pale green	Dark green	Dark green	Pale green	Light yellow	Pink

11.	Drug powder+ Alcohol	Green	Dark green	Dark green	Green	Green	Orange
12.	Drug powder+ Water	Green	Green	Green	Green	Green	Green

Preliminary phytochemical analysis

In the present study, the phytochemical analysis of ethanolic extract of *Ionidium suffruticosum* showed the presence of alkaloids, flavonoids, phenol, steroids, amino acids, tannins, saponins and the absence of terpenoids, coumarine, quinines compound (table 4). The significant hepatoprotective, nephroprotective and antioxidant effect of *Ionidium suffruticosum* may be presence of alkaloids and it is inhibitory effect on lipid peroxidation. [20]

Table 4: Phytochemical analysis

S.No.	Test	Extracts					
		Dry Powder	**Hexane**	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
1.	Saponins	+	+	_	_	_	_
2.	Tannins	+	+	+	+	_	+
3.	Steroids	+	+	+	_	+	_
4.	Terpenoids	_	_	_	_	_	_
5.	Flavonoids	+	+	+	+	+	+
6.	Coumarine	_	_	_	_	_	_
7.	Quinones	_	_	_	_	_	_
8.	Lignin	_	_	_	_	_	_
9.	Alkaloids	+	+	+	+	_	+
10.	Sugar	+	+	+	+	+	_

(+) Indicate present

(-) Indicate absent

Quantitative analysis

The quantitative analysis of *Ionidium suffruticosum* were analysed for its carbohydrate, protein, phenol contents. Results (Table 5) revealed that the plant has considerable amount of carbohydrate (0.4g), protein (4.705mg) and phenol (1.538mg)

Table 5: Quantitative analysis

S.No.	Parameters	Amount
1.	Carbohydrate	0.4g
2.	Protein	4.705mg
3.	Phenol	1.538mg

***In vitro* antioxidant activity**

DPPH radical scavenging assay

Good stability, credible sensitivity, simplicity and feasibility are the advantages of DPPH assay. [21, 22, and 23] The DPPH radical scavenging activity was analyzed for ethanol extract of *Ionidium suffruticosum*. The results of antioxidant activity were presented in Table 6. The scavenging activity was determined to be increased with the increase in the concentration of extract from 5 to 25µg/ml. The percentage of inhibition of the DPPH radical was varying from 2.63% (in 5µg/ml of extract) to 44.73% (in 25µg/ml of extract). The IC_{50} value of the plant was 27µg/ml. All the concentration of plant extracts showed higher percentage of inhibition. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the plant extract in a concentration-dependent manner.

Table 6: DPPH Radical scavenging Assay

S.No	Concentration(µg\dl)	% Inihibition
1.	5	2.631
2.	10	13.15
3.	15	23.68
4.	20	34.21
5.	25	44.73
6.	10mg(Standard)	21.56

IC_{50} Value=27µg/ml

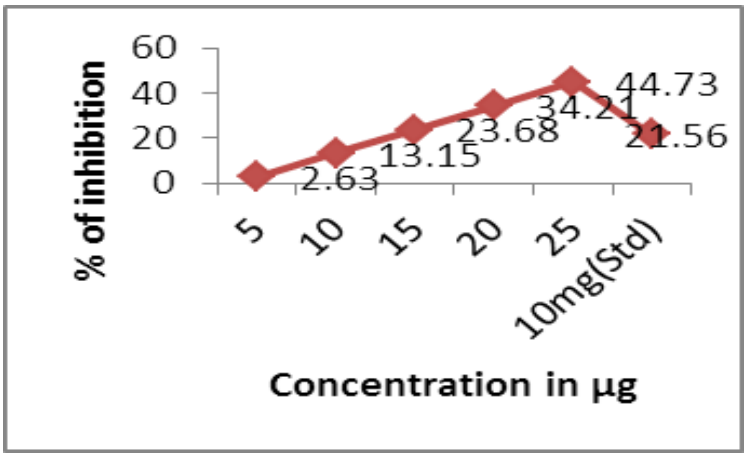

Figure: 2

ABTS radical scavenging assay

The results of antioxidant activity of the ethanolic extract of *Ionidium suffruticosum* based on ABTS radical scavenging activity were presented in Table 7. The dose dependent response was observed from 100 (30%) to 500µg/ml (53.3%). The IC_{50} value was 400µg/ml. 500µg of

sample extracts showed highest percentage of inhibition of ABTS radicals than standard ascorbic acid (25.21%). ABTS radical scavenging assay involves a method that generates a blue/green ABTS+ chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 745 nm. [24]

Table 7: ABTS Radical Scavenging Assay

S.No	Concentation(µg\dl)	%Inhibition
1.	100	30
2.	200	36.6
3.	300	43.33
4.	400	50
5.	500	53.33
6.	10mg(Standard)	25.21

IC_{50} value=400µg/ml

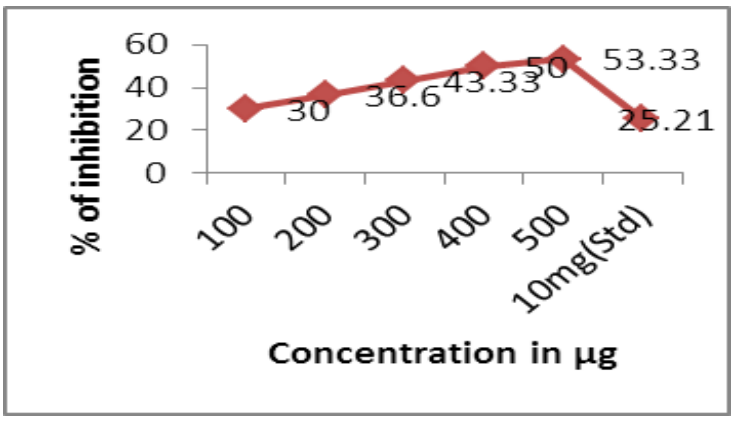

Figure: 2

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 8 was illustrated the reducing power values of ethanolic extracts of various concentration (50, 100, 150, 200, 250µg/ml) of *Ionidium suffruticosum* and ascorbic acid. The IC_{50} value was recorded as 230µg/ml. When the extract concentration increases from 50µg/ml to 250µg/ml, inhibition percentage was also increased from 13.04% to 56.52%. The standard ascorbic acid exhibited 34.04% of inhibition.

The reducing capacity of compounds may serve as a significant indicator of its potential antioxidant activity. [25] However the antioxidant activity of putative antioxidants have been

attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging activity.[26, 27] For the measurement of the reductive ability, we investigated Fe^{3+} to Fe^{2+} transformation in the presence of the ethanol extract of *Ionidium suffruticosum* using the method of Oyaizu, 1986.

Table 8: Reducing Power Assay

S.No	Concentration (µg/dl)	Reducing Power in %
1.	50	13.04
2.	100	23.07
3.	150	28.57
4.	200	41.17
5.	250	56.52
6.	10mg(Standard)	34.04

EC_{50} value=230µg/ml

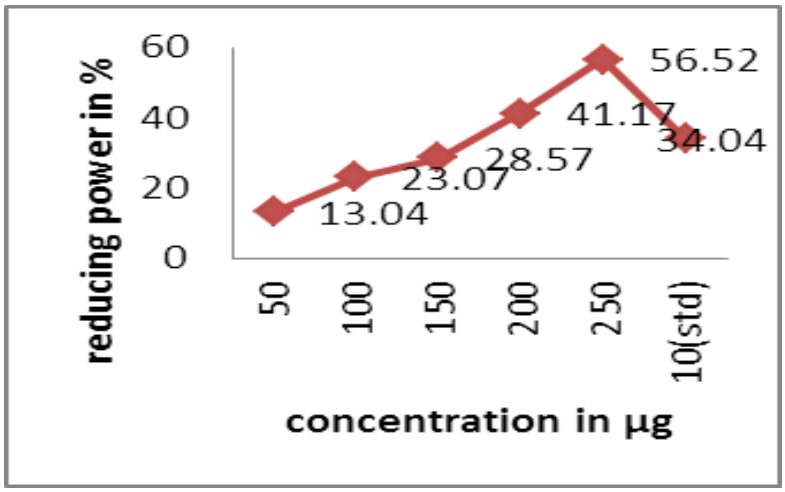

Figure 3

Nitric oxide scavenging assay

Scavenging of nitric oxide radical is based on the generation of nitric oxide. Sodium nitroprusside in buffered saline reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. In the present study, *Ionidium suffruticosum* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside. The reduction of nitric oxide radical can be the decrease in absorbance at 546nm. The results of antioxidant activity of the ethanol extract of *Ionidium suffruticosum* based on nitric oxide radical scavenging activity are presented in Table 9. The scavenging activity was determined to be increased with the increase in the concentration of extract from 100 to 500µg/ml. The

percentage of inhibition of the nitric oxide radical was varying from 30% (in 100µg/ml of extract) to 85% (in 500µg/ml of extract). The IC_{50} value of the ethanol extract of *ionidium suffruticosum* was 260µg/ml. All the concentration of sample extracts showed higher percentage of inhibition of nitric oxide radicals than that of standard ascorbic acid. The result indicated that the extracts might contain compounds able to compete with oxygen to react with NO and thus inhibit the generation of the nitrite and peroxy nitrite anions. [28]

Table 9: Nitric oxide Scavenging Assay

S.No.	Concentration(µg/ml)	%Inhibition
1.	100	30
2.	200	40
3.	300	60
4.	400	75
5.	500	85
6.	10mg(Standard)	35

IC_{50} value =260µg/ml

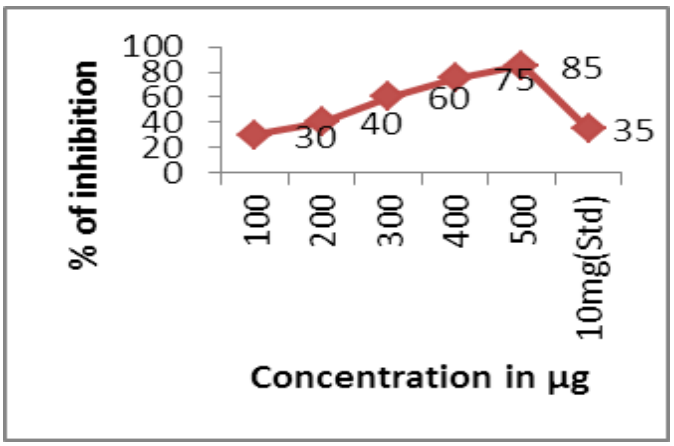

Figure: 4

SUMMARY AND CONCLUSION

Free radical produced from oxygen to form reactive oxygen species such as the singlet oxygen, superoxide, peroxyl, hydroxyl and peroxy nitrite radicals, are constantly produced within living cells for specific metabolic purposes. Living cells have complex mechanisms that act as antioxidant systems to counteract the damaging effects of reactive species. *Ionidium suffruticosum* (Ging.) it belongs to the family Violaceae, was selected for this study. The phytochemical analysis and *In vitro* antioxidant activity were evaluated using ethanol extract. Phytochemical analysis confirmed the presence of steroids, flavonoids, saponins,

alkaloids and sugar. The quantitative analysis revealed the presence of considerable amount of carbohydrate (0.4g), protein (4.70mg), phenol (1.53mg).

The experiments were also performed for antioxidant potential with different concentration. The percentage inhibition (IC_{50} values) were calculated for DPPH radical scavenging assay (27µg/ml), ABTS radical scavenging assay (400µg/ml), reducing power assay (230µg/ml), nitric oxide radical scavenging assay (260µg/ml). The above data has shown that this demonstrated a significant antioxidant activity.

The present investigation revealed that the ethanol extract of whole plant of *Ionidium suffruticosum* was found to contain a noticeable amount of flavonoids, which may play a major role in antioxidants activities. Therefore, further works should be performed on the isolation and identification of the antioxidant components in ethanol extract of whole plant of *Ionidium suffruticosum.*

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