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Research Article

ISSN:2230-7346
Journal of Global Trends in Pharmaceutical Sciences

Volume .4, Issue 1, pp -1034-1038, January–March 2013

IN -VITRO ANTIOXIDATIVE ACTIVITY OF AQUEOUS EXTRACT OF *IONIDIUM SUFFRUTICOSUM* (GING.) ENTIRE PLANT

Ashok Kumar C.K.*, G.Devivani, V.G. Sharmila, P. Mounika, Johnson. K, Satheesh Kumar. D

Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy, Tirupati-517 102, (A.P) INDIA.

*Corresponding Author E-mail: **ashokkumarck@yahoo.com**

ABSTRACT

The phytochemical investigation of *Ionidium suffruticosum* revealed that greater amount of phenols and flavonoids are found in aqueous extract. The free radical scavenging activity were determined by Hydroxyl radical scavenging activity, FRAP assay and Iron chelating activity. The aqueous extract of *Ionidium suffruticosum* showed significant free radical scavenging activity in comparison with standard drugs and found to be concentration dependent.

Key words: *Ionidium suffruticosum,* 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]. *Ionidium suffruticosum* (Ging.) (Syn: *Hybanthus enneaspermus*) it belongs to the family Violaceae [6]knowns as Orilaitamarai, Amburuha , Charati. It is an important plant in the Indian system of medicine. Various phytoconstituents viz. Leucine, isoleucine, tryptophan and phenylalanine have been reported (*Proc.Indian Acad.Sci.Plant Ser.,* 1986, 96,41).Various phytoconstituents viz. dipeptide alkaloids, aurantiamide acetate, isoarborinol, and β- sitosterol have been isolated from different parts of this plant[3,4] . It is a small suffrutescent perennial herb, found in the regions of warmer parts of

India from Uttar Pradesh, southwards to the Deccan peninsula.Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, leucorrhoea, dysuria, sterility, diabetes, bowel complaints[8]. The plant is also attributed to its antimicrobial and antiplasmodial action[9,10] anti gonorrhoeac, anti-inflammatory, anti tussives, anti convulsant and freeradicals scavenging activity,and in the treatment of jaundice and aqueous extract possessed hypoglycaemic activity [6,7].

Therefore, the aim of the present investigation was to evaluate the antioxidant potential of aqueous extract from whole plant of *Ionidium suffruticosum* through various *in vitro* models.

MATERIAL AND METHODS

Collection and identification of the 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 dried powder of the whole plant was extracted by maceration, using water as a solvent 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. This dry extract powder is used for various studies.

EVALUATION OF ANTIOXIDANT ACTIVITY BY *IN VITRO* METHODS

Determination of Hydroxyl radical scavenging activity[15]

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, P^H 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 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

FRAP assay[16]

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.

Iron chelating activity [17]

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.

RESULTS AND DISCUSSION

Hydroxyl radical scavenging activity

The percentage of Hydroxyl radical scavenging activity of various extracts of *Ionidium suffruticosum* was presented in Table 1. The IC_{50} values of aqueous extract of *Ionidium suffruticosum* was found to be 120µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml.

Table 1: Hydroxyl radical scavenging activity of various extracts of *Ionidium suffruticosum*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Aqueous extract	51.03±0.19	54.89±0.06	63.54±0.07	75.18±0.11	120
Standard (Ascorbate)	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[18]. Iron binding capacity of the aqueous extracts of *Ionidium suffruticosum* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were summarized in Table 2.The IC_{50} values of aqueous extract of *Ionidium suffruticosum* was found to be 163µg/ml respectively. Whereas, the IC_{50} value of standard EDTA was observed 80µg/ml.

Table 2: Iron-chelating activity of various extracts of *Ionidium suffruticosum*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Aqueous extract	43.13±0.31	62.52±0.60	65.57±0.13	61.96±0.17	163
Standard (EDTA)	58.68±0.01	65.87±0.02	83.83±0.01	97.90± .02	80

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

FRAP Assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 3 was depicted the FRAP values of aqueous extract of *Ionidium suffruticosum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The IC_{50} values of aqueous extract of *Ionidium suffruticosum* was found to be 430µg/ml. Where as, the IC_{50} value of standard ascorbate was observed 410µg/ml. The aqueous extract of *Ionidium suffruticosum* was showed significant antioxidant activity than that of other extracts.

Table 3: FRAP Assay of aqueous extracts of *Ionidium suffruticosum*

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Aqueous extract	28.08±0.02	39.27±0.09	57.73±0.29	63.28±0.08	430
Standard (Ascorbate)	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

CONCLUSION

The results of the present study were clearly indicated that the aqueous extract of *Ionidium suffruticosum* shows anti oxidant activity which is comparable with that of standard drugs. The aqueous extract of *Ionidium suffruticosum* was found high content of flavonoids and phenolic compounds. The anti oxidant activity of the plant may be due to the presence of the flavonoids and phenolic compounds. The plant can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry.

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