

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article.....!!!

Received: 17-12-2012; Revised; Accepted: 25-10-2013

***IN VITRO* ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *ACALYPHA INDICA* LINN**

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Keywords:

Antioxidant, *Acalypha indica* , DPPH, Nitric Oxide scavenging assay

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ABSTRACT

The present study was carried out to evaluate the antioxidant potential of *Acalypha indica* Linn. The ethanolic extract of aerial parts of *A.indica* was screened for its antioxidant activity by DPPH scavenging assay and Nitric oxide scavenging assay. The results showed that the selected plant extract was found to possess excellent antioxidant activity which may be attributed to the presence of bioactive constituents of the plant.

INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including Atherosclerosis, Arthritis, Ischemia, Reperfusion injury of many tissues, Central nervous system injury, Gastritis, Cancer and AIDS(1). The most effective way to eliminate free radicals which cause oxidative stress is with the help of antioxidants(2). Recently, there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury (3).The phenolic compounds and flavonoids distributed in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging activities, anti-inflammatory, anti-carcinogenic activities etc (4). *A.indica* is an annual herb found throughout various parts of India, Bangladesh, Srilanka, Philippines and Tropical Africa. The plant is commonly known as Indian Acalypha and belongs to the family Euphorbiaceae(5).This plant is locally known as khokali or kuppi in Hindi, Kuppaimeni in Tamil and grows in Indian gardens and backyards of houses and waste places throughout the plains of India (6).Traditionally, the plant is used as a laxative, anthelmintic, cathartic and the juice is used as a speedy emetic for children and as expectorant. The whole plant is useful in treating Chronic Bronchitis, Asthma, Scabies and other Skin diseases, Rheumatism,Congestive heart failure etc (7).

Chemical constituents reported from this plant include acalyphamide(as acetate), aurantiamide and its acetate, succinimide calypho-lactate, 2 methyl anthraquinone, tri-O-methyl ellagic acid, β - sitosterol and its β -D glucoside (leaves), a cyanogenic glucoside, acalyphine, alkaloids namely acalyphine and triacetoneamine, an essential oil n- octacosanol, kaempferol,quebrachitol, β -sitosterol acetate and tannin (whole plant) and stigmaterol(root) (8).Recently four kaempferol glycosides namely mauritianin, clitorin, nicotiflorin and biorobin have been isolated from the flowers and leaves of this plant (9).

MATERIALS AND METHODS

1. COLLECTION OF PLANT MATERIAL

The aerial parts of *A. indica* were collected from Indian Ayurvedic Hospital and Research Limited, Coimbatore and identified and authenticated by Botanical Survey of India, Coimbatore, whose voucher specimen number is BSI/SRC/5/23/2011-12/Tech.245.

2. PREPARATION OF PLANT EXTRACT

The dry powder of the plant was extracted with 95 % ethanol for 48 hours after defatting with petroleum ether for 72 hours. The extract was filtered and concentrated in vacuum under reduced pressure and dried in desicator.

3. EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY

The antioxidant activity of ethanolic extract of aerial parts of *A. indica* was studied with different concentration from 100 µg to 500 µg /ml. *In vitro* methods namely DPPH scavenging and Nitric oxide scavenging assays were used for screening the antioxidant activity.

(i) DPPH RADICAL SCAVENGING ASSAY

A stock solution of 0.1 ml of DPPH was prepared in ethanol. This solution was mixed with equal volume of solution (different concentrations) of ethanolic extract in ethanol. The reaction was allowed to complete in dark for about 20 minutes. The absorbance was measured at 517 nm. The experiment was repeated three times. The difference in absorbance between the test and the control was calculated and expressed as percentage scavenging of DPPH radical. IC₅₀ values denote the concentration of sample which is required to scavenge 50% of DPPH free radical (10).

ii) NITRIC OXIDE SCAVENGING ASSAY

The ethanolic extract of aerial parts of *A. indica* was dissolved in different concentrations of PBS and Sodium nitro-prusside was added in each tube and the tubes were incubated at 25°C for 5 hours. Control experiments without test compound were carried out with identical conditions. After 5 hours, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess reagent. The absorbance was measured at 546nm and the experiment was repeated three times (11).

RESULTS AND DISCUSSION

Table 1 shows the free radical scavenging activity of ethanolic extract of aerial parts of *A. indica* by DPPH and Nitric oxide radical scavenging assays. DPPH is a relatively stable free radical and the assay determines the ability of ethanolic extract of *A. indica* to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones (12). Nitric oxide is an important chemical mediator involved in the regulation of various physiological processes (13). Oxygen reacts with excess nitric acid to generate nitrite and peroxy nitrite anions, which act as free radicals (14). In the

present study, the ethanolic extract of *A.indica* competes with oxygen to react with nitric oxide and thus inhibits the generation of anions.

TABLE -I FREE RADICAL SCAVENGING ACTIVITY OF ACALYPHA INDICA

CONCENTRATION (µg)	DPPH RADICAL SCAVENGING ASSAY		NITRIC OXIDE RADICAL SCAVENGING ASSAY	
	% ACTIVITY	IC- 50 /TRV*	% ACTIVITY	IC -50 /TRV*
100	10.04 ± 0.729	476.19	11.40 ± 0.147	427.35
200	21.04 ± 0.317		23.98± 0.564	
300	33.10 ± 0.449		36.63 ± 0.250	
400	41.88 ± 1.421		47.67 ± 0.306	
500	52.37 ± 0.400		56.87 ± 0.278	

* TRV – Total Reaction Volume

Values are means of three independent analysis of the extract ± Standard Deviation (n = 3).

Based on the results obtained, the ethanolic extract of aerial parts of *A. indica* is found to have an excellent antioxidant and free radical scavenging activity with IC -50 value of 476.19 and 427.35 against the synthetic free radicals DPPH and Nitric oxide respectively. Hence the ethanolic extract of *A.indica* could be useful for the preparation of nutraceuticals as potent antioxidant to treat oxidative stress related degenerative diseases. Plants belong to Euphorbiaceae showed high concentration of flavonoids, phenols and alkaloids (15). As *A.indica* belongs to Euphorbiaceae family, the bioactive compounds present in the plant extract may be responsible for the potent antioxidant activity of *A.indica* in the current study. However further studies must be performed to identify the specific principles responsible for the antioxidant activity of *A.indica*.

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