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# EVALUATION OF *IN VITRO* ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *ACALYPHA INDICA* LINN

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## ABSTRACT

Free radicals have been implicated in the causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity etc(1). Antioxidant compounds may function as free radical scavengers, complexes of pro- oxidant metals, reducing agents and quenchers of singlet oxygen formation (2).The present study estimated the *in vitro* antioxidant and free radical scavenging activity of ethanolic extract of *Acalypha indica* using hydroxyl and superoxide radical scavenging assays. The findings of the present study suggested that *A.indica* could be a potential natural source of antioxidants and could be used as a therapeutic agent in preventing oxidative stress related diseases.

## INTRODUCTION

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 reactions which may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals.(3).Active oxygen and free radicals exist in human body in the form of superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($^{\cdot}OH$) and so on. If they reach higher levels, oxidative stress in human body would be created, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death. (4).Hydroxyl radical ($^{\cdot}OH$) is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (5).Superoxide anions are the precursors to active free radicals which have the potential of acting with biological macromolecules and thereby inducing tissue damage (6) Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infections and various disease status. During normal metabolic processes, human body generates more than 2 kg of $O_2^{\cdot-}$. per year(7). *Acalypha indica* of the family Euphorbiaceae is a common weed in many parts of Asia including India, Pakistan, Yemen and Srilanka and throughout the tropical Africa and South America (8).It is an annual herb, commonly found in waste places or fields (9).The plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (10).This plant produces a diverse range of bioactive molecules making them a rich source of different types of medicines. The most important of the bioactive constituents of the plant include alkaloids, tannins, flavonoids and phenolic compounds (11).Various chemical constituents namely kaempherol glycoside, mauritianin, clitorin, nicotiflorin and biorodin have been isolated from flowers and leaves of *A.indica.* (12).

## MATERIALS AND METHODS

### 1. COLLECTION OF PLANT

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.

The freshly collected leaves of *A.indica)* were dried in shade at room temperature (32 - 37ºC) for 5 days. The dried leaves were ground into powder using homogenizer.

## 2. PREPARATION OF PLANT EXTRACT

About 500 grams of dried and powdered aerial parts of *A.indica* was weighed and moistened with sufficient amount of 95% ethanol. After 72 hours, it was filtered and the powder was resuspended in ethanol and filtered again. The filtrate was evaporated in a boiling waterbath and the extract was used for the study.

## 3.EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF PLANT EXTRACT

The antioxidant and free radical scavenging activity of ethanolic extract of aerial parts of *A.indica* was investigated with different concentration from 100 µg to 500 µg / ml. *In vitro* methods namely hydroxyl and superoxide radical scavenging assays have been employed for the study.

### (i) HYDROXYL RADICAL SCAVENGING ASSAY

Hydroxyl radical scavenging activity was measured by studying the interaction between deoxyribose and test extracts for hydroxyl radicals which were obtained by Fenton's reaction. The damage on deoxyribose due to free radicals was determined colorimetrically at 532 nm. Percentage of inhibition was calculated and recorded (13).

### (ii) SUPEROXIDE RADICAL SCAVENGING ASSAY

Superoxide radical scavenging activity of plant extract was measured based on light induced superoxide generated by riboflavin and the subsequent reduction of Nitroblue tetrazolium. All the solutions were prepared in phosphate buffer at pH 7.8 and the measurement is done at 560 nm and the percentage inhibition was calculated and recorded accordingly (14).

## 4.RESULTS AND DISCUSSION

Table I showed the free radical scavenging activity of ethanolic extract of aerial parts of *A.indica* by Hydroxyl and Superoxide radical scavenging assays.

**TABLE I : FREE RADICAL SCAVENGING ACTIVITY OF *ACALYPHA INDICA* EXTRACT.**

| CONCENTRATION (µg / ml ) | HYDROXYL RADICAL SCAVENGING ASSAY | | SUPEROXIDE RADICAL SCAVENGING ASSAY | |
|---|---|---|---|---|
| | % ACTIVITY | IC $_{50\ /TRV*}$ | % ACTIVITY | IC $_{50\ /TRV*}$ |
| 100 | 8.92±0.308 | 574.71 | 8.96±0.394 | 568.18 |
| 200 | 17.45±0.178 | | 19.27±0.333 | |
| 300 | 28.27±0.122 | | 28.26±0.658 | |
| 400 | 35.74±0.216 | | 35.66±0.441 | |
| 500 | 42.16±0.394 | | 42.62±0.500 | |

*TRV - Total Reaction Volume, Values are means of three independent analysis of the extract ± Standard Deviation ( n = 3 ).

The results of the present study indicated that the aerial parts of the plant *A.indica* is found to have $IC_{50}$ value of 574.71 and 568.18 for hydroxyl radical and superoxide radical scavenging activity respectively. Among the reactive oxygen species, the hydroxyl radical is the most reactive radical which induces severe damage to adjacent biomolecules by abstracting hydrogen atoms from membrane lipids and brings about peroxidation of lipids (15).The results showed that the ethanolic extract of aerial parts of *A.indica* exhibited scavenging activity against hydroxyl radicals generated in the Fenton reaction system. Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reactions such as autooxidation (16) In the present study, the probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of ethanolic extract of *A.indica* towards the generation of superoxides in the *in vitro* reaction mixture.

Preliminary phytochemical analysis of ethanolic extract of A.indica leaves showed the presence of alkaloids, tannins, steroids, saponins, flavonoids, glycosides and phenolic compounds(17).It may be concluded that the ethanolic extract of aerial parts of *A.indica* was found to possess antioxidant and free radical scavenging activity against hydroxyl and superoxide radicals which may be due to the presence of bioactive constituents in the plant extract. However further work is required on the isolation and identification of the antioxidant components present in the plant.

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