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# Antioxidant Activity of *Acalypha indica*

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## Abstract

The present study was carried to explore the antioxidant activity of leaves and roots of *Acalypha indica*. The study was designed to evaluate the enzymic and non enzymic antioxidant status and *in vitro* free radical scavenging effects of *Acalypha indica*. The leaves and roots of *Acalypha indica* were found to have appreciable amounts of enzymic and non-enzymic antioxidants. The benzene, chloroform, acetone and methanol extracts exhibited potent *in vitro* free radical scavenging activity. Therefore, *Acalypha indica* leaves and roots can be used for treatment of diseases caused by free radicals (**Keywords:** Enzymic antioxidants, non-enzymic antioxidants, free radical scavenging activity).

## Introduction

Plants have been an important source of medicine for thousands of years. They constitute one of the main source of new pharmaceutical and healthcare products (Demiray *et al.*, 2009). The improper balance between reactive oxygen metabolite production results in oxidative stress (Deepa *et al.*, 2009). Oxidative stress is the major causative factors for the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression and neurodegenerative disease (Souri *et al.*, 2007). Free radicals are the compounds generated from normal body processes and they tend to attack the cells of our body causing them to deteriorate (Sofidiya *et al.*, 2006). The antioxidants must be constantly replenished since they are used up in the process of neutralizing free radicals. The leaves, root, stem and flowers of *Acalypha indica* Linn. (*Euphorbiaceae*) are used in traditional medicine (Mohamed *et al.*, 2009). It can be used for the treatment of snakebites, pain relief and wound healing. *Acalypha indica* is commonly known as Indian copperleaf and Indian nettle. All the parts of the plants are used in various traditional systems among them roots seems to be much useful (Balakrishnan *et al.*, 2009). Hence, the present study was contemplated to investigate the antioxidant activity of the leaves and roots of *Acalypha indica* with the following objectives: (i) to evaluate the enzymic and non enzymic antioxidant status of *Acalypha indica* and (ii) to analyze the *in vitro* free radical scavenging effects of *Acalypha indica*.

## Materials and Methods

### *Sample collection*

The leaves and roots of *Acalypha indica* were collected from the nearby village of Coimbatore district, Tamilnadu, India and dried in shade. They were powdered and stored in airtight container at room temperature until use.

### *Assessment of the activities of the enzymic and non enzymic antioxidants*

The leaf and root sample of *Acalypha indica* was analysed for the enzymic and non enzymic antioxidants such as catalase (Luck, 1974), superoxide dismutase (Misra and Fridovich, 1972),

glutathione reductase (David and Richard, 1983), glutathione S-transferase (Habig *et al.*,1974), glutathione peroxidase (Rotruck *et al.*, 1973), ascorbic acid (Roe and Kuether, 1953), α-tocopherol (Rosenberg, 1992), total carotenoids (Zakaria *et al.*, 1979), flavonoids (Cameroon *et al.*, 1943), polyphenols (Malick and Singh, 1980) and reduced glutathione (Moron *et al.*, 1979).

***Free radical scavenging activity***

The leaf and root of *Acalypha indica* powder were subjected to extraction with solvent like petroleum ether, benzene, chloroform, acetone, methanol and water in soxhlet apparatus for 18 h - 20 h. The solvents were evaporated to dryness and resulting solid residue was dissolved in dimethyl sulfoxide (DMSO) and used for further studies. Each extracts were used for the determination of free radical scavenging activity such as DPPH (Mensor *et al.*, 2001), inhibition of *in vitro* lipid peroxidation (Okhawa *et al.*, 1979), inhibition of superoxide generation (Mc Cord and Fridovich, 1968), nitric oxide generation (Green and Hill, 1984) and hydrogen peroxide scavenging (Ruch *et al.*, 1989) activity.

***Statistical analysis***

Statistical analysis was performed according to student t-test procedure. The values ($p < 0.05$) were considered to be significant.

## Results and Discussion

***Enzymic and non-enzymic antioxidants***

Activities of the enzymic antioxidants such as catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase and glutathione peroxidase and levels of non enzymic antioxidants such as ascorbic acid, α- tocopherol, carotenoids, flavonoids, polyphenols and reduced glutathione were analysed in the leaves and roots of *Acalypha indica* and the results obtained were depicted in Table 1.

It is clear that among the leaves and roots of *Acalypha indica*, the roots showed higher amounts of superoxide dismutase and catalase activity when compared to its leaves. The leaves of *Acalypha indica* were found to exhibit higher activity of glutathione-S-transferase and glutathione peroxidase than that of roots (Table 1). Jaleel (2009) stated that the roots of *Withania somnifera* have maximum activities of antioxidant enzymes like superoxide dismutase, ascorbate peroxidase compared to fresh leaves.

The Table 1 reveals that the leaves and roots of *Acalypha indica* were found to show moderate levels of flavonoids, polyphenols and reduced glutathione. Appreciable amounts of carotenoids were found to be present in the leaves than that of roots. In case of vitamin C, the leaves possess higher activity (7.3 ± 0.39) when compared to that of roots. The leaves of *Acalypha indica* showed higher amounts of α tocopherol (15.5 ± 11.8) than that of roots 1.8 ± 0.3. The activity of flavonoids in leaves of *Acalypha indica* was found to be high (1.6 ± 0.1) compared to the roots. The total flavonoid content and antioxidant activity of methanolic extract of *Sargassum wightii* was found to be 2.02 ± 0.07 mg $g^{-1}$ and 1.16 ± 0.11 mg $g^{-1}$. The capacity of flavonoids to act as antioxidants depends upon their molecular structure (Meenakshi *et al.*, 2009). Reduced glutathione underlies its potent antioxidant action and enzyme cofactor properties and supports a complex thiol exchange system which hierarchially regulates cell activity. It is involved in the synthesis and repair of DNA, blocks free radical damages and enhances the antioxidant activity of vitamin C (Chavan *et al.*, 2005).

**Table 1.** Effect of enzymic and non- enzymic antioxidant in *Acalypha indica*

| Antioxidants | Leaf | Root | t value* |
|---|---|---|---|
| Enzymic antioxidants (U $g^{-1}$) | | | |
| Catalase[1] | 187.00 ± 17.00 | 234.9 ± 20 | 3.15 |
| Superoxide dismutase[2] | 12.60 ± 0.49 | 18.9 ± 0.33 | 18.47 |
| Glutathione reductase[3] | 65.00 ± 1.05 | 37.0 ± 1.0 | 11.3 |
| Glutathione-S- transferase[4] | 0.04 ± 0.00 | 0.01 ± 0.00 | 22.34 |
| Glutathione peroxidase[5] | 7.70 ± 2.58 | 1.98 ± 0.66 | 862.59 |
| Non-enzymic antioxidants (mg $g^{-1}$) | | | |
| Ascorbic acid | 7.25 ± 0.39 | 2.1 ± 0.19 | 9.694 |
| Locopherol | 15.50 ± 11.75 | 1.8 ± 0.28 | 2.015 |
| Carotenoids | 84.00 ± 2.24 | 7.5 ± 1.16 | 57.67 |
| Flavonoids | 1.60 ± 0.10 | 0.5 ± 0.04 | 16.81 |
| Polyphenols | 0.90 ± 0.04 | 0.8 ± 0.04 | 84.12 |
| Reduced glutathione | 0.30 ± 0.00 | 0.2 ± 0.00 | 22.53 |

Values are mean ± SD of triplicates ns- not significant *Significant at 0.001 level

*Note :*
1. Amount of enzyme that brings about decrease in absorbance of 0.05 at 240 nm.
2. Amount of SOD that cause 50 per cent reduction in the extent of NBT oxidation.
3. Millimoles of NADPH oxidized $min^{-1}$ $g^{-1}$ sample.
4. Millimoles of CDNB - GSH conjugates $min^{-1}$ $g^{-1}$.
5. Change in absorbance $min^{-1}$ $g^{-1}$ sample.

From the results, it is clear that *Acalypha indica* was found to contain significant levels of non-enzymic antioxidants and therefore it can be used for treating diseases caused by free radicals such as cancer, cardiovascular diseases and carcinogenesis.

***Inhibition of in vitro free radical scavenging activity***

Free radical often exists in the form of peroxyl (ROO), alkoxyl (RO), hydroxyl (OH.) and nitric oxide (NO) all of which retain surplus non-paired electron being highly reactive to macromolecular compounds including proteins and nucleic acids (Devi *et al.*, 2010). The efficacy of a plant extract as an antioxidant is best evaluated based on results obtained by the following assays such as DPPH scavenging assay, inhibition of *in vitro* lipid peroxidation, the inhibition of superoxide radical generation, inhibition of nitric oxide generation and hydrogen peroxide scavenging activity. The free radical scavenging activity of *Acalypha indica* is depicted in Table 2.

The extract of *Acalypha indica* exhibits good antioxidant activity against DPPH radical. In the leaves and roots of *Acalypha indica*, petroleum ether, chloroform and aqueous extracts were found to be below 50 per cent inhibition. The acetone extract of the leaves and benzene extract of the roots exhibits below 50 per cent of inhibition. DPPH, a stable free radical, to decolorize the

**Table 2.** Percentage inhibition of *in vitro* free radical scavenging activity of *Acalypha indica*

| Extracts / % inhibition | | Petroleum ether | Benzene | Chloroform | Acetone | Methanol | Aqueous |
|---|---|---|---|---|---|---|---|
| DPPH Radical | Leaf | 44 | 68 | 21 | 7 | 50 | 11 |
| | Root | 34 | 18 | 17 | 50 | 44 | 7 |
| Lipid peroxidation | Leaf | 32 | 12 | 25 | 26 | 51 | 13 |
| | Root | 13 | 25 | 64 | 7 | 16 | 25 |
| Superoxide generation | Leaf | 81 | 85 | 73 | 54 | 93 | 53 |
| | Root | 89 | 44 | 75 | 84 | 97 | 84 |
| Nitric oxide generation | Leaf | 46 | 29 | 24 | 7 | 19 | 21 |
| | Root | 14 | 25 | 28 | 30 | 92 | 34 |
| $H_2O_2$ | Leaf | 80 | 52 | 94 | 96 | 88 | 88 |
| | Root | 80 | 60 | 82 | 4 | 22 | 88 |

presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Hasan *et al.*, 2009).

Superoxide radical generation inhibition of the leaves and roots of *Acalypha indica* was assessed and the methanol and petroleum ether extracts of roots of *Acalypha indica* was found to show good inhibition against superoxide generation as 97 per cent and 89 per cent respectively than that of the leaves. *Bergia suffruticosa* is a medicinal plant which is traditionally used to repair bones and applied on sores. The methanolic extract (13.1 µg) of whole plant of *Bergia suffrutisco* showed a very good DPPH radical scavenging activity and superoxide scavenging activity (139.4 µg) in a dose dependent manner (Anandjiwale *et al.*, 2007).

The methanol extract of roots of *Acalypha indica* highlights the maximum nitric oxide scavenging activity of 92 per cent and all the other extracts have moderate activity. The petroleum ether extract of the leaves of *Acalypha indica* was found to have highest inhibition of 46 per cent against nitric oxide radical generation. The crude, boiled and ethanolic extracts of *Armillaria mellae* showed significant increase in nitric oxide production and the results were found to be 711.46 ± 61.1, 188.31 ± 8.6, 104.6 ± 23.1 respectively (Rai *et al.*, 2009).

The *in vitro* lipid peroxidation of *Acalypha indica* showed that the chloroform extract of the roots showed maximum inhibition of 64 per cent whereas other extracts shows lesser inhibition against *in vitro* lipid peroxidation. Rao *et al.* (2009) reported that the per cent of inhibition of hydroxyl radical in *Clitoria ternatea* and *Eclipta prostrata* was 59.0 per cent and 18.1 per cent respectively and the per cent inhibition of lipid peroxidation in *Clitoria ternatea* and *Eclipta prostrata* was 57.8 per cent and 18.4 per cent respectively.

The chloroform and acetone extracts of leaves of *Acalypha indica* were found to have maximum hydrogen peroxide scavenging activity (94 per cent and 96 per cent) respectively whereas the aqueous extracts of leaves and acetone extracts of roots were found to shows least activity. Hydroxyl radicals are one of the quick initiators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply autoxidation of polyunsaturated fatty acids, found primarily in membranes (Kappus, 1991).

## Conclusion

The results from the present study reveal that both the leaves and roots of *Acalypha indica* were found to have appreciable amount of enzymic and non-enzymic antioxidants. The results for *in vitro* free radical scavenging activity of *Acalypha indica* were also appreciable. Therefore, the extracts of *Acalypha indica* could be used to treat various diseases caused by free radicals, ROS and RNS.

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