



## Antioxidant Potential of *Tinospora cordifolia* Climber from *Eucalyptus hybrid*, *Ficus religiosa* and *Roystonea regia*

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### Abstract

Stems of *Tinospora cordifolia* were collected from different hosts namely, *Eucalyptus hybrid*, *Ficus religiosa* and *Roystonea regia*. To evaluate antioxidant potential, various parameters such as total reducing power, DPPH radical scavenging activity, lipid peroxidation inhibitory activity and superoxide radical scavenging assay were performed. Stems collected from the host *Ficus religiosa* showed highest phenolic content 5.25 mg/gm and hence higher reducing ability. Highest antioxidant activity of 41.73  $\mu\text{M Fe (II)/g}$  and  $\text{EC}_{50}$  value for DPPH radical scavenging activity was also observed in stems collected from *Ficus religiosa* (0.6 mg/ml).

**Keywords:** Antioxidant potential, *Ficus religiosa*, *In vitro*, *Tinospora*

### Introduction

Nowadays, nutraceuticals are gaining popularity for commercial use by pharmaceutical industries. Beyond the pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people with minimum side effects<sup>1</sup>. *Tinospora cordifolia* (Miers.) is an extensively spreading, glabrous, succulent, climbing shrub with several elongated twining branches belongs to the family *Menispermaceae*. It is known as Gulancha in English, Guduchi in Sanskrit, and Giloya in Hindi. The plant is distributed throughout the tropical region of India up to 1,200 m above the sea level from Kumaon to Assam, in north extending through West Bengal, Bihar, Deccan, Konkan, Karnataka and Kerala. The extracts of the various parts of the plant show different activities viz; the anti-inflammatory activity, anti-oxidase activity<sup>2</sup>, anti-stress activity, anti-ulcer, digestive activities<sup>3</sup> and hypolipidemic effect<sup>4</sup>. Studies are available on antioxidant activity of *Tinospora cordifolia*. In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants.

Free radicals are the normal products of cellular aerobic metabolism. Superoxide ( $\text{O}_2^-$ ) and hydroxyl species ( $\text{OH}^\cdot$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and peroxynitrite ( $\text{ONOO}^-$ ) include in the prominent list of free radicals the cell and together referred as reactive oxygen species (ROS). Although not themselves, these molecules contribute importantly to cellular redox state. Moreover, a powerful antioxidant system of the cells takes care of ROS<sup>5</sup>. When the balance between ROS production and antioxidant defense is disturbed, an oxidative stress is resulted. Oxidative stress refers to cytological consequences of a mismatch between the production of free radicals and the ability

of the cell to defend against them. This oxidative stress can damage cells by causing lipid peroxidation, altering protein and nucleic acid and further resulting in various diseased states. Free radicals released during the oxidative stress pose the major endogenous damage in the biological system<sup>6</sup>. More recent studies have shown the involvement of these radicals in cancer<sup>7</sup>, AIDS<sup>8</sup>, hepatitis<sup>9</sup>, atherosclerosis<sup>10</sup> and multiple system organ failure<sup>11-12</sup>. There is also growing evidence to show that production of ROS at the site of inflammation can contribute to tissue damage<sup>13</sup>. This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular disease, immuno function decline and aging<sup>6,14</sup>. There are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities to lower incidence of various human morbidities or mortalities.

The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents<sup>15</sup>. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins<sup>10</sup>, carotenoids<sup>16</sup>, flavonoids<sup>17</sup> and other phenolic compounds<sup>18</sup>.

It is a traditional belief among the Ayurvedic practitioners that Guduchi Satva obtained from Guduchi plant growing on Neem tree (*Azadirachta indica*) is more bitter and more efficacious and is said to incorporate the medicinal virtue of Neem also<sup>19</sup>. *Tinospora cordifolia* has been reported to prefer Neem and Mango trees as it climbs. This existing study prompted us to undertake present investigation as a preliminary study to evaluate antioxidant potential of *Tinospora Cordifolia* procured from different sources namely *Ficus religiosa*, *Eucalyptus hybrid* and *Roystonea regia* with the objective of extension of further research on screening the best host to procure *Tinospora* for ayurvedic formulations and drug development.

## Material and Methods

**Sample Collection and Preparation of Extract:** *Tinospora Cordifolia* stem samples were collected from IIM Jammu, from three different hosts viz, *Ficus religiosa*, *Eucalyptus hybrid* and *Roystonea regia*. The samples were dried in oven at 40°C for 72 hours. The dried samples were ground into fine powder followed by defatting in petroleum ether by soaking the samples in solvent for 90 minutes and the step was repeated thrice for each sample. Defatted dried samples were extracted to exhaustion (soxhlet) with ethanol. The extracts were filtered through and concentrated using rotary evaporator, the extracts were preserved at 4°C in airtight bottles for future usage<sup>20</sup>.

**Estimation of Phenolic Content:** Folin-Ciocalteu reagent method, Malick and Singh<sup>21</sup> was used for estimation of total phenolic content using gallic acid as standard.

**Antioxidant Activity Assays: Total reducing power:** The determination of reducing power was performed as described by Duh and Yen<sup>22</sup>.

**Ferrous reducing antioxidant power assay (total antioxidant activity assay):** The method already reported by Benzie and Strain for FRAP assay was followed<sup>19</sup>.

**Lipid peroxidation inhibitory activity:** The lipid peroxidation inhibitory activity of the stem extracts was determined according to Duh and Yen<sup>22</sup>.

**DPPH Radical Scavenging Activity:** The free radical scavenging activity of stem extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). The DPPH scavenging assay performed in the present study was a modification of the procedure of Moon and Terao<sup>23</sup>.

**Superoxide Radical Scavenging Assay:** To measure the superoxide anion scavenging activity method of Fontana et al.<sup>24</sup>,

was followed. Superoxide radical is generated in phenazine methosulphate–nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT) to a purple formazan.

## Results and Discussion

Stems of *Tinospora cordifolia* were collected from various hosts to evaluate their effect on antioxidant potential (figure 1). Existing studies on humans demonstrate a convincing effect of antioxidant polyphenols on some aspects of health<sup>25</sup>. Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activities and their effects on human nutrition and health are considerable<sup>26</sup>.

**Total Phenolic Content:** The preliminary phytochemical testing reveals that the level of polyphenols in the sample obtained from the host *Ficus religiosa* was higher as compared to *Eucalyptus hybrid* and *Roystonea regia*. The amount of phenolic compounds in the sample obtained from the host *Ficus religiosa* had phenolic content of 5.25 mg/g, followed by the sample obtained from *Eucalyptus hybrida* 4.25 mg/g and that of *Roystonea regia* had 4.0 mg/g respectively (table 1). The importance of antioxidant plant phenols is also witnessed by the researchers: (a) to increase the content of phenolics in plants<sup>27</sup>, (b) to elucidate the quantitative structure–activity relationships of various phenol classes<sup>28-29</sup>. As per literature available medicinal plants have been shown as an important source of antioxidants. Phenolics and flavonoids are the chemical constituents possessing the anti-oxidant activity<sup>30</sup>.

**Total Reducing Power Assay:** The results indicate that the reducing potential of the extracts increased with the increased concentration of samples. Among the extracts tested the ethanolic extract of *Tinospora cordifolia* from the host *Ficus religiosa* showed the maximum absorbance (0.068nm) at 1.0 mg/ml concentration at 700 nm followed by *Eucalyptus hybrida* and *Roystonea regia* (figure 2).



**Figure-1**  
Stems of *Tinospora cordifolia* from Different Hosts

**Table-1**  
Total Phenolic Content of *Tinospora cordifolia* stems collected from different hosts

S. No.	Hosts	Total Phenolics (%w/w)*
1	<i>Eucalyptus hybrid</i>	5.0±0.55
2	<i>Ficus religiosa</i>	5.25±0.12
3	<i>Roystonea regia</i>	4.25±0.65

\*Mean % SD (n=3)

**Total Antioxidant Activity:** In the current study ethanolic extracts of the stems of *Tinospora cordifolia* from different hosts were used for estimating total antioxidant activity and it was observed that the sample from the host *Ficus religiosa* (41.7 uM Fe (II)/g) possess slightly higher total antioxidant activity compared to *Eucalyptus hybrid* (41.0 uM Fe (II)/g) followed by *Roystonea regia* samples (39.7 uM Fe (II)/g) (table 2).

**Lipid Peroxidation Inhibitory Activity:** The results revealed that EC 50 value of ethanolic extract of the stem sample of *Ficus religiosa* showed inhibition value of 52.05%, followed by *Eucalyptus hybrida* with inhibition value of 51.03 % and *Roystonea regia* of 50.06 % inhibition. Sharp decline was observed at 0.7 mg/ml for all the samples (figure 3).

**Table-2**  
**Total Antioxidant Activity of *Tinospora cordifolia* stems collected from different hosts**

Sr. No.	Hosts	Total Antioxidant Activity (uM Fe (II)/g)
1	<i>Eucalyptus hybrid</i>	41.0±1.22
2	<i>Ficus religiosa</i>	41.7±0.852
3	<i>Roystonea regia</i>	39.7±0.421

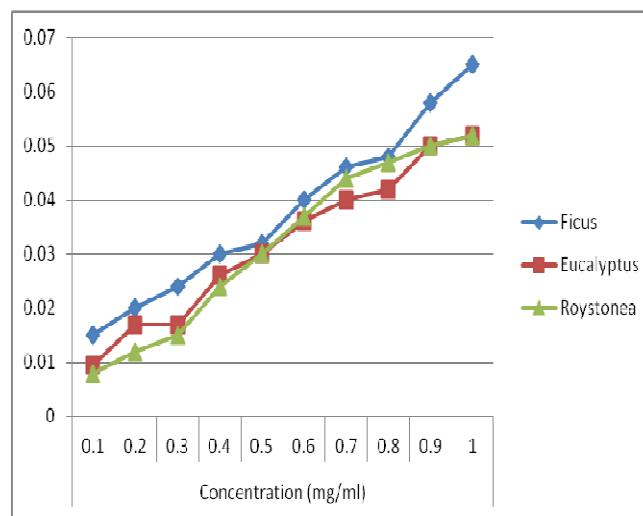
**DPPH radical scavenging activity:** In the present study DPPH radical scavenging activity of different stem extracts of *Tinospora cordifolia* revealed that the sample obtained from the host *Ficus religiosa* at 0.5 mg/mL showed highest scavenging activity of 47.8, which was followed by *Eucalyptus hybrida* having 42.2 and that of *Roystonea regia* 40.09. The EC<sub>50</sub> value of all extracts was achievable at the concentration between 0.5-0.6 mg/ml (figure 4). The stems collected from *Ficus religiosa* were noted with slightly higher total phenolic contents, reducing capability, antioxidant activity, DPPH radical scavenging activity and EC50 value for lipid peroxidation inhibitory activity when compared with *Eucalyptus hybrid* and *Roystonea regia*.

*Ficus* species have also been reported as a rich source of polyphenolic compounds, flavanoids which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases<sup>31</sup>. It is reported that the phenolics are responsible for the variation in the anti-oxidant activity of the plant<sup>32</sup>. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals<sup>33-34</sup>.

**Superoxide radical scavenging assay:** The results revealed that quenching power was negligible with all the samples. The 50% inhibition could not be achieved at any concentration from 0.1-0.9 mg/ml. The decrease in the absorbance at 562 nm with the plant extracts indicated their abilities to scavenge superoxide radicals in the reaction mixture. Several studies on antioxidant potential of *Tinospora cordifolia* are available<sup>35</sup> however, no pertinent study is available to justify the traditional belief on efficacy of *Tinospora* species from Neem host over other host trees hence systematic study needs to be done with variety of hosts of *Tinospora cordifolia* to validate the fact.

**Conclusion**

To conclude, study indicates that even though the differences in antioxidant potential were meager between the samples but the varied antioxidant activities of the *Tinospora cordifolia* collected from *Ficus* species are slightly higher than other two samples studied. In order to be able to exploit excellent medicinal potential of already in use *Tinospora* species, evaluation of effects to add more efficacy in medicinal properties by host plants have to be understood. *Tinospora* species with multiple medicinal properties may be a novel candidate for development of drug where satisfactory cure is still lacking.



**Figure-2**  
**Total Reducing Power Assay at varying concentration of samples from 0.1 to 1.0 mg/ml**



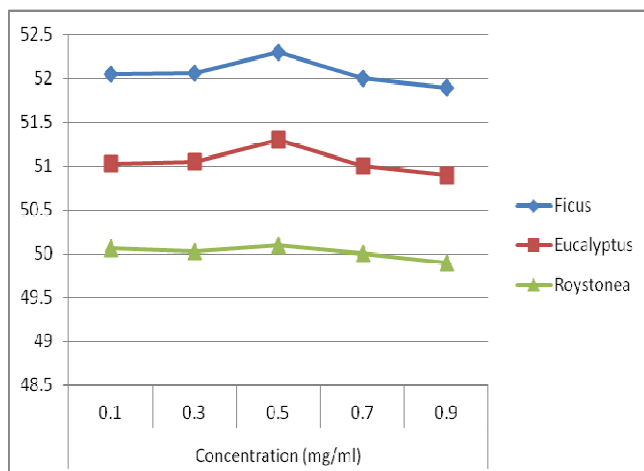


Figure-3

% Lipid Peroxidation inhibitory activity of *Tinospora cordifolia* at different concentrations (mg/ml)

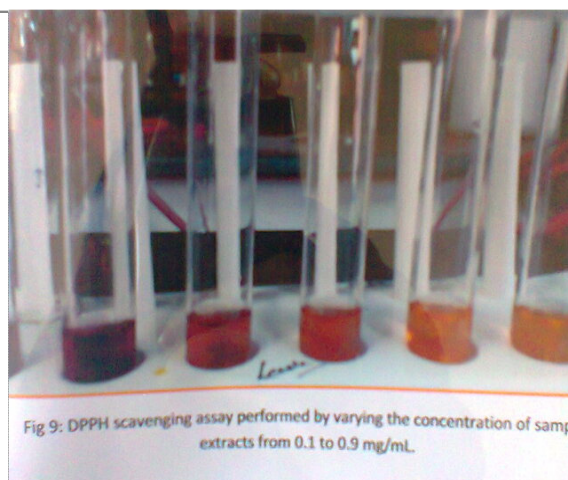
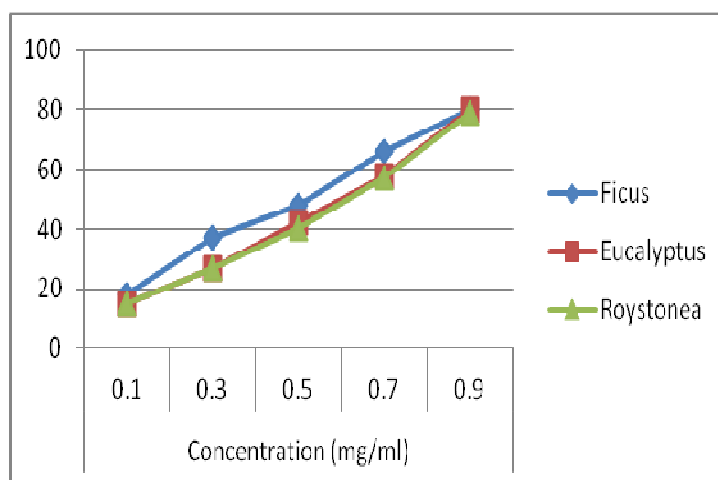


Figure-4

DPPH Radical scavenging activity of *Tinospora cordifolia* at different concentrations (mg/ml)

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