

# Anti-Oxidant and Membrane Stability Studies in *Tinospora cordifolia*

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**Abstract:** *Tinospora cordifolia* plant extracts were selected for this study to establish Anti oxidant and membrane stability activities, with estimation of total phenolic content (TPC), antioxidant (AOA) and their total flavanoid content (TFC), the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) screening assay and DNA Nicking Assay. The amount of TPC varied from 15.42 mg/g gallic acid in *Tinospora cordifolia*. It also showed high TFC (30.40µg/mg) and high AOA (82.50%), DPPH: IC<sub>50</sub> were 30.40µg/ml. In membrane stability assay *Tinospora cordifolia* revealed at the dose of 400mg/ml showed 24.2%, when compared to Standard drug at 40mg/ml showed (Aspirin) 21.5%.

*Tinospora cordifolia* plant metabolites possess TFC, TPC, DNA nicking assay, and heat induced hemolysis in human RBC membrane models respectively. Since this plant extract were the potential source of natural favonoids and polyphenols with a wide range of biological activities, with a promising AOA and further, the active components can serve as a beneficial sources for anti inflammatory activities

**Keywords:** DPPH assay, TFC, TPC and DNA damage estimation, membrane stability assay, phytopharmacology

## 1. Introduction

Guduchi *Tinospora cordifolia* (Wild) is widely used in veterinary folk medicine and ayurvedic system of medicine for its general tonic; antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties [1-4]. The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The root of this plant is known for its antistress, anti-leprotic and anti-malarial activities [5, 6].

Dry barks of *T. cordifolia* have anti-spasmodic, antipyretic [7], anti-allergic [8], anti inflammatory [9, 10] and anti-leprotic [11] properties. The dried stem of *T. cordifolia*

produced significant anti-inflammatory effect in both acute and subacute models of inflammation. *T. cordifolia* was found to be more effective than acetylsalicylic acid in acute inflammation. Jagetia *et al.*, have found that guduchi killed the HeLa cells very effectively *in vitro* and thus it indicates that guduchi needs attention as an anti-neoplastic agent [12].

Antioxidants interfere with the production of free radicals and also play a key role to inactivate them [13]. Phytochemicals like carotenoids, tocopherols, ascorbates and phenols present in plants are strong natural antioxidant and have an important role in health care system. Phenols, a major group with antioxidant properties, comprise subclasses such phenolicacids, flavonoids, biflavonoids, anthocyanins and isoflavonoids and act against allergies, ulcers, tumours, platelet aggregation, cardiovascular diseases and can reduce the risk of cancer [14, 15].

Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power) [16-18]. Polyphenols possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals. In generally, polyphenols all share the same chemical patterns; one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals [19-24].

Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species [25]. Erythrocyte membrane is structurally analogous to the lysosomal membrane.

Therefore, the use of erythrocyte membrane is good model to study the protective effect of medicinal plant extracts [26]. Previously,

stabilization of hypo toniccy induced human red blood cell membrane (HRBC) was used as an *in vitro* model to study the cytoprotective activity of medicinal plant extracts [27].

To find potential natural resources of antioxidants, extracts of different parts of some plants were studied for their total phenolic content, antioxidant and free radical scavenging activities. Medicinal and biological properties of the investigated plants have already been reported [28-33]. On the other hand, oxidative stress is intricately linked with ageing related diseases (ARDs) and longevity: antioxidant activity were recently studied in relation to CNS disorders [34].

In this study (i) IC<sub>50</sub> of the radical scavenging activity of five selective medicinal plants was evaluated against the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and the activity of the plants was compared with *Standard Rutin* [35] (ii) the total flavanoid content (TFC) of each herbal extracts were determined by AlCl<sub>3</sub> and then (iii) Total Phenolic Contents (TPC) were measured by Folin-Ciocalteu's reagent are expressed as gallic acid equivalents ( $\mu\text{g}$  of GAE)/mg dry weight of the samples. (iv) DNA Nicking Assay was performed using supercoiled pBR322 plasmid DNA [36] and HRB membrane stabilization test was performed by the following described method [37].

## 2. Materials and Methods

### 2.1 Chemicals

All of the chemicals used in this work were purchased are the 1,1 -diphenyl-2-picryl-hydrazil (DPPH) and rutin S. D. Fine Chem. Ltd - (Mumbai - India), and solvents and other reagents of analytical grade were from E. Merk (Mumbai , Maharashtra, India).

### 2.2 Plant material

Plant materials of *Tinospora cordifolia* (stem) (Menispermaceae), collected from different natural habitats in south Karnataka. India, in March 2015. Voucher specimens were deposited in the Herbarium of R&D, C.D. Sagar Centre. Dept of P.G. Studies Biotechnology Department. Dayananda Sagar College. India, chopped, dried powdered (40- mesh) and stored in polythene bags at 4°C.

### 2.3 Preparation of extracts and solutions

Plant materials were air dried at room temperature and finely grounded. Each sample (100 g) was macerated with ethanol 90% (500 ml) three times. Solvent was evaporated under reduced pressure at approximately 40 °C. The

dried extracts were dissolved in ethanol 90% to a final concentration of 1000  $\mu\text{g ml}^{-1}$  (sample stock solution), and then the different concentrations of each sample (100, 50, 40, 30, 20, 10,  $\mu\text{g ml}^{-1}$ ) were prepared.

### 2.4 Antiradical activity test

The antiradical activity of the extracts was estimated [35]. Briefly, a 0.3mM solution of DPPH radical solution in ethanol 90% was prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations of each extract (sample). After 30 min incubation in dark and at room temperature, absorbance (A) was measured at 518 nm in a SHIMADZU Multispect 1501 spectrophotometer.

The percentage of the radical scavenging activity (RSA) was calculated by the following Equation: 
$$\text{RSA}\% = \frac{[\text{A control} - (\text{A sample} - \text{A blank})]}{\text{A control}} \times 100$$

Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. Rutin Solution (at the concentrations of 100, 50, 25, 10, 5, 2.5  $\mu\text{g /ml}$ ) was used as a positive control. The IC<sub>50</sub> value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract ( $\mu\text{g /ml}$ ) against the mean percentage of the radical scavenging activity.

### 2.5 Amount of total flavanoid content

The determination of the total flavanoid content (TFC) [35]. Briefly, 2.5 ml of each extract solution was mixed with 2.5 ml AlCl<sub>3</sub> reagent in ethanol 90% and allowed to stand for 40 min at room temperature. After that, the absorbance of the mixture at 415 nm was measured with a SHIMADZU Multispect-1501 spectrophotometer.

Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound. The TFC for each extract [as  $\mu\text{g}$  rutin equivalents (RE) / mg of extract] was determined on the basis of the linear calibration curve of rutin (absorbance versus rutin concentration).

### 2.6 Estimation of the Total Phenolic Contents

The method [38, 39] downscaled to 1 mL final volume was followed to determine the amounts of total phenolics in the test samples. The test samples (each 100 /L) were mixed with 500 /L of

1:10 Folin-Ciocalteu's reagent followed by the addition of Na<sub>2</sub>CO<sub>3</sub> (400 / L, 7.5%). After incubating the reaction mixture at 24 °C for 2 h, the absorbance at 765 nm was recorded. Gallic acid monohydrate was used as the standard. The total phenolic contents as gallic acid equivalents (µg of GAE)/mg dry weight of the samples.

### 2.7 DNA Nicking Assay

The DNA nicking assay was performed using supercoiled pBR322 plasmid DNA [27], plant extracts (10µl) of different concentrations (0.2-20 µg/ml) and DNA (0.5 µg) were incubated for 10 min at room temperature followed by the addition of 10 µl Fenton's reagent (30 µl of 30 µM H<sub>2</sub>O<sub>2</sub>, 500 µl of 50 µM ascorbic acid, and 800 µl of 80 µM FeCl<sub>3</sub>). The reaction mixture was incubated for 30 min at 37°C and analysed on 1 % agarose gel by using ethidium bromide [40].

### 2.8 HRB membrane stabilization test

HRB membrane stabilization test was performed by the following described method [22]. Fresh whole human blood (10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline. The reaction mixture 2ml consists of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30min. At the end of the incubation the tubes were cooled under running tap water.

The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula:  
Percentage Inhibition = (A of Control – A of Sample) / A of Control x100.

### 2.9 Statistical analysis

All of the experiments were carried out in triplicate. The IC<sub>50</sub> (µg ml<sup>-1</sup>) values were presented by their respective 95% confidence limits. The TFCs (µg mg<sup>-1</sup>) were shown as mean ± SEM.

One-way analysis of variance (ANOVA) followed by Tuckey's post test was used to assess significant differences ( $p < 0.05$ ) between extracts. All of the statistical analyses were accomplished using the

computer software Graph Pad Prism 3.02 for Windows (Graph Pad Software, USA).

## 3. Results and Discussion

Phenols, a major group of phytochemical, have profound importance due to their AOA. To determine their potential sources,

**Table 1. Antioxidant activity (AOA %) and total phenolic content (TPC) expressed as mg/g gallic acid equivalent on a dry weight basis**

| Plant  | Plant part | AOA%          | TPC mg/g gallic acid equivalent (5 --- 500 mg/g gallic acid) |
|--|------------|---------------|--|
| <i>Tinospora cordifolia</i> (Menispermaceae) | stem       | 82.50 ± 0.042 | 15.42± 0.024 --- 20.52 ± 0.064                               |

Note: The AOA% and TPC mg/g gallic acid equivalent (Conc. of doses in triplicate 5 --- 500 mg/g gallic acid Mean+ SEM) P < 0.005

**Table 2. IC<sub>50</sub> by DPPH method (µg /ml) and total flavanoid content (TFC (µg/mg) ± SEM)**

| Plant  | Plant part | IC <sub>50</sub> by DPPH method (µg/ml) | TFC(µg/mg) ±SEM |
|--|------------|---|-----------------|
| <i>Tinospora cordifolia</i> (Menispermaceae) | stem       | 46.80*                                  | 30.40 ± 0.60*   |

\*Note: The IC<sub>50</sub> value of the positive control, rutin, was measured as 32.60 (34.50 -36.20) µg ml<sup>-1</sup> P < 0.005\*

The IC<sub>50</sub> values are presented with their respective 95% confidence limits.

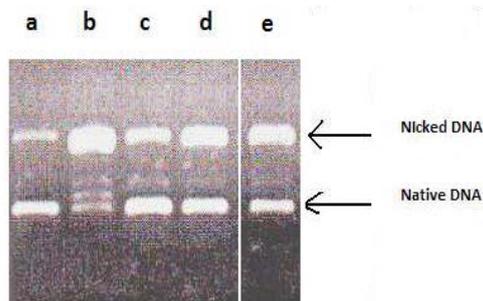
The TFC values are mean ± SEM of three determinations.

Stem of *Tinospora cordifolia* Antioxidant activity (AOA %) (82.50 ± 0.042) (Table. 1) The highest amount of the total flavanoid was found in the extract of *Tinospora cordifolia* (30.40 ± 0.60µg mg<sup>-1</sup>) (Table. 2) DNA nicking assay was showed a significant reduction in the formation of nicked DNA and an increase in native DNA (super coiled). Extracts (2.0 µg /10 µl) of *Tinospora cordifolia* were effectively prevented DNA nicking (figure 1)

**Table 3: Membrane stability assay of HRBCs incubated with different concentrations of methanolic extract of *Tinospora cordifolia* and Std drug (Aspirin)**

| Sl No. | Membrane stability assay             |   |
|--------|--------------------------------------|---|
|        | Std drug (Aspirin) / (Concentration) | <i>Tinospora cordifolia</i> extract / (Concentration) |
| 1.     | 6% (20mg/ml)                         | 8% (200mg/ml)   |
| 2.     | 21.5% (40mg/ml)                      | 24.2% (400mg/ml)                                      |
| 3.     | 32% (60mg/ml)                        | 36 % (600mg/ml)                                       |
| 4.     | 46% (80mg/ml)                        | 48% (800mg/ml)  |

|    |                   |                      |
|----|-------------------|----------------------|
| 5. | 58%<br>(100mg/ml) | 62.5%<br>(1000mg/ml) |
|----|-------------------|----------------------|



**Fig: 1 Inhibitory effects of plant extract on DNA nicking caused by hydroxyl radicals.**

Lane a. native pBR322 plasmid DNA;

Lane b, Fenton's reagent + DNA;

Lane c, Fenton's reagent + DNA+ 5 U catalase;

Lane d, Fenton's reagent + DNA+ 2 U superoxide dismutase

Lane e, Fenton's reagent + DNA+ (2.0µg/10µl) extract of *Tinospora cordifolia* (stem)

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. The use of DPPH radical provide an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers [48, 49, 50 and 51]. Therefore, in this study, the methanolic Extracts of *Tinospora cordifolia* showed the possible antioxidant and radical scavenging activity by DPPH method.

TPCs ranging from 2.12 to 64.4 g/100g indifferent parts of *Cassia fistula* have been reported [30]. In our study the amount of TPC varied from 15.42 mg/g gallic acid in *Tinospora cordifolia*. In general, fruits were found to have high amount of phenols, while rhizomes, stems, twigs, aerial parts and leaves had moderate levels, whereas roots were poor sources of TPC. Phenols, a major group of phytochemicals, have profound importance due to their AOA, in *T. cordifolia* AOA were 82.50%.

Phenolic compounds, especially flavonoids, constitute one of the most divers and widespread group of natural compounds. These compounds possess a broad spectrum of biological activities including antioxidant and radical scavenging properties [20, 17, 24], therefore the TFC in the extracts was determined (Table 2), the TFC, 30.40µg/mg, when compared Rutin to 32.60 (34.50 -36.20) µg/ml. It is known that only

flavanoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [41, 16].

Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities. The total phenolics in the all five extracts were determined spectrophotometrically by the Folin-Ciocalteu method and expressed as µg of GAE/mg of the test samples (Table. 1).

Thus, the previous results clearly established plant extracts showed best antioxidant due to its higher reducing power as well as higher content of phenolics, especially AOA by DPPH method and DNA nicking assay. It was noted that methanolic extract *Tinospora cordifolia* which gave positive test for Flavonoids exhibited potential membrane stabilities of 24.2% and 62.5% at the concentration of 400mg/ml and 1000mg/ml respectively. Moreover the standard anti-inflammatory drugs (Aspirin) at 40mg/ml and 100 mg/ml exerted maximum membrane stabilities of 21.5% and 58% respectively (table-3).

*Tinospora cordifolia*, leaf extract characterized as saponarin (apigenin-6-C-glucosyl-7-Oglucoside) had appreciable antioxidant and hydroxyl radical scavenging activities and contained the flavanoid, Saponarin showed mixed competitive inhibition on activities of alpha-glucosidase and sucrase of different origins [42].

Extract of *Tinospora cordifolia* has been shown to inhibit the lipid peroxidation and superoxide and hydroxyl radicals in vitro. Moreover, administration of the extract partially reduced the elevated lipid peroxides in serum and liver as well as alkaline phosphatase and glutamine pyruvate transaminase. This indicates the use of *Tinospora c.* extract in reducing the chemotoxicity induced by free radical forming chemicals [43].

The decoction of *T. cordifolia* showed anti-inflammatory activity on carrageenin-induced hind paw oedema in rats [44]. The effect of extract of stem of *T. cordifolia* was studied on the contractile response due to various agonists (such as histamine, 5-HT, bradykinin, prostaglandin E<sub>1</sub> and F<sub>2a</sub>, cholinomimetics and KCl) on smooth muscles of rat in the dose of 100 to 600 µg/mg. The possible mechanism of antagonistic action of *T. Cordifolia* has been discussed in the light of involvement of various autocooids in the pathophysiology of clinical joint inflammation.

The mechanism of potentiating effects of *T. cordifolia* on NA induced responses is suggested to be due to an uptake blocking effect of *T. cordifolia* or to an inhibition of metabolism by COMT since MAO inhibition would also produce potentiation of 5-HT responses [45]. Antioxidant activity and amelioration of cyclophosphamide-induced toxicity has been reported [46]. It has an ameliorative effect in aflotoxicosis of duck [47].

#### 4. Conclusion

*Tinospora cordifolia* methanolic extract exhibited significant cytoprotective activities against standards. The results also indicate that plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progression of various oxidative stress mediated disorders.

The extract therefore could be regarded as a natural source of membrane stabilizes and was capable of providing an alternative remedy for the management and treatment of inflammatory conditions

#### 5. References

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