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# Evaluation of Antinociceptive and Antioxidant Properties of the Ethanolic Extract of Tinospora cordifolia Stem from Bangladesh

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## Abstract

The crude ethanolic extract of the stem of *Tinospora cordifolia* (*T. cordifolia*) (Family: Menispermaceae) was evaluated for its possible antinociceptive and antioxidant properties growing in the most of the part of Bangladesh. The ethanolic extract of stems of *T. cordifolia* exhibited statistically significant ($p>0.001$) writhing inhibition in acetic acid induced writhing model in white albino mice (Swiss-webstar strain). The crude extract produced 40 % inhibition of writhing at the dose of 500 mg/kg body weight while the standard drug diclofenac inhibition was found to 45.22% at a dose of 25 mg/kg body weight. The antioxidant property of ethanolic extract of *T. cordifolia* was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. In DPPH scavenging assay the $IC_{50}$ value was found to be (83.52 µg/ml) which was comparable to the standard ascorbic acid (98.16 µg/ml). Phytochemical nature (group determination of plant constituent) and selected phytochemical analysis of the ethanolic extract of the stems of *T. cordifolia* indicated the presence of steroid, reducing sugars, tannin & saponin types of compounds. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

KEY WORDS: TINOSPORA CORDIFOLIA, ANTINOCICEPTIVE, ACETIC ACID INDUCED WRITHING MODEL, DPPH FREE-RADICAL SCAVENGING

## Introduction

*Tinospora cordifolia* (Family: Menispermaceae) is popularly known as "Gulancha", "Gulancha lata", "Gurach" and "Gadancha" found throughout tropical India, Burma, Andaman, Ceylon and all most everywhere in our country [1]. Leave and stem act as febrifuge and blood purifier and are also used in the treatment of acidity jaundice, burning urination and fatigue. Juice of fresh leaves is useful in fever, cough, cardiac problems, rheumatism, haemdysis, colic, dropsy, gonorrhoea and skin infections. Infusion of powdered aerial parts is used as alternative and aphrodisiac [1]. In the previous study, methanolic crude extracts obtained from the *T. cordifolia* were evaluated for the antifungal and HPLC analysis [2]. *T. cordifolia* alcoholic extract shows immunomodulator activity [3]. The antibacterial activity of the aqueous, ethanol and chloroform extracts from the stems of *T. cordifolia* was studied using disc diffusion method against *Escherichia coli*, *Proteus vulgaris*, *Enterobacter faecalis*, *Salmonella typhi* (Gram-negative), *Staphylococcus aureus* and *Serratia marcesenses* (Gram-positive) [4]. *T. cordifolia* root extract showed antioxidant action in alloxan diabetic rats [5]. Same authors also showed the hypoglycaemic and other related actions of *T. cordifolia* roots in alloxan-induced diabetic rats [6] and hypoglycaemic and hypolipidaemic action of alcohol extract of *T. cordifolia* roots in chemical induced diabetes in rats [7]. The aerial part of *T. cordifolia* Miers. was evaluated for analgesic and neuropharmacological properties of in mice [8]. Evaluation of antimicrobial activity and phytochemical screening of extracts of *T. cordifolia* was done against some pathogenic microbes [9]. The results of a preliminary antimicrobial screening of the methanol extracts of *Zingiber officinale*, *Asteracantha longifolia*, *Citrus acida*, *Salacia microsperma* and *T. cordifolia* are reported [10]. A standardized extract from *Tinospora* known as Tinofend has been studied clinically. One small study in 75 patients with allergic rhinitis showed statistically significant reduction of symptoms compared to placebo [11]. Recent research has demonstrated that a combination of *T. cordifolia* extract and turmeric extract is effective in reducing the hepatotoxicity which is induced by the combination of isoniazid, rifampicin, pyrazinamide and ethambutol for treating tuberculosis [12]. *T. cordifolia* showed the antitumor activity of tumor-associated macrophages-derived dendritic cells [13] and immunomodulatory and antitumor actions through activation of tumor-associated macrophages [14]. Immune stimulating properties found from a novel polysaccharide from *T. cordifolia* [15]. *T. cordifolia* exerted neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation [16]. *T. cordifolia* showed hepatoprotective and immunomodulatory properties of in $CCl_4$ intoxicated mature albino rats [17], immunomodulatory role of *T. cordifolia* as an adjuvant in surgical treatment of diabetic foot ulcers [18] and a polysaccharide from this plant showed Antioxidant properties against iron-mediated lipid damage and gamma-ray induced protein damage [19]. *T. cordifolia* showed alteration of lethal effects of gamma rays in Swiss albino mice [20] and antifertility effect in male rats [21].

Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases in human. Reactive oxygen species (ROS) have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive includes the hydroxyl radical (OH.), the superoxide radical (O.2), the nitric oxide radical (NO.) and the lipid peroxyl radical (LOO.) cause severely deleterious effects on the human body [22]. Enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P450 system and oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria [23]. ROS are the products of normal cellular metabolism, having both deleterious and beneficial effect in the body [24]. The balance between the production of free radicals and the antioxidant defenses in the body has important health implications. If there are too many free radicals produced and too few antioxidants, a

condition of "oxidative stress" develops which may cause chronic damage body [24]. Antioxidants play an excellent role in preventing cell damage. They donate their own electrons to free radicals. Free radical accepts the electron from antioxidant and they do not attack the cell and the chain reaction of oxidation is inhibited [25]. Phenolic compounds, flavonoid and triterpenoids containing foods and beverages with antioxidant activity have been reported [26]. Very recent, health risks and toxicity have been reported using synthetic antioxidants restricted [27]. Some well known natural antioxidants like rosemary and sage are already exploited commercially either as antioxidant additives or as nutritional supplements stipulating the antioxidant potential of plant species [28]. In recent years, the interest in natural antioxidant, especially of plant origin, has greatly increased [29].

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [30]. Analgesic activities are commonly exhibited by the non-steroidal anti-inflammatory drugs (NSAIDS). These NSAIDs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin [31].

Since no literature is currently available to substantiate antinociceptive and antioxidant activities from ethanolic extract of *T. cordifolia* stem, therefore the present study is a part of our on-going pharmacological and chemical screening of selected *T. cordifolia* stem and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antioxidant activities.

## Materials and methods

### 2.1. Collection and identification of plant materials

For this present investigation the *T. cordifolia* was collected from Khulna region, Bangladesh in October, 2004. The plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka.

### 2.2. Preparation of ethanolic extract

About 600 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

### Drug

Drug employed in the study was: diclofenac sodium (Opsonin Pharmaceuticals Ltd, Bangladesh).

### Chemicals

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA) and other chemicals were of analytical grade.

### Preparation of plant extract

The plant material was shade dried with occasional shifting and then powdered with a mechanical grinder, and stored in a tight container. The dried powder (1.5 kg) was refluxed with ethanol for three hours. The total filtrate was concentrated to dryness, in vacuo at 40°C to render the ethanol extract for investigation.

### Animal

For the experiment, twenty swiss albino mice of either sex, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: (24.0 ± 1.0°C), relative humidity: 55-65% and 12 h light/ dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

### Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, steroids with Libermann-Burchard reagent and reducing sugars with Benedict's reagent [32-34].

### Antinociceptive activity

Antinociceptive activity of the crude extract was tested using the model of acetic acid induced writhing in mice [35-36]. The experimental animals were randomly divided into three groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 mL/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III was test groups and was treated with the extracts at dose 500 mg/kg of body weight respectively. Each mouse was weighed properly and the dose of the test samples and control materials were adjusted accordingly. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection in peritoneum. Then after an interval of 10 min, the number of writhes (squirms) was counted for 5 min.

### Screening for In-vitro Anti-oxidant Activity

### Free radical scavenging activity by DPPH Method

Quantitative assay was performed on the basis of the modified method of Choi Y et al. 2007 [37]. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph $IC_{50}$ was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

## Results

### Preliminary phytochemical analysis

Results of different chemical tests on the methanol crude stems extract of *T. cordifolia* showed the presence of Steroid, Reducing sugars, Tannins and Alkaloids (**Table-1**).

### Antinociceptive

The results of the test showed that *T. cordifolia* ethanolic stems extract 500 mg/kg exhibit highly significant ($P<0.001$) inhibition of writhing reflex by 40 % while the standard drug diclofenac inhibition was found to be 45.22% at a dose of 25 mg/kg body weight. The result is showed in Table-2 and percent of writhing of standard and ethanolic extract of stems of *T. cordifolia* is showed in Figure-1.

From the above observation it can be suggested that the ethanolic extract of stems of *T. cordifolia* is an effective analgesic that supports the claim about the stem being used as an analgesic in traditional practice. However further study should be done for its isolated, purified active principles

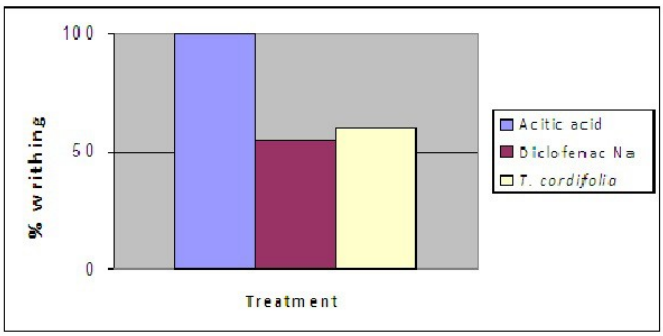


Figure-1: Effect of *T. cordifolia* on acetic acid induced writhing of mice. Each Bar represents % writhing.

**Antioxidant**

DPPH applied TLC plates ware observed under UV detector both in short (254 nm) and long (360 nm) wavelength. Antioxidant components in the ethanolic extract of *T. cordifolia* were identified. Ethanol extract of *T. cordifolia* showed potential antioxidant activity (Figure-1) where the $IC_{50}$ was 83.52 µg/mL ($P < 0.001$), as compared to that of ascorbic acid ($IC_{50}$ 98.16 µg/mL) ($P < 0.001$) which is a well known antioxidant. The extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Figure-2).

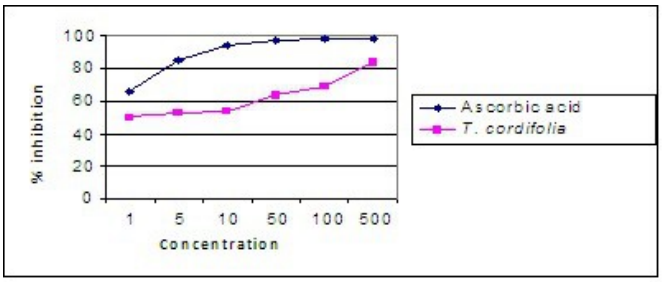


Figure-2: DPPH radical scavenging activity of the ethanolic extract of stem of *T. cordifolia* and standard.

## Discussion

Preliminary phytochemical screening showed the presence of Steroids, Reducing sugars, Tannins and Alkaloids in the stem extract. Multiple biological effects, including antioxidant activity commonly found in plants containing Polyphenolic compounds, like flavonoids, tannins and phenolic acids [38]. Tannic acid present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. It was shown that the percentage (%) scavenging of DPPH radical was increased significantly with increasing dose, $P< 0.001$. $IC_{50}$ value of the stem extract was found to be very fairly significant (83.52 µg/ml) when compared to the $IC_{50}$ value of the reference compounds ascorbic acid (98.16 µg/ml).

Antinociceptive activity of the ethanol extract of *T. cordifolia* was tested by acetic acid induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [39]. Increased levels of $PGE_2$ and $PGF_{2á}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [39]. The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanol extract of stems of *T. cordifolia* might possess antinociceptive activity. Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with antiphlogistic activity [40].

## Conclusion

In conclusion it can be revealed that the crude ethanolic extract of *T. cordifolia* stem possess significant antinociceptive as well as antioxidant activities. The potential of the extract of *T. cordifolia* as antinociceptive and antioxidant agents may be due to the presence of phytoconstituents like tannins, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, extensive researches are necessary to search for active principles responsible for these activities.

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| Plant Extract | Alkaloid | Reducing Sugars | Tannins | Gums | Flavonoids | Saponin | Steroid |
|---|---|---|---|---|---|---|---|
| **EE** | + | + | + | - | - | - | + |

*EE: Ethanol extract of T. cordifolia; +: Positive result; - : Negative result*

Table 1: Results of different group tests of ethanolic extract of *T. cordifolia* stems

| Treatment | Dose | Mean Writhing ± SEM | % writhing | % writhing inhibition |
|---|---|---|---|---|
| Test group1 (1%tween-80 solution in water) | ------ | 22.4± 1.51 | 100 | -- |
| Test group 2 (Diclofenac sodium as Positive control) | 25 mg/kg | 12.6± 0.20* | 54.78 | 45.22 |
| Et extract of *T. cordifolia* | 500 mg/kg | 10.4± 0.50* | 60 | 40 |

*Values are expressed as mean ± SEM (Standard Error Mean); Et.: Ethanolic; * indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control.*

Table 2: Effects of *T. cordifolia* stem extract on writhing effect on acetic acid induced mice