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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF *TINOSPORA CORDIFOLIA*

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Abstract: The crude ethanol extract of the stem of *Tinospora cordifolia* (Family Menispermaceae) was evaluated for its antioxidant and analgesic activities. The antioxidant property of the ethanol extract of *T. cordifolia* was assessed by 1, 1-diphenyl –2- picryl hydrazyl (DPPH) free radical scavenging assay. The extract showed potential antioxidant activity (IC_{50} about 52.80 µg/ml), which was comparable to standard drug ascorbic acid (IC_{50} about 4.12 µg ml^{-1}). The ethanolic extract of *T. cordifolia* also exhibited statistically significant ($p<0.001$) writhing inhibition in aceitic acid induced white albino mice (Swiss-webstar strain). It produced 40% inhibition of writhing at the dose of 500 mg kg^{-1} body weight while the standard drug (diclofenac sodium) inhibition was found to 45.22 % at a dose of 25 mg kg^{-1} body weight. The results tend to suggest that the crude ethanolic extract of *T. cordifolia* might possess antioxidant and analgesic activities.

Key words: *Tinospora cordifolia,* phytochemical properties, pharmacological activities

Introduction

Tinospora cordifolia (Family: Menispermaceae), commonly known as 'Gulancha', is a large glabrous climber with succulent, corky, grooved stems and is distributed throughout tropical India (Kiritikar and Basu, 1999). A wide variety of chemical constituents have been isolated from *T. cordifolia* such as alkaloids, glycosides, diterpenoid lactones, steroids, sesqui terpenoids etc. (Ghani, 2003). *T. cordifolia* is widely used in veterinary folk medicine / ayurvedic system of medicine for its general toxic, antiperiodic, antispasmodic, anti-inflammatory, antiallergic and anti-diabetic properties (Jahan, 2005). Leaves and stems 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, caugh, cardiac problems, rheumatism, haemdysis, colic, dropsy, gonorrhoea and skin infections (Ghani, 2003).

Materials and Methods

Collection of plant: The stem of *T. cordifolia* was collected from the Khulna region, during the month of October, 2004 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka.

Extraction: The collected plant part (stem) was separated from undesirable materials or plants or plant parts and sun-dried for one week. The plant part was ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 250 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

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accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a filtration through Whatmann filter paper. The filtrate thus obtained was evaporated under normal environment by electric fan to get the dried extract to provide a gummy concentrate of brown color.

Phytochemical analysis: The crude extract was subjected to preliminary phytochemical testing for the detection of major chemical groups (Evans, 1989). In each test 10% (w/v) solution of the extract in solvent was taken unless otherwise mentioned in individual test.

Animals: Young Swiss-albino mice of either sex, weighing 20-25gm, purchased from the Animal Resources Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for analgesic activity test. The animals were kept at animal house (Pharmacy Discipline, Khulna University, Khulna) for adaptation after their purchase under standard laboratory conditions (relative humidity 55- 65%, room temperature 25.0±20 C and 12 hours light: dark cycle) and fed with standard diets (ICDDR,B formulated) and had free access to tap water.

Antioxidant activity: Antioxidant activity of the ethanol extract was determined on the basis of their scavenging activity of the stable DPPH free radical.

Qualitative assay: A Suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.,* 2003).

Quantitative assay: Quantitative assay was performed on the basis of the modified method of Gupta *et al.* (2003). 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 µg/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 at the same concentrations of extract was used as positive control (Gupta *et al*., 2003).

Analgesic activity: Analgesic activity of the ethanolic extract of *T. cordifolia* was tested using the model of acetic acid induced writhing in mice (Whittle, 1964; Ahmed *et al*., 2001). The experimental animals were randomly divided into four groups, each consisting of eight animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 solution in water; 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 ethanolic extract of *T. cordifolia* at the doses of 500 mg/kg of body weight. Control vehicle, standard drug and extracts were administered orally; 30 min prior to acetic acid (0.7 %) injection, then after interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Statistically analysis: Student's t-test was used to determine a significant difference between the control group and experimental group.

Results

The crude ethanolic extract of the stem bark of *T. cordifolia* when tested for its different chemical groups showed the presence of alkaloids, reducing sugars, tannins and steroids (Table 1).

Table 1. Results of different group tests of *T. cordifolia.*

Ethanolic Plant extract	Alkaloid	Reducing Sugars	Tannins	Gums	Flavonoids	Saponin	Steroid
Tinospora cardifolia	+	+	+	-	-	-	+

+: present; - : absent

The extract showed excellent antioxidant activity (IC_{50} about 52.80 µg ml^{-1}) against DPPH free radical comparable to that of standard drug, ascorbic acid (IC_{50} about 4.12 µg ml^{-1}) (Table 2).

Table 2. Antioxidant activity of *T. cordifolia* (Values are expressed as mean ± S.D.).

Sample	Concentration (µg/ml)	% inhibition	IC_{50} (µg ml^{-1})
Ethanolic extract of *T. cordifolia*	1	43.83	52.80
	5	45.45	
	10	46.66	
	50	48.90	
	100	55.25	
	500	75.86	
Ascorbic acid	1	39.78	4.12
	5	53.82	
	10	61.40	
	50	77.47	
	100	93.96	
	500	96.84	

The ethanolic extract of *T. cordifolia* produced about 40% writhing inhibition at 500 mg kg^1 body weight, which was comparable to standard drug diclofenac sodium where the inhibition was about 45.22% at the dose of 25 mg kg^1 (Table 3).

Table 3. Effect of ethanolic extract of *Tinospora cardifolia* on acetic acid induced writhing in mice.

Animal group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 solution in water, p.o.	22.4±1.01 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	12.6±1.68* (54.78)	45.22
Test group Et. Extract 500 mg/kg, p.o.	13.8±0.61** (60.00)	40.00

Values are expressed as mean ± S.E.M; *: P <0.001; **: p <0.001 vs. control; Et.: Ethanolic; %: percentage. p.o.: per oral.

Discussion

Since *T. cordifolia* belongs to the coastal forests, part of the plant constituents may be polar in nature. Ethanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness.

The free radical scavenging property may be one of the mechanisms by which the plants are effective in traditional medicine. Further study is necessary to identify the constituents which are responsible for this property as well as studies with other models, such as lipid peroxidation and in vivo assays are essential to characterize them as biological antioxidants.

The analgesic activity of *T. cordifolia* was tested by using the 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 then excite the pain nerve endings (Taesotikul *et al.,* 2003). Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.,* 1980). On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanolic extract of T. cordifolia might possess an analgesic activity.

Conclusion

In conclusion, it can be concluded that the crude ethanolic extract of the stem of *T. cordifolia* may possess antioxidant and analgesic activities, which correlate well with the traditional use of the plant. Therefore, further researches are essential to find out the active principles responsible for these activities.

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