

Full Length Research Paper

Phytochemical and antinociceptive study of leaves of *Tabernaemontana divaricata* (L)

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The crude ethanol extract of leaves of *Tabernaemontana divaricata* (L). (Apocynaceae) was screened for its preliminary antinociceptive activity. The extract produced significant writhing inhibition in acetic acid induced mice at the oral dose of 250 and 500 mg/kg body weight respectively ($P < 0.05$, $P < 0.01$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The result tends to suggest the antinociceptive activity of the extract.

Key words: *Tabernaemontana divaricata*, antinociceptive, writhing.

INTRODUCTION

Tabernaemontana divaricata (L). (Apocynaceae) (synonym-*Tabernaemontana coronaria*, *Ervatamia coronaria*), commonly known as Togor, Dudhphul in Bangladesh and Wax flower, Crepe flower, Crepe jasmine in India, is an evergreen shrub to 6 feet (1.8 m) distributed in Coast forests of Bengal, Myanmar, mangrove forests of China and Japan. *T. divaricata* extract inhibits neuronal acetylcholinesterase activity in rats (Chattipakorn et al., 2007). Although, a number of chemical investigations have been performed and some constituents have been reported as alkaloids, carotenoids, flavonoids, glycosides, lipids, triterpenes, polyphenols, saponins, etc. (Basak et al., 1996; Ghosh et al., 1985; Sharma and Garg, 1996). The objective of the present study was to investigate the antinociceptive activity of the crude ethanol extract of the leaves of *T. divaricata*.

MATERIALS AND METHODS

Plant material collection and extraction

The leaves of *T. divaricata* were collected from Dighinala Thana, Khagrachari District, Bangladesh on 8th October, 2007, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession no. 32077). About 275 gm of powdered

soaked in 900 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 14 days 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 and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get the crude extract (% Yield = 2.91%).

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh),

Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in ethanol was used unless otherwise mentioned in individual test (Evans, 1989; Ghani, 1998).

Reagents used for the different chemical group test

- i) Mayer's reagent: 1.36 gm mercuric iodide in 60 ml of water was mixed with a solution containing 5 gm of potassium iodide in 20 ml of water.
- ii) Dragendroff's reagent: 1.7 gm basic bismuth nitrate and 20 gm tartaric acid were dissolved in 80 ml water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.
- iii) Fehling's solution A: 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.
- iv) Fehling's solution B: 176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide were dissolved in sufficient water to

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produce 500 ml. Equal volume of above solution were mixed at the time of use.

v) Benedict's reagent: 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

vi) Molish reagent: 2.5 gm of pure α -naphthol was dissolved in 25 ml of ethanol.

Tests procedure for identifying different chemical groups

Test for Steroids

i) Sulphuric acid test: 1 ml solution of chloroform extract was taken and then added 1ml sulphuric acid. Red color indicates the presence of steroid.

Test for alkaloids

i) Mayer's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow color precipitate was not formed and that was indicated as the absence of alkaloids.

ii) Dragendorff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendorff's reagent was added. Orange brown precipitate was not formed and that was indicated as the absence of alkaloids.

Tests for reducing sugar

i) Benedict's test: 0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of benedict's solution was added to the test tube, boiled for 5 min and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar.

ii) Fehling's test (Standard Test): 2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few minutes. A red or brick red color precipitate was formed in the presence of a reducing sugar.

Tests for tannins

i) Ferric Chloride test: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins.

Test for gums

Five milliliter solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums.

Test for Flavonoids

Added a few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids.

Test for Saponins

One milliliter solution of the extract was diluted with distilled water to

20 ml and shaken in a graduated cylinder for 15 min. One centimeter layer of foam indicates the presence of saponins.

Animals

Young Swiss-albino mice of either sex, weighing 20 to 25 gm, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 to 65%, room temperature $25.0 \pm 2.0^\circ\text{C}$ and 12 h light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies

Antinociceptive activity

Antinociceptive activity of the crude ethanol extract of *T. divaricata* (L) was tested using the model of acetic acid induced writhing in mice (Whittle, 1964; Ahmed et al., 2004; Sharker et al., 2009). The experimental animals were randomly divided into four groups, each consisting of five 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 and group IV were test groups and were treated with the extracts at dose of 250 and 500 mg/kg of body weight, respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Statistical analysis

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

RESULTS

Preliminary phytochemical analysis

Results of different chemical tests on the ethanol extract of *T. divaricata* (L) showed the presence of steroids, tannins, saponins, gums and reducing sugar (Table 1).

Antinociceptive activity

Table 2 showed the effect of the ethanol extract of *T. divaricata* (L) on acetic acid induced writhing in mice. At the dose of 250 and 500 mg/kg of body weight, the extract produced about 19.80 and 31.68% writhing inhibition in test animals, respectively. The results were statistically significant ($P < 0.05$, $P < 0.01$) and were comparable to the standard drug diclofenac sodium, which showed 54.45% writhing inhibition at the dose of 25 mg/kg ($P < 0.001$).

Table 1. Results of different chemical group tests of *T. divaricata*.

Plant extract	Steroids	Alkaloids	Reducing sugars	Tannins	Gums	Flavonoids	Saponins
Ethanol extract of <i>Tabernaemontana divaricata</i>	+	-	+	+	+	-	+

+: Positive result; -: Negative result.

Table 2. Effect of ethanol extract of *T. divaricata* on acetic acid induced writhing in mice.

Animal group/treatment	Number of writhes (% writhing)	Inhibition (%)
Group-I (Control) 1% tween-80 10 ml/kg, p.o.	20.2 ± 1.8	----
Group-II (Positive control) Diclofenac sodium 25 mg/kg, p.o.	9.2 ± 1.24*	54.45 ^a
Group-III Ethanol extracts 250mg/kg, p.o.	16.2 ± 0.48*	19.80 ^b
Group-IV Ethanol extracts 500 mg/kg, p.o.	13.8 ± 0.37*	31.68 ^c

Values are expressed as mean ± SEM (Number of animals, n = 5); p-values were determined using student t-tests, a>0.001, b>0.05, c>0.01, vs. control; p.o.: per oral.

DISCUSSION

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. Antinociceptive activity of the ethanol extract of *T. divaricata* (L) 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 algia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul et al., 2003). Increased levels of PGE₂ and PGF_{2α} in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt et al., 1980).

The ethanol extract of *T. divaricata* (L) produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). On the basis of this result it can be concluded that the ethanol extract of *T. divaricata* (L) might possess antinociceptive activity. In conclusion, it could be suggested that the crude ethanol extract of *T. divaricata* (L) might possess antinociceptive activity. However, further studies are necessary to find out the active principles responsible for this activity.

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