International Journal of Life Science and Engineering
Vol. 1, No. 4, 2015, pp. 202-206
http://www.aiscience.org/journal/ijlse

Cytotoxic, Anthelmintic and Analgesic Activities of Methanol Extracts from Different Plant Parts of *Tabernaemontana Corymbosa* (Family: Apocynaceae)

Sumit Kumar Das, Sadia Afreen Chowdhury*

Department of Pharmacy, Stamford University Bangladesh, Dhaka, Bangladesh

Abstract

The aim of the present study was to evaluate the cytotoxicity, anthelmintic activity, and analgesic properties of the leaf, flower and steam of *Tabernaemontana corymbosa* (Family: Apocynaceae). The cytotoxic activity of the plant extracts were performed by brine shrimp lethality bioassay in which, the methanol extracts of the leaf, flower and steam got LC_{50} value of 0.571, 0.350 and 0.517µg/ml respectively, while LC_{50} value of vincristin sulphate was 0.35µg/ml. Anthelmintic activity was performed by observing the time of paralysis and the time of death of earthworms *Pheretima posthuma* compared with the standard drug, albendazole. The range of minimum to maximum time of paralysis and death of albendazole at the conc. 100µg/ml was 2min. 6sec. to 9min. 23sec. whereas, the range of minimum to maximum time of paralysis and death of the fresh leaf juice was 2min. 52sec. to 6min. 32sec., respectively. The results confirmed the presence of significant anthelmintic activity. Analgesic potential of the extracts were evaluated using tail-flick and hot plate tests to understand the central analgesic activity in mice (Swiss Albino mice) at 200 and 400mg/kg body weight doses. The results of the present study suggested that the extracts possess significant analgesic activity in mice, which is comparable to the standard drug, diclofenac-Na.

Keywords

Tabernaemontana Corymbosa, Brine Shrimp Lethality Bioassay, Anthelmintic Activity, Analgesic Activity, Vincristine Sulphate, Albendazole, Diclofenac-Na

Received: May 6, 2015 / Accepted: June 3, 2015 / Published online: July 31, 2015

1. Introduction

The plant *Tabernaemontana corymbosa* belongs to Apocynaceae family and commonly cultivated for its ornamental and fragrant flowers. It has been used traditionally to treat cancer, wound and inflammations, uterine stimulant, antispasmodic and hypotensive (Dhar *et al*., 1968).

T. corymbosa is a very common traditional medicinal plant in Brunei, China, Indonesia, Laos, Malaysia, Myanmar, Singapore, Thailand, and Vietnam. The plant has been used traditionally to treat fever, diabetes, rheumatism and sinusitis. Its aerial parts are commonly used as antimalarial, analgesic and against other diseases (Mathappan *et al*., 2013).

Flower is used as expectorant. Leaves are used as emollient, demulcent, vulnerary, against gonorrhea, as a hemostate, contraceptive, for liver disease and applied as a poultice to ulcer. Bark juice is used against dysentery and the seeds contain fatty oil and supposedly a 3% infusion of them is a potent vermifuge. Stem are used to treat tuberculosis, dyspepsia, syphilis and also the whole plant is used as decoction against cough (Austin, 1999).

* Corresponding author
E-mail address: sadia_afreen_chy@yahoo.com (S. A. Chowdhury)

A new ervatamine alkaloid, 5-oxo-19, 20-dehydroervatamine, in addition to N1-methoxy-19, 20-dehydroervatamine, 19, 20-dehydroervatamine and methuenine has been isolated from the leaves of *T. corymbosa*. Two seco-tabersonine alkaloids, jerantiphyllines A and B, in addition to a tabersonine hydroxyindolenine, jerantinine H, and a recently reported vincamine alkaloid 7, were isolated from the leaf extract of the Malayan *T. corymbosa* and the structures were established using NMR and MS analysis. Biomimetic conversion of jerantinines A and E to their respective vincamine and 16-epivincamine derivatives were also carried out (Lim *et al*., 2009).

2. Materials and Methods

2.1. Chemicals

Drugs and chemicals used in the study include: vincristine sulphate (Gedeon Richter), albendazole, diclofenac-Na, acetic acid (Merck, Germany), methanol (Merck, Germany), DMSO (Merck, Germany), DMF (Merck, Germany).

2.2. Plant Materials and Extraction

T. corymbosa was collected from Baniyachong, Habigaj, Bangladesh. The time of collection was March, 2013. Later the plant was identified by the respective scientist of Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 38687) has been deposited in the herbarium for future reference. *T. corymbosa* leaves, flower, steam were first separated from undesirable materials. They were dried for one and half week in a shaded place. After drying, the plant part was grinded by blender machine (NOWAKE, JAPAN). Coarse powder was obtained after grinding. Following the method of cold extraction, 100g of powder from each plant part of *T. corymbosa* were separately soaked in 300 ml of methanol for 20 days and then all the extracts were filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50)°C and reduced pressure which provide greenish color extracts from each plant part respectively.

2.3. Animals

For the experiment Swiss Albino mice of either 3-4 weeks of age, weighing between 20 to 25g were collected from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0°C), relative humidity (55-65% and 12hrs. light /12hrs. dark cycle). Husk and excreta were removed from the cages every day. Adult earthworm *Pheretima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil. For anthelmintic study, all the worms were washed with normal saline to remove fecal matters. In brine shrimp lethality bioassay, brine shrimp nauplii is used as a favourable monitor for screening and fractionation in the discovery of new bioactive natural products (Hasan, 2009).

2.4. Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops were used as the test organism. Seawater was taken in the small tank. Shrimp eggs were added to one side of the tank, and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to each of the vials containing 5ml of seawater (Mayer *et al*., 1982).

2.4.1. Preparation of Positive Control Group

Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40µg/ml from which serial dilutions were made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.0781µg/ml respectively. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

2.4.2. Preparation of Negative Control Group

100µl of DMSO was added to each of three pre-marked glass vials containing 5ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

2.4.3. Preparation of Test Groups

4mg of sample was dissolved in 100µl of DMSO. From that test solution different volumes were added to premarked glass vials or test tubes containing 5ml of seawater and 10 shrimp nauplii, so as to make the final concentration of samples in the vials or test tubes as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781µg/ml respectively.

2.4.4. Counting of Nauplii

After 24 hours, the vials were inspected using a magnifying

glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

2.5. Anthelmintic Activity

Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Anthelmintics or antihelminthics are the drugs or the agents that destroy or cause the expulsion of such parasitic intestinal worms and helps to treat helminthiasis, one of the most common infections in humans and cattle. Resistant worms accumulate and finally treatment failure occurs. To overcome the resistance, plant derived drugs can serve as prototype to develop more effective and less toxic medicines (Singh *et al*., 2002). Fresh juice extract of the leaves of *T. corymbosa* were dissolved in minimum amount of DMF and the volume was adjusted to 10ml with saline water. All drug and extract solutions were freshly prepared before starting the experiment. In each case, 6 earthworms released into 10ml of desired formulations as follows: vehicle (5% DMF in normal saline), abendazole (25, 50 and 100mg/ml) and fresh juice extract of the leaf (5% DMF in normal saline). Observation was made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in saline solution (Patil *et al*., 2009).

2.6. Analgesic Activity

The study of analgesic activity of the *T. corymbosa* was performed in animal models for central mechanism of pain. To evaluate the analgesic activity against centrally mediated pain tail-flick and hot plate tests were used.

2.6.1. Tail-Flick Test

The procedure is based on the observation that morphine like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test groups were treated with diclofenac-Na (5 mg/kg body weight), water (10ml/kg body weight) and test samples at the doses of 200 and 400mg/kg body weight respectively. 1 to 2cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30, 60 and 90min after the administration of the test drugs and standard (Hasan, 2009).

2.6.2. Hot Plate Test

The paws of mice are very sensitive to temperature at 55±0.5°C, which are not damaging to the skin. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15sec (Franzotti, 2000), was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped at 0, 30, 60 and 90min after oral administration of the samples (Eddy, 1953). The animals of test groups received test samples at the doses of 200 and 400mg/kg body weight. Positive control group and vehicle control group were treated with diclofenac-Na (5mg/kg b.w.) and in water (0.1ml/mouse) respectively (Kulkarni, 2005).

3. Results and Discussion

3.1. Brine Shrimp Lethality Bioassay of *T. corymbosa*

Following the procedure of Meyer, 1982**,** the lethality of all the methanol extracts of *T. corymbosa* to brine shrimp were determined and the regression analysis data is given in the Table 1.

Table 1. Regression analysis data for Vincristine Sulphate and different plant extracts of *T. corymbosa.*

Sample		LC_{50} (µg/ml)	Regression equation	R^2
Vincristine sulphate (positive control)		0.348	Y=32.032x+64.676	0.979
Methanol extracts	Leaf	0.571	Y=4.248x+34.18	0.955
	Flower	0.350	Y=20.58x+42.79	0.951
	Steam	0.517	Y=17.29x+41.06	0.959

The plant extracts of *T. corymbosa* showed significant cytotoxic activity against brine shrimp nauplli with LC_{50} value, 0.571, 0.35 and 0.517µg/ml respectively when compared with the standard vincristine sulphate (LC_{50} value: 0.35µg/ml). Comparison with positive control vincristine sulphate signifies that cytotoxicity exhibited by methanol extract of all plant parts possess significant cytotoxic activity.

3.2. Anthelmintic Activity

The results of anthelmintic activity of *T. corymbosa* are given in Table 2.

It is evident from experimental data (Table 2) that the fresh juice extract of the leaf of *T. corymbosa* showed potent anthelmintic activity. Results are comparable with standard drug, albendazole.

Table 2. In vitro anthelmintic activity of *T. corymbosa*.

Test Samples	Conc. (mg/ml)	Time Taken for Paralysis (minutes)	Time Taken for Death (minutes)
Fresh juice extract of leaves	25	6 min. 37 sec.	10 min. 33 sec.
	50	5 min. 22 sec.	9 min. 19 sec.
	100	2 min. 52 sec.	6 min. 32 sec.
Albendazole	25	5 min. 56 sec.	12 min. 25 sec.
	50	4 min. 31 sec.	10 min. 13 sec.
	100	2 min. 6 sec.	9 min. 23 sec.

3.3. Analgesic Activity

3.3.1. Tail-Flick Test

The Analgesic Activity Tests were carried out in the laboratory on five groups of mice by Tail-flick Method. Time interval for the test was 30 minutes. The tail withdrawal reflex time after administration of the *T. Corymbosa* was found to increase with increasing dose of the extract.

The tail immersion test is widely used for assessing analgesic activities. In our experiments, *T. corymbosa* exhibited significant analgesic activity in tail immersion test. It seems possibly that the higher doses of the extract have more potent analgesic effect (Table 3).

Table 3. Effect of *T. corymbosa* on tail withdrawal reflex in mice.

Test Group	Dose mg/kg	Plant parts	Response Times (in seconds)			
			0 min	30 min	60 min	90 min
Control			1.29±0.12	1.76±0.15	2.37±0.14	1.66±0.23
Positive Control	5.0		1.87±0.21	5.33±0.26	7.12±0.18	6.94±0.14
Group-I	200	Leaf	1.61±0.12	5.80±0.63	8.16±0.66	6.76±1.24
		Flower	1.85±0.43	7.40±1.85	5.39±1.04	4.48±0.37
		Steam	3.68±1.69	3.15±0.17	2.93±0.21	3.15±0.29
Group-II	400	Leaf	1.47±0.05	2.89±0.24	4.85±0.24	5.11±0.39
		Flower	1.14±0.06	2.71±0.11	5.45±1.30	5.87±0.49
		Steam	3.23±1.71	3.55±0.33	3.53±0.35	3.55±0.14

Values are expressed as mean±SEM (n=5); Control (water, 0.1ml/mouse), Positive Control (Diclofenac-Na, 5mg/kg), Group I = *T. corymbosa* (200mg/kg), Group II = *T. corymbosa* (400mg/kg).

Table 4. Effect of *T. corymbosa* on hot plate test.

Test Group	Dose mg/kg	Plant parts	Response Times (in seconds)			
			0 min	30 min	60 min	90 min
Control			1.29±0.12	1.76±0.15	2.37±0.14	1.66±0.23
Positive Control	5.0		1.87±0.21	5.33±0.26	7.12±0.18	6.94±0.14
Group-I	200	Leaf	4.79±0.61	7.65±0.99	7.51±0.63	4.46±0.38
		Flower	4.12±0.39	3.19±0.35	3.36±0.26	3.29±0.23
		Steam	3.73±0.42	3.29±0.27	3.78±0.31	3.18±0.30
Group-II	400	Leaf	5.33±0.60	7.65±0.52	7.51±0.63	1.95±0.87
		Flower	4.15±0.45	8.42±1.00	7.68±1.22	7.48±1.27
		Steam	3.13±0.30	3.09±0.13	3.52±0.35	2.98±0.34

Values are expressed as mean±SEM (n=5); Control (Water, 0.1 ml/mouse), Positive Control (Diclofenac-Na, 5mg/kg), Group I = 200mg/Kg, Group II = 400mg/kg.

3.3.2. Hot Plate Test

Based on the findings of two thermal pain models employed in this study, *T. corymbosa* was found to possess higher analgesic activities. The hot plate test is widely used for assessing analgesic activities. In our experiments, *T. corymbosa* exhibited significant analgesic activity in hot plate test. It seems possibly that the higher doses of the extract have more potent analgesic effect (Table 4).

3.4. Statistical Analysis

All experiments were performed thrice and the results averaged Data were expressed as mean ± SD.

4. Conclusion

Based on the result of the present study, it can be concluded that the crude plant extracts of *T. corymbosa* possess significant cytotoxic and anthelmintic effects and remarkable analgesic potential. The leaf, flower and steam extracts showed promising analgesic properties compared to respective standard drug. At higher dose, notable analgesic activity was observed from hot plate and tail-flick test. Significant cytotoxic activity can be suggested from the results of brine shrimp lethality bioassay. Dose dependant activity was also identified by all the performed pharmacological investigations.

Acknowledgement

The authors are grateful to the authority of Stamford University Bangladesh for necessary funding and logistics.

References

[1] Austin F. (1999). Impresario of Texas,Yale University Press, USA.

[2] Dhar M. L., Dhar M. M., Dhawan B. N., Mehrotra B. N. and Ray C. (1968). Screening of Indian plants for biological activity: Part I. *Indian J. Exp. Bio*. 6: 232-247.

[3] Eddy N. B. and Leimbach D. Synthetic analgesics II.(1953) Dithienylbutenyl- and dithienylbutylamines. *J. Pharmacol. Exp. Ther*. 107(3): 385–393.

[4] Franzotti E. M., Santos C. V. F., Rodrigues H. M. S., Mourão R. H. V., Andrade M. R. and Antoniolli A. R. (2000) Anti-inflammatory, analgesic and acute toxicity of *Sida cordifolia* L. *J. Ethnopharmacol*. 72: 273–277.

[5] Hasan S. M. R. (2009) Phytochemical and Pharmacological Investigations of *Commelina benghalensis* Linn. M. Pharm. Thesis. Department of Pharmacy, Jahangirnagar University.

[6] Kulkarni S. K. (2005). Handbook of Experimental Pharmacology. 3rd Revised and Enlarged Edn, Vallabh Prakashan, Delhi, India.

[7] Lim K. H., Thomas N. F., Abdullah Z. and Kam T. S. (2009). Seco-tabersonine alkaloids from *Tabernaemontana corymbosa*. *Phytochemistry*. 70: 424-429.

[8] Mayer B. N., Ferrigni N. R., Putnam J. E., Jacobsen L. B., Nichols D. E. and Mclaughlin J. L. (1982). Brine shrimp: A convenient bioassay for active plant constituents. *Planta Medica*. 45: 31-34.

[9] Mathappan R., Prasanth V. V., Jolly C. I. and Somanath M. (2010) Comparative study on the antibacterial activity of the methanolic extract of *Urena lobata* root and a standard marketed herbal formulation. *Journal of Pharmacy Research*. 3(5): 953-955.

[10] Patil S, Dave A. K., Singh V. K. and Jagannadham M. V. (2009). In-vitro Anthelmintic Activity of Indian Medicinal Plant. *J. Agric. Food Chem*. 55(14): 5809-5818.

[11] Singh D., Swarnka R. C. P. and Khan F. A. (2002) Anthelmintic resistance in Gastrointestinal nematodes in livestock in India. *J. Vet. Parasit*. 16: 115-1130.