

ANALGESIC AND CYTOTOXIC ACTIVITIES OF *JASMINUM SAMBAC* (L.) AITON

Md. Atiqur Rahman^{1*}, Md. Shamim Hasan¹, Md. Anwar Hossain¹, N. N. Biswas¹

¹Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

* **Corresponding Author:** Md. Atiqur Rahman

Present Address: Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

Phone: +8801717584231, E-mail: satikrahman@gmail.com

Summary

The ethanol extract of the dried leaves of *Jasminum sambac* (L.) Aiton (Family - Oleaceae) was investigated for its possible analgesic and cytotoxic activities in animal models. The extract produced significant ($P < 0.001$) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The crude ethanolic extract also produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* ($LC_{50} = 50 \mu\text{g/ml}$ and $LC_{90} = 100 \mu\text{g/ml}$). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: analgesic activity, cytotoxic activity, *Jasminum sambac* (L.) Aiton.

Introduction

Jasminum sambac (L.) Aiton (English Name: Jasmine, Arabian Jasmine; Family: Oleaceae; Synonym: *Nyctanthes sambac* L., *Nyctanthes undulata* L.). Locally known as 'Beli or Bel' in Bangladesh. It is also known as Full (Arabic), Mogra (Hindi and Marathi), Sampaguita (Spanish), Mallikā (Sanskrit), Juhi Mahli (Oriya), Kampupot, Melati putih (Malay and Indonesian Language), Kampupot (Tagalog), Mallepuvvu (Telugu), Mallikaipu (Tamil), Dundu Mallige (Kannada), Kaliyan (Urdu), Pikake (Hawaiian), Sampaguita (Philippines), Yasmin (Persian), Mo li hua (Transcribed Chinese), Jasmin d'Arabie (French) and Bogarim (Portuguese). It is an evergreen vine or shrub reaching up to 1-3 m tall, widely distributed and cultivated throughout Bangladesh. It is native to southwestern, southern and southeastern Asia especially in Bangladesh, India and Myanmar. It is the national flower of the Philippines where it is known as Sampaguita. It is also distributed and cultivated more or less throughout Srilanka, Pakistan, Nepal, Malaysia, China, Indonesia, France, Spain, Hawaii and tropical Australia. It is also widely cultivated in other parts of the world¹⁻².

The traditional use of this plant suggests analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant, anti-spasmodic, galactagogue and tonic (uterine) effects. Essential oil of *Jasminum sambac* (L.) Aiton is used as fragrance for skin care products.

Leaves and flowers are used as antipyretic and decongestant; roots as analgesic, flowers as lactifuge, flower extract as deodorant. In Bangladesh and India, it is traditionally used for skin disorders; in ancient Baghdad, it is used with opium for gangrenous ulcers of the gums; in Jordan, infusion of flowers is used for ulcerations, dermatoses and fever; the Chinese, Arabians and Indians used Jasmine medicinally as an aphrodisiac and for ceremonial purposes. The root is used in China to treat headaches, insomnia, and pain due to dislocated joints and broken bones; it is reported to have anesthetic properties as well. Several *Jasminum* species have been used in cancers^{1,3,4}.

Literature study shows that essential oil and methanol extract from *Jasminum sambac* have in-vitro antimicrobial and antioxidant activities which could support the use of the plant by traditional healers to treat various infective diseases⁵. Another study shows that Jasmine flowers, applied to the breasts showed suppression of puerperal lactation comparable to Bromocriptine, with significant reduction of serum prolactin (greater than bromocriptine) and Jasmine flowers seem to be an effective and inexpensive method of suppressing puerperal lactation and can be used as an alternative in situations where cost and nonavailability restrict the use of bromocriptine⁶. On the other hand, studies on chemical constituents in roots of *Jasminum sambac* shows that it contains dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol and hesperidin⁷.

From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the analgesic and cytotoxic activities of the ethanol extract of dried leaves of *Jasminum sambac* (L.) Aiton.

Materials and Methods

Plant Material

Leaves of *Jasminum sambac* (L.) Aiton were collected from Khulna University campus, Khulna, Bangladesh in June 2009 and were authenticated by the experts at National Herbarium (Accession Number: 34406). After collection, leaves were sun dried for several days to remove moisture. After drying, the dried leaves were ground into course powder by 'Hammer' mill. About 400 gm of powdered leaves was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. And this crude ethanolic extract was used for all phytochemical and pharmacological screening.

Animals

For analgesic activity study, young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), were used. After purchase, the animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12h light-dark cycle) and fed with standard diets and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics

Committee (IAEC) approved the experimental protocol. All the experiments were conducted on an isolated and noiseless condition.

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh).

Preliminary Phytochemical Analysis

The ethanol extract of leaves of *Jasminum sambac* (L.) Aiton was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test⁸⁻⁹.

Tests for Reducing Sugar

Benedict's Test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Tests for Tannins

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

Test for Saponins

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Test for Gums

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Tests for Steroids

Liebermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Liebermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

Tests for Alkaloids

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Tests for Glycosides

A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added. Yellow precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath. Brick-red precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath. Brick red precipitate is considered as an indication for the presence of glycosides.

Pharmacological Studies

Analgesic Activity

Analgesic activity of the ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton was tested using the model of acetic acid induced writhing in mice¹⁰⁻¹¹. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control' 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 and group IV were test groups and were treated with ethanolic extracts of leaves of *Jasminum sambac* (L.) Aiton at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and the ethanolic extracts were administered orally 30 min prior to the intra-peritoneal injection of 0.7 % acetic acid, then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Cytotoxicity Test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Six doses of plant extract (20, 40, 50, 80, 100 and 120 µg/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 µl/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette¹². The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

Statistical Analysis

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

Results**Chemical Group Test**

Results of different chemical group tests on the ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton showed the presence of Reducing Sugar, Alkaloids, Tannins, Flavonoids and Glycosides (Table 1).

Table 1: Results of different chemical group tests of the extract of leaves of *Jasminum sambac* (L.) Aiton

Extract	Reducing Sugar	Steroids	Alkaloids	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanolic extract of leaves of <i>Jasminum sambac</i> (L.) Aiton	+	-	+	+	-	+	+	-

Key: + = Presence, - = Absence

Antinociceptive Activity

Table 2 showed the effect of leaves of *Jasminum sambac* (L.) Aiton on acetic acid-induced writhing model in mice. The extract produced about 42.86% and 70.94% writhing inhibition at the dose of 250 and 500 mg/kg of body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 82.27% at the dose of 25 mg/kg of body weight (Table 2).

Table 2: Effect of ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton on acetic acid induced writhing in mice

Animal Group / Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	20.3±1.76 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	3.6±1.17* (17.73)	82.27
Test group-I Ethanolic extract 250 mg/kg, p.o.	11.6±1.19* (57.14)	42.86
Test group-II Ethanolic extract 500 mg/kg, p.o.	5.9±1.87* (29.06)	70.94

Values are expressed as Mean±S.E.M (n=10), * $P < 0.001$, % = Percentage, p.o. = per oral.

Cytotoxic Activity

In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50} and LC_{90} were deduced ($LC_{50} = 50 \mu\text{g/ml}$; $LC_{90} = 100 \mu\text{g/ml}$) (Table 3).

Table 3. Brine shrimp lethality bioassay of the ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton

Test sample	Concentration ($\mu\text{g/ml}$)	Log (concentration)	Number of alive shrimp	Mortality (%)	LC_{50} ($\mu\text{g/ml}$)	LC_{90} ($\mu\text{g/ml}$)
Ethanolic Extract	20	1.30	08	20	50	100
	40	1.60	06	40		
	50	1.69	05	50		
	80	1.90	2	80		
	100	2.00	1	90		
	120	2.07	0	100		

Discussion

Analgesic activity of the ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton tested by acetic acid induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings¹³. The extract produced significant writhing inhibition comparable to standard drug diclofenac sodium. Based on this, it could be concluded that it might possess analgesic activity.

The cytotoxic activity of the ethanol extract of leaves of *Jasminum sambac* (L.) Aiton was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc¹⁴. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it could be suggested that the crude ethanolic extract of leaves of *Jasminum sambac* (L.) Aiton might possess analgesic and cytotoxic activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential to confirm its pharmacological properties.

Acknowledgements

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Dr. Asish Kumar Das, Associate professor, Pharmacy Discipline, Khulna University; Ahmed Ayedur Rahman, Assistant professor, Pharmacy Discipline, Khulna University; Nripendra Nath Biswas, Lecturer, Pharmacy Discipline, Khulna University, Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia, for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

References

1. Ghani A. Medicinal Plants of Bangladesh, 1st edition, Dhaka, Bangladesh, The Asiatic Society of Bangladesh, 1998: 266-70.
2. Kirtikar, K. R. and Basu, B. D. In: Indian medicinal plants, 2nd edition, Dehradun, India, International Book Distributors and Book sellers, 1987: 372–75.
3. Nadkarni KM, Basu BD. *Jasminum sambac*, In: Indian Materia Medica, 1st edition, Mumbai, India, Popular prakashan Ltd, 1996: 120-23.
4. Khare C.P. Indian Herbal Remedies, 1st edition, Germany, Springer-Verlag Berlin Heidelberg, 2004: 269-71.
5. Fatouma Abdoul-Latif, Prosper Edou, François Eba, Nabil Mohamed, Adwa Ali, Samatar Djama, Louis-Clément Obame, Ismael Bassolé and Mamoudou Dicko. Antimicrobial and antioxidant activities of essential oil and methanol extract of *Jasminum sambac* from Djibouti. African Journal of Plant Science 2010; 4 (3): 038-043.

6. Pankaj Shrivastav, Korula George, N. Balasubramaniam, M. Padmini Jasper, Molly Thomas, A. S. Kanagasabhapathy. Suppression of Puerperal Lactation Using Jasmine Flowers (*Jasminum Sambac*). Australian and New Zealand Journal of Obstetrics and Gynaecology 1988; 28 (1): 68–71.
7. Zhang ZF, Bian BL, Yang J, Tian XF. Studies on chemical constituents in roots of *Jasminum sambac*. Zhongguo Zhong Yao Za Zhi. 2004; 29 (3): 237-39.
8. Evans WC. Trease and Evan's Textbook of Pharmacognosy, 13th edition, London, Cambridge University Press, 1989: 546.
9. Ghani A. Medicinal Plants of Bangladesh, 1st edition, Dhaka, Bangladesh, The Asiatic Society of Bangladesh, 1998: 134-135.
10. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br. J. Pharmacol. Chemother. 1964; 22: 246-253.
11. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie 2004; 59: 329-330.
12. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982; 45: 31-34.
13. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol. 2003; 84: 31-33.
14. Anderson JE, Chang CJ, McLaughlin JL. Bioactive components of *Allamanda schottii*. J. Nat. Prod. 1988; 51: 307-308.