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Thin layer chromatographic profiling and evaluation of analgesic activity of *Nelumbo nucifera* leaf extracts in Swiss mice

Sumita Bera[1], Sanjib Bhattacharya[2*], J. N. Pandey[1], Moulisha Biswas[1]

1. Institute of Pharmaceutical Sciences, Kalyani, Nadia 741235, West Bengal, India
2. Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India

*Corresponding author: sakkwai@yahoo.com

ABSTRACT:

Nelumbo nucifera Gaertn. (Nelumbonaceae), commonly known as lotus in English, is an aquatic perennial plant grown and cultivated commercially in India for its ornamental flowers. The present study assessed the different solvent extracts of *N. nucifera* leaf for thin layer chromatography (TLC) and also evaluated their analgesic potential by acetic acid induced writhing assay in Swiss albino mice. The chloroform extract yielded maximum numbers of spots in TLC. All the test extracts exhibited significant analgesic activity. The methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts respectively. The present preliminary study demonstrated marked analgesic activity of *N. nucifera* leaf in Swiss mice.

Key words: Analgesic, *Nelumbo nucifera*, writhing, leaf

INTRODUCTION:

Nelumbo nucifera Gaertn. (Nelumbonaceae), commonly known by a number of names including Indian lotus, sacred lotus or simply Lotus, may also be referred to by its former botanical names, *Nelumbium speciosum* (Willd.) or *Nymphaea nelumbo*. This is an aquatic perennial plant grown and cultivated commercially in India for its ornamental flowers. It has reported that *N. nucifera* leaves extract showed anti-diabetic and anti-inflammatory effects, [1, 2] stalks extract showed antipyretic effect, [3] leaves and stamens extracts showed antioxidant effect, [4, 5] and seeds extract showed hepatoprotective and free radical scavenging effects. [6] The leaf of *N. nucifera* is bitter, sweet and neutral. It is aromatic and blue-green in color. It is best for cleaning heat, resolving summer heat and stop bleeding. It contains several flavonoids and alkaloids, [7] and has been also used as an effective drug for hematemesis, epistaxis, hemoptysis, hematuria and metrorrhagia. [8] However, the planar chromatographic profile and analgesic assessment of petroleum ether, chloroform and methanol extract from *N.*

nucifera leaf are still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *N. nucifera* grown in India.

MATERIALS AND METHODS

Plant material: The mature leaves of *Nelumbo nucifera* Gaertn. (Nelumbonaceae), were collected during August 2011 from Kolkata, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/96/2011/Tech II/592) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material was defatted with petroleum ether (60-80°C), the percentage extractive value was 0.73 % w/w. The defatted powdered material thus obtained was further extracted successively with chloroform and methanol for 72 h. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 1.43 % w/w and 4.20 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts. [9]

Chemicals: Acetyl salicylic acid (aspirin) and glacial acetic acid from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals: Adult male albino mice of Swiss strain weighing 20 ± 2 g were procured from registered breeders (Rita Ghosh & Co., Kolkata, India) and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark and light circle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee.

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using

silica gel G as stationary phase. The mobile phases, results and chromatograms are depicted in Figures 1-3.

Analgesic evaluation: acetic acid-induced writhing test: Swiss albino mice were divided into five groups (*n* = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes. Group II received aspirin (100 mg/kg b.w. p.o.) Groups III, IV and V received the petroleum ether, chloroform and methanol extracts at the doses of 200 mg/kg b.w., p.o. respectively. Thirty minutes after aspirin and extracts administration, group II to V received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes [10, 11]. The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:-
(Control mean - Treated mean/ Control mean) ×100 %.

Statistical analysis: The data are represented as mean ± standard error of mean (SEM). Degree of significance was assessed by Student's '*t*' test.

RESULTS AND DISCUSSION

Preliminary phytochemical studies showed the presence of triterpenoids and steroids in the petroleum ether extract; triterpenoids in the chloroform extract; and triterpenoids, alkaloids, phenolic compounds, glycosides and carbohydrates in the methanol extract of *N. nucifera*.

Among the various methods for separating plant constituents, the thin layer chromatographic procedure is the one of the most commonly used techniques of general application. [12] Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations. [13] The chloroform extract yielded maximum spots in TLC (Figures 1-3). All of these TLC profiles may serve as characteristic fingerprint of *N. nucifera* leaf. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions. [14]

The analgesic activity of *N. nucifera* leaf extracts was evaluated by acetic acid induced writhing method in mice to assess peripheral (non-narcotic) type of analgesic activity.

[15] Acetic acid induced writhing is chemically induced nociception by intraperitoneal injection of dilute acetic acid solution to mice. The chemical agents can produce nociceptive reactions in mice. Intra-peritoneal injection of phenyl para quinone, bradykinin or dilute acetic acid (1-3% v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain. [15, 16]

Table 1. Analgesic effect of *N. nucifera* leaf extracts on acetic-acid induced writhing in mice.

Treatment	Dose	Number of writhes	% Protection
Acetic acid (1% v/v)	10 ml/kg	52.83 ±1.400	-
Acetic acid + Aspirin	100 mg/kg	17.66 ±1.606*	66.57
Acetic acid + Pet. ether extract	200 mg/kg	24.07 ±1.241*	54.43
Acetic acid + Chloroform extract	200 mg/kg	21.25±1.391*	59.77
Acetic acid + Methanol extract	200 mg/kg	17.29±1.471*	67.27

Values are mean ± SEM (n = 6). *p < 0.001 when compared to control.

Fig. 1. TLC profile of the pet. ether extract of *N. nucifera* leaf. Solvent system: benzene: chloroform: ethyl acetate (5: 3: 2). R_f values: 0.54, 0.84, 0.89, 0.93.

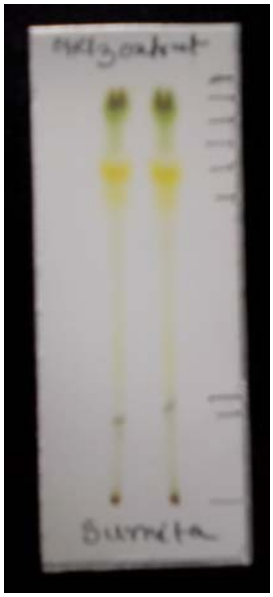

Fig. 2. TLC profile of the chloroform extract of *N. nucifera* leaf. Solvent system: benzene: chloroform: ethyl acetate (2: 3: 5). R_f values: 0.20, 0.24, 0.79, 0.77, 0.83, 0.88, 0.94

Fig. 3. TLC profile of the methanol extract of *N. nucifera* leaf. Solvent system: chloroform: ethyl acetate (3: 7). R_f values: 0.41, 0.81, 0.89, 0.95

Acetic acid induced writhing test is known as a visceral pain model nociception. Several mediators like kinins, acetylcholine, substance P, calcitonin-gene-related peptide and prostaglandins (PG) take part in visceral pain model nocicepotion and transmission of the nociception from the viscera. In this test both central and peripheral analgesics are

detected. Analgesics of narcotic (central) e. g. morphine, pentazocin, pethidine etc and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin etc can inhibit the writhing response in mice. [15-18]

Based on the results obtained from the present preliminary study, it can be inferred that all the test extracts had effective peripheral analgesic actions. The methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts respectively (Table 1). The present preliminary study confirms marked analgesic activity of *Nelumbo nucifera* leaf in Swiss mice which may be due to presence of multitude of constituents as revealed by TLC profile.

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