THIN LAYER CHROMATOGRAPHIC STUDIES AND EVALUATION OF ANALGESIC ACTIVITY OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACTS IN MICE

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Summary

Andrographis paniculata (Acanthaceae) (Chuan Xin Lian in Chinese) is a traditional medicinal herb which grows widely in many regions of Asia, including China, Thailand, India and Sri Lanka. This herb is also known as "king of bitters" due to its bitterness. The present study assessed the different solvent extracts of *A. paniculata* leaf for thin layer chromatography (TLC) and also evaluated their analgesic potential by acetic acid induced writhing assay in Swiss albino mice. All of 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 demonstrates marked analgesic activity of *A. paniculata* leaf.

Key words: Analgesic, *Andrographis paniculata* , writhing, leaf.

Introduction

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. The rich wealth of plant kingdom can represent a novel source of new compounds with therapeutic activity. *Andrographis paniculata* (Chuan Xin Lian in Chinese) is a traditional medicinal herb which grows widely in many regions of Asia, including China, Thailand, India and Sri Lanka. This herb is also known as "king of bitters" due to its bitterness, and is prescribed for the treatment of numerous ailments and diseases, including bacterial dysentery, diarrhea, and fever (1). Its fresh and dried leaves as well as the juice of the whole plant are used in various Asian traditional medicines, often in herbal combinations (2).

Extensive research in the last few decades had revealed that the plant extract of *Andrographis paniculata* contains diterpenes, flavonoids and stigmasterols. The predominant and well-studied active component of *Andrographis paniculata* is found to be diterpenoid lactone, such as andrographolide (Andro), 14- deoxyandrographolide, deoxy-di-de-hydro-andrographolide, and neo-andrographolide (3). Importantly, Andro and its derivatives are considered to be the main bioactive compounds with immunostimulant, antipyretic, anti-inflammatory (4) and anti-diarrhea properties in *Andrographis paniculata* (5). However, its chromatographic profile and analgesic assessment are still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *A. paniculata.*

Materials and methods

Plant material: The mature leaves of *Andrographis paniculata* (Acanthaceae) were collected during November 2010 from Murshidabad, 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/I-I/(78)/2010/Tech.II/347] 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 (350 g) was defatted with petroleum ether (60-80 °C), the percentage extractive value was 7.10 % w/w. The defatted powder material thus obtained was further extracted with chloroform and methanol in soxhlet extractor for 8 hours. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 12.00 % w/w and 32.11 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts (6, 7).

Chemicals: Acetyl salicylic acid (aspirin) and glacial acetic acid from Sigma Chemical Co. Ltd. (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.

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per standard one dimensional ascending method. The 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.) Group III, IV and V received the petroleum ether, chloroform and methanol extracts at the doses of 200 mg/kg b.w., p.o. respectively. 30 min 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 min (10). 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 %.

Table 1. Analgesic effect of extracts from *M. paradisiaca* 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	17.26 ±1.606*	67.32
Acetic acid + Pet. ether extract of *A. paniculata*	200 mg/kg	24.05 ±1.261*	54.48
Acetic acid + Chloroform extract of *A. paniculata*	200 mg/kg	19.15±1.371*	63.75
Acetic acid + Methanol extract of *A. paniculata*	200 mg/kg	17.29±1.471*	67.27

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

Results and discussion

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract; steroids, triterpenoids, saponins, glycosides and carbohydrates in the chloroform extract; and steroids, saponins, glycosides and carbohydrates in the methanol extract.

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 (8). 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 aluminium sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations (9). The methanol

extract yielded maximum spots in TLC, followed by chloroform and petroleum ether extracts respectively (Figures 1-3). All of these TLC profiles may serve as characteristic fingerprint of *A. paniculata* leaf. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions.

The analgesic efficacy of *A. paniculata* leaf extracts was evaluated by acetic acid induced writhing method in mice to assess peripheral (non-narcotic) type of analgesic activity (10). Acetic acid induced writhing is chemically induced nociception by intraperitonial 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 (10, 11).

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 both narcotic (central) e. g. morphine, pentazocin, pethidine and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin can inhibit the writhing response in mice (10-12).

Based on the data obtained from the present study, all the 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 *A. paniculata* leaf which may be due to presence of multitude of constituents as revealed by TLC profile.

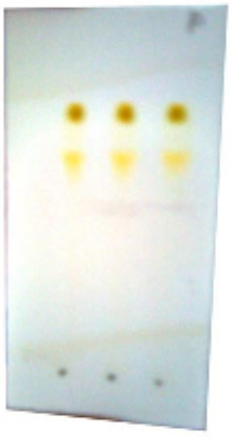

Fig. 1. TLC profile of the pet. ether extract of *M. paradisiaca* leaf. Solvent system: benzene: chloroform: ethyl acetate (5: 3: 2). R_f values: 0.86, 0.70.

Fig. 2. TLC profile of the chloroform extract of *M. paradisiaca* leaf. Solvent system: benzene: chloroform: ethyl acetate (2: 3: 5). R_f values: 0.78, 0.69.

Fig. 3. TLC profile of the methanol extract of *M. paradisiaca* leaf. Solvent system: chloroform: ethyl acetate: methanol (5: 3: 2). R_f values: 0.88, 0.81, 0.74, 0.48.

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