



Evaluation of analgesic activity of methanol extract from the *Spondias pinnata*

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ABSTRACT

This Present study was surpassed to investigate the methanolic fruit extracts of *Spondias pinnata* for analgesic activities in rats using Acetic acid induced writhing test & Formalin induced licking test. *S. pinnata* is a plant of Anacardiaceae family, a species growing in Bangladesh. The crude methanolic fruit extract of *Spondias pinnata* were screened for Analgesic activities using Acetic acid induced writhing reflex method (*P<0.05, significant) and formalin induced licking method (*P<0.05, significant). A reputed acidic agent acetic acid used as standard. In conclusion, the methanol extract of the fruits of *Spondias pinnata* displayed analgesic activity.

Keywords: *Spondias pinnata*, Anacardiaceae, Analgesic.

INTRODUCTION

Pain is a excruciating feeling triggered in nervous system often caused by intense or damaging stimuli. In medical diagnosis, Pain is the most common symptom of disease and the most frequent complaint presented to physicians. While the International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage ^[1]. For pain management acetaminophen, morphine and aspirin are commonly used drugs. Among current pain therapies, mostly non-steroidal, anti-inflammatory drugs (NSAID) and opioids often have limited efficiency, can only relieve 50% of the pain in about 30% of patients ^[2].

Besides, many of these drugs cause serious side effects; studies have shown that prolong use of acetaminophen cause kidney and liver damage, opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders ^[3,4]. In case of morphine acute morphine poisoning, hypotension, drug dependence, etc occurs. As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel analgesic agents have been used for centuries for therapeutic purposes. Many of these herbs with analgesic activity had been used without any adverse effects. . *Spondias*

pinnata^[5] is also known as Hogplum or Aamra in Bengali is a deciduous tree which is distributed throughout Bangladesh, India, Srilanka and South-East Asian countries. Its accession number is 40254 as per the Bangladesh National Herbarium. Fruits, leaves, bark of *S. pinnata* are strong anti-scorbutic agents. Roots of the plants are traditionally used for regulating menstruation. All parts of this plant have been used in folkloric medicine as an anti-tubercular agent, while the unripe fruits were used as an aphrodisiac.^[5] Studies have been shown that *S. pinnata* is rich of gallic acid 76 ± 15.8 mg/gm, ascorbic acid 92 ± 19.7 mg/gm, quercetin 189.5 ± 15.2 mg/gm in methanolic extract^[6]. *S.pinnata* was tested for many important pharmacological activities such as: hypoglycemic activity^[5], anthelmintic activity^[5], anti-cancer activity^[5], anti-microbial activity^[5], anti-oxidant activity^[5], cytotoxic activity^[5], hepatoprotective activity^[5].

However, no activity of the plant parts has been examined for its analgesic effects. As a part of our continuing studies on medicinal plants of Bangladesh the analgesic activity of the crude methanolic extract and its chromatographic fraction as well as the purified compound itself was evaluated by the acetic acid induced writhing method and the formalin induced licking test in swiss albino mice ^[7-10]. The purpose of this present study is to evaluate the methanolic fruit extract of *Spondias pinnata* for analgesic activities in animal model.

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MATERIALS & METHODS

Collection of plant materials: The fruits of *Spondias pinnata* (Family: Anacardiaceae) was collected from Chaumuhani, Noakhali, Bangladesh on April, 2015. The Local Name of *S. pinnata* is Amra, Amna, Deshi-amra; Piala, Pjal (Chittagong). Its accession number is 40254 as per the Bangladesh National Herbarium.

Extraction: After collection fruits were thoroughly washed with water. The collected plant parts (fruits) were separated from undesirable materials or plants or plant parts. Then the collected plant materials were cut, dried, and pulverized. About 500g of the powdered materials was soaked in 1.5 liter of 99% methanol at room temperature for 16 days with occasional stirring. Then the solution was filtered using filter cloth and Whatman's filter paper and evaporated by using traditional spontaneous natural vaporization method at room temperature covered with aluminum foil with small pores to facilitate evaporation of methanol. It was kept there for 3 months which rendered a gummy concentrate of dark greenish color. The gummy concentrate was designated as crude methanolic extracts.

Experimental Animal: Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. They were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 2^\circ\text{C}$; relative humidity 60-70%) in a 12 h light-dark cycle and fed ICDDR; B formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Test of Analgesic

Writhing test: Pain is induced by injection of noxious chemical as Acetic acid 0.1% at volume 0.3 ml. Writhing means stretching behavior of the abdominal and at least one hind limb. The animals react with ar-acteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity al-though some psychoactive agents also show activity.

Mechanism of pain induction in acetic acid induced writhing method

Writhing method: Acetic acid is a pain stimulus. Intraperitoneal administration of acetic acid (0.7%) causes the release of free arachidonic acid from tissue phospholipids' by the action of

phospholipase A2 and other acyl hydrolases. There are three major pathways in the synthesis of eicosanoids from arachidonic acid. All the eicosanoids with ring structures that is the prostaglandins, thromboxanes and oristacyclines are synthesized via the cyclooxygenase pathway. The leucotrienes, HETE (hydroxyl eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids) are hydroxylated derivatives of straight chain fatty acids and are synthesized via the lipooxygenase pathway [11]. The released prostaglandins, mainly prostacyclin (PGI2) and Prostaglandin-E have been reported to be responsible for pain sensation by exciting the Aδ fibres. Activity in the Ad fibres cause a sensation of sharp well localized pain [12]. Diclofenac, used as the positive control in this method, acts by inhibition of prostaglandin synthesis. Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.

Principle: Dried power of *Spondias pinnata* fruits was extracted with MeOH. The analgesic activity of the crude extract and its chromatographic fraction as well as the purified compound itself was evaluated by the acetic acid induced writhing method and the formalin induced licking test in Swiss albino mice.

Materials & Apparatus: Test sample, Albino mice, Basket, Weight machine, Beaker, Glass rod, Spatula, Syringe, Gloves.

Preparation of test sample: At first two 10ml beaker was taken and methanol extracts of fruits *Spondias pinnata* were weight & kept at amount of 200mg & 400mg in separated beaker. Then 10 ml distilled water was poured in each beaker & properly mixed. Then they were used as test samples of different concentrations (200-400mg/10ml).

Preparation of reference standard solution: 6ml acetic acid was measured by pipette and dissolved in 94 ml distilled water to make a concentration of 0.6% glacial acetic acid (10ml/kg) solution. A controlled group was established with distilled water to ensure that the test was a validate one.

Acetic Acid Induced Writhing Reflex Method:

Twenty five Albino mice were divided into four groups of five mice per group. They were fasted for 18 hours and later treated as follows: Group I mice were given distilled water 10 ml/kg, I,p (negative control group), group I mice were given 100mg/kg acetylsalicylic acid, s.c (positive control group) while groups III and IV received 200 and 400

mg/kg of methanolic extract i.p respectively. 1 hour after administration of drug and extract, 0.6% glacial acetic acid (10 ml/kg) was given intraperitoneally (I.p) to all the mice to induce pain. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was then counted at 5 min. interval for 30 min. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula (1, 2).

Formalin induced licking test

Principle: The formalin test is used to determine the potential analgesic effects of compounds for states of persistent pain in which tissue damage occurs.

Material: Animal: 20-25 g. ,Test sample, Weighing machine, Formalin(2.5%), 0.5 ml syringe with 26 G needles, Micro – pipette, Hand held counters, Clock or timers.

Preparation of reference standard solution: 2.5 ml 0.1% formalin solution was taken in 100 ml measuring flask & 98.5 ml distilled water was poured in the flask to make 2.5% standard formalin solution.

Formalin induced Licking test Method: Twenty five albino mice (20-25 gm) were divided into five groups of five mice per group. Mice were fasted for 18 hours and later treated as follows: Group I mice were given distilled water (10 mg/kg per body weight, *i.p.*), Group II mice were given acetylsalicylic acid (100 mg/kg per body weight, *s.c*) as standard drug while groups III and IV received methanolic extract (200 and 400 mg/kg per body weight, *i.p*) as sample. One hour after this treatment, 20 μ L of 2.5% formalin was injected subcutaneously under the plantar surface of the left hind paw of each mice^[13]. The time (in second) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 20–30 minutes after formalin injection^[14, 15].

RESULT AND DISCUSSION

Acetic acid induced writhing reflex method: The results of the test showed that the fruit of *Spondias pinnata* methanolic extract 400 mg/kg exhibit inhibition of writhing reflex by 90.64% while the standard inhibition was found to be 57.22% at dose of 100mg/kg body weight. The result is showed in table 1 and figure 1. The analgesic activity of the extract *Spondias pinnata* was slightly in comparison with control animals. The extract, at doses of 400 mg/kg showed significant decrease in acetic acid induced writhing reflex of mice.

Formalin induced licking test: The result of test showed that the methanolic extract of *Spondias pinnata* exhibit 400mg/kg inhibition of licking by 50% while the standard inhibition was found to be 25.58% in (0-5) mins of induction at dose of 100mg/kg. Inhibition of licking in(20-30) mins for 400mg/kg at dose 100mg/kg is 89.79% while standard inhibition was 67.54% . The result is shown in table 2 and figure 2. The analgesic activity is slightly in comparison with control animals. The extract, at doses of 400 mg/kg showed significant decrease in acetic acid induced writhing reflex of mice.

CONCLUSION

The fruit extract of *Spondias pinnata* has been tested for secondary metabolites as Analgesic test. Two complementary test systems, namely Acetic acid induced writhing test & Formalin induced licking test. On the basis of above result and available reports, methanolic fruit extracts of *Spondias pinnata* had potent analgesic activity. Due to the analgesic activity; it will be useful for the treatment of pain induced diseases. In the end, it can be concluded that the experimental evidence obtained in the laboratory test model could provide a rationale for the traditional use of this plant along with fruit. Further studies are necessary to reveal the active compound contained in the crude extracts of *Spondias pinnata* responsible for different biological activities, and to establish the mechanism of action.

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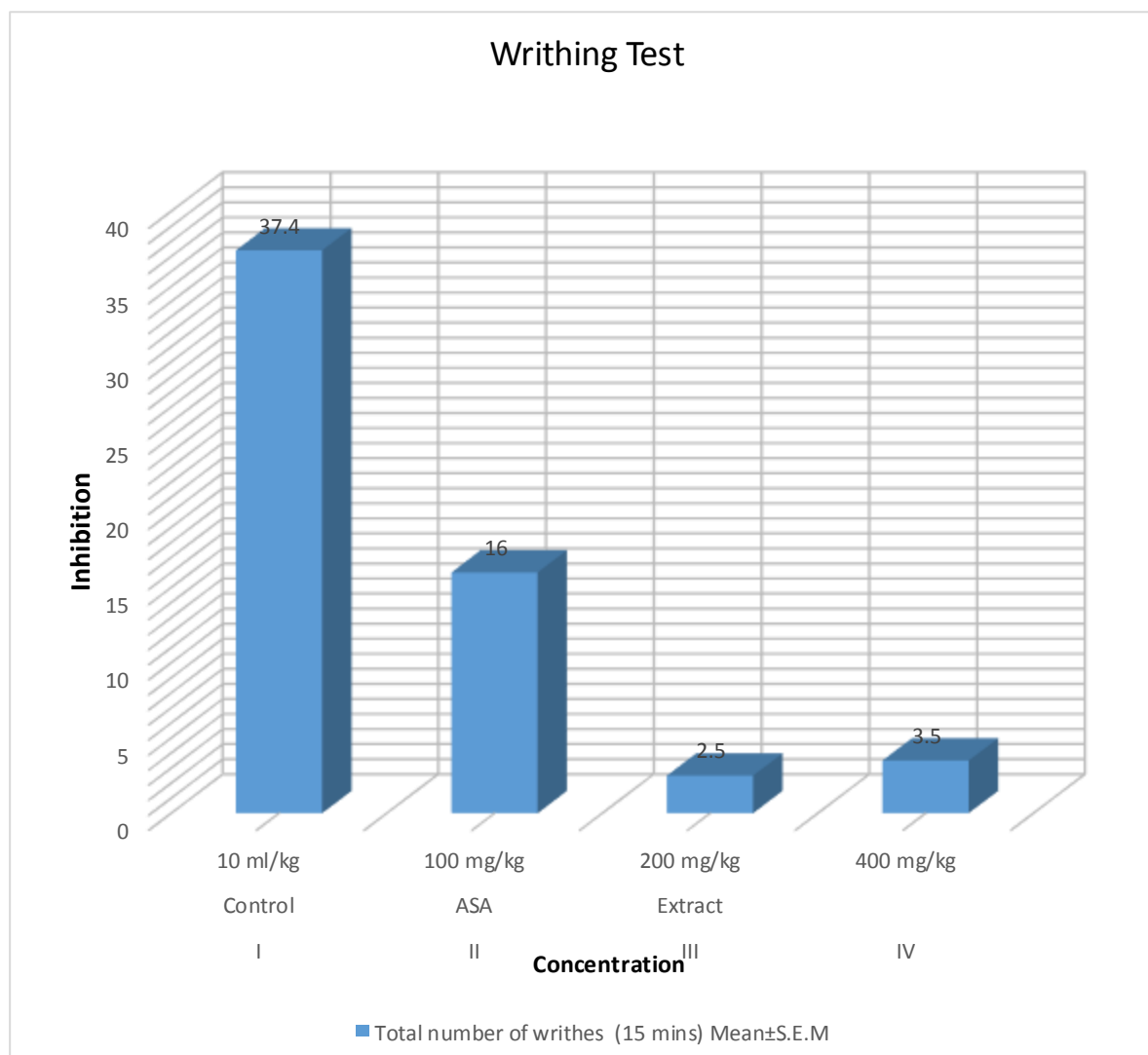


Figure 1: Percentage of inhibition of writhing for *Spondias pinnata* showed that the fruit of *Spondias pinnata* methanolic extract 400 mg/kg exhibit inhibition of writhing reflex by 90.64% while the standard inhibition was found to be 57.22% at dose of 100mg/kg body weight.

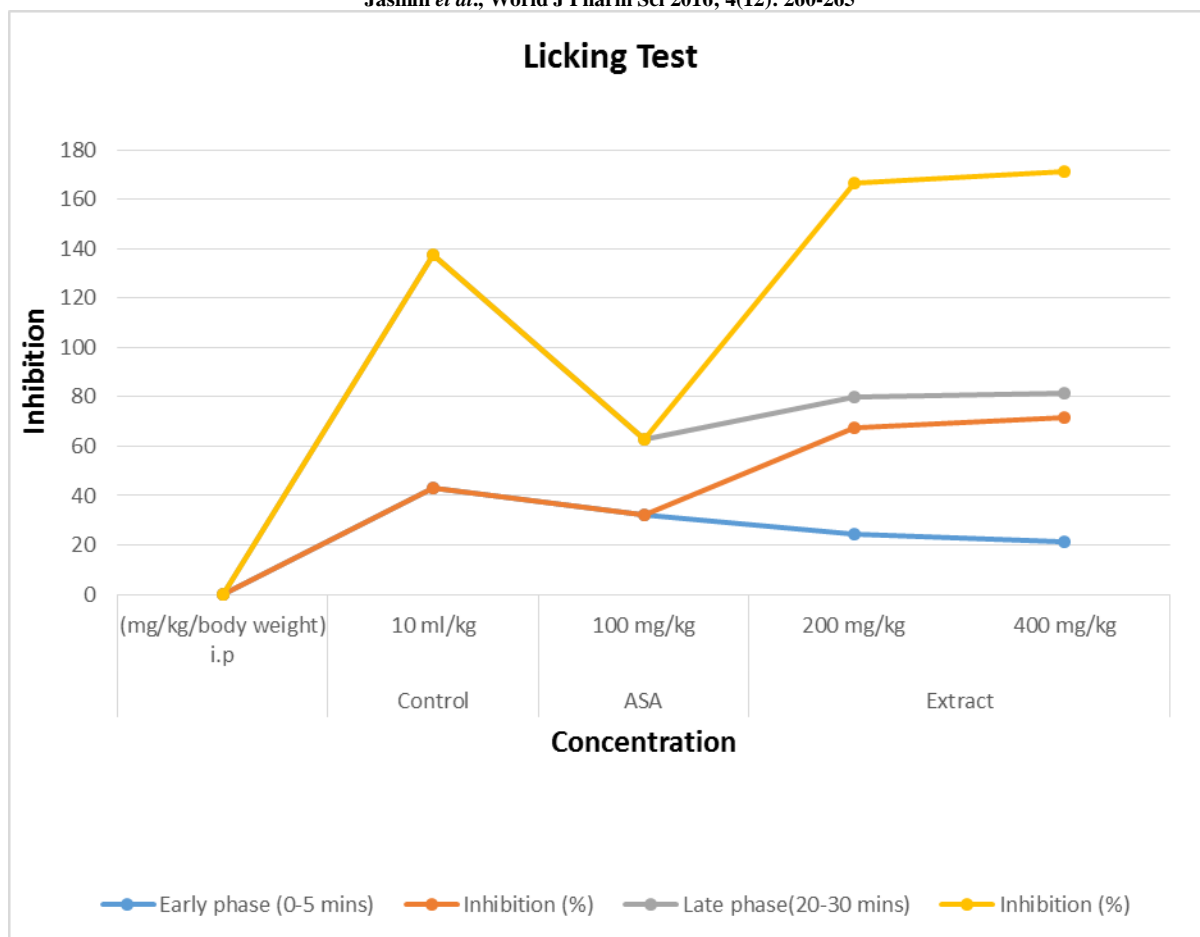


Figure 2: Percentage inhibition of formalin induced licking test showed that the methanolic extract of *Spondias pinnata* exhibit 400mg/kg inhibition of licking by 50% while the standard inhibition was found to be 25.58% in (0-5) mins of induction at dose of 100mg/kg

Table 1: Analgesic activity test of fruit extract by acetic Acid induced writhing method.

Groups	Drugs	Dose (mg/kg/body weight) i.p	Total number of writhes (15 mins) Mean±S.E.M	Inhibition (%)
I	Control	10 ml/kg	37.4±4.2	---
II	ASA	100 mg/kg	16.0±4.5*	57.22%
III	Extract	200 mg/kg	2.5±0.28*	93.31%
IV		400 mg/kg	3.5±0.47*	90.64%

Values are expressed as mean±S.E.M (n=4). *P<0.05 (significant) and **P<0.01 (more significant), compared with vehicle control (ANOVA followed by Dunnet's t-test)

Table 2 Analgesic activity test of fruit extract of *Spondias pinnata* by formalin induced licking method.

	Drugs	Dose(mg/kg/body weight) i.p	Early phase (0-5 mins) Mean±S.E.M	Inhibition (%)	Late phase(20-30 mins) Mean±S.E.M	Inhibition (%)
	Control	10 ml/kg	43±5.1		94.6±14.6	
II	ASA	100 mg/kg	32±7.1*	25.58%	30.7±7.8	67.54%
III	Extract	200 mg/kg	24.5±3.79*	43%	12.25±8.26*	87.05%
IV		400 mg/kg	21.5±3.18*	50%	9.75±6.0*	89.79%

Values are expressed as mean±S.E.M (n=4). *P<0.05 (significant) and **P<0.01 (more significant), compared with vehicle control (ANOVA followed by Dunnet's t-test)

REFERENCES

1. Merskey H. "Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy," Pain 1979; 6: 249–252
2. Hewitt D J *et al.* Challenges in analgesic drug development, Clinical Pharmacology & Therapeutics 2009; 86(4): 447–450.
3. Hanson GR, Venturelli PJ, Fleckenstein AE. Drugs and Society, 10th ed., Jones and Bartlett: Boston, Mass, USA, 2009.
4. Ahmadiani A. Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J Ethnopharmacol 1998; 61: 229–235.
5. Bora NS. Ethno-medicinal claims, phytochemistry and pharmacology of *Spondias pinnata*: A Review. Int J Pharm Sci Res 2014; 5(4): 1138-1145.
6. Anahita TZ. Evaluation of the antioxidant and in vitro cytotoxic activity of *Averrhoa carambola*, *Aegle marmelos* and *Spondias pinnata* leaf extracts. Master's Thesis, BRAC University: Bangladesh, April 2015.
7. Importance and problems in natural regeneration of *Spondias pinnata*. [Report and Opinion. 2009; 1(5):12-13. (ISSN: 1553-9873)].
8. Ezeja MI *et al.* Evaluation of the Analgesic Activity of the Methanolic Stem Bark Extract of *Dialium Guineense* (Wild). Ann Med Health Sci Res. 2011 Jan-Jun; 1(1): 55–62.
9. Akindede A J *et al.* Analgesic and Antipyretic Activities of *Drymaria Cordata* (Linn.) Willd (Caryophyllaceae) Extract. Afr J Tradit Complement Altern Med. 2011 Oct 2; 9(1): 25-35.
10. Ahmed M *et al.* Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. Pharmazie 2001; 56(8): 657-660.
11. Tateson JE *et al.* Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: biochemical assessment in vitro and ex vivo. Br. J. Pharmacol. (1988), 94: 528-539.
12. Rang HP, Dale MM, Ritter JM, Moore PK. In: Pharmacology, 5th edition. Churchill Livingstone, London, 2003: 515.
13. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987 Jul; 30(1):103-14.
14. Shibata M *et al.* Modified formalin test: characteristic biphasic pain response. Pain 1989; 38: 347-352.
15. Arif Ullah HM. Evaluation of antinociceptive, in-vivo & in-vitro anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complement Altern Med. 2014; 14: 346.