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Research Article

# Assessment of the analgesic activity of methanolic leaves extract of *Spondias mombin* Linn (Anarcadiaceae)

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**Background:** *Spondias mombin* belongs to the family of Anacardiaceae. It is a fruitiferous tree and all parts of the plant are reported to be used in traditional medicine. It is also a well-known febrifuge, treats spasms, relieves stomach cramps and reduces pain during childbirth.

**Objective:** The present study was designed to investigate the analgesic properties of methanolic leaves extract of *Spondias mombin* in male and female mice.

**Methodology:** the analgesic activity of the methanolic leave extract of *Spondias mombin* at a dose range of 420 mg/kg, 210 mg/Kg and 105 mg/Kg was evaluated in mice using, as standard drugs, paracetamol at a dose of 100 mg/Kg for the writhing test and morphine at a dose of 10 mg/Kg for the tail flick test

**Results**: The methanolic leaves extract of *Spondias mombin* injected intraperitoneally to mice at a dose range of (420 mg/Kg b.wt; 210 mg/kg b.wt and 105 mg/Kg b.wt) respectively decreased abdominal writhing induced by the acetic acid at a rate of 65.75%; 50.97% and 33.07%. However, the extract of *Spondias mombin* showed no effect using the Tail flick test.

**Discussion:** The methanolic extract of *Spondias mombin* at the doses of (420 mg/kg; 210 mg/kg and 105 mg/kg) caused a dose dependant inhibition of pain. The methanolic extract of *Spondias mombin* showed no effect on tail withdrawal. The analgesic activity of the methanolic extract of *Spondias mombin* may be peripheral and could be due to the bioactive metabolites it contains.

**Conclusion:** The methanolic leaves extract of *Spondias mombin* might have a peripheral analgesic property.

**Keywords:** *Spondias mombin*, Writhing test, Tail flick test, methanolic extract

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## 1. Introduction

The pharmacological treatment of disease began long ago with the use of herbs (Schulz et al, 2001). In Africa, up to 80% of the population uses traditional medicine for primary health care [WHO, 2003]. On this account, a few years earlier the world health organisation was firmly committed to the research and the promotion of traditional herbal to meet the health needs of populations (WHO, 1978). *Spondias mombin* a fruitiferous tree belongs to the family of anacardiaceae, all parts of the plant are reported to be used in

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traditional medicine (Abbiw, 1990).The tree is 15-20 meters long with thick and rough bark, it is also recognized by its large flowering panicles with yellow fruits (Aubreville, 1950). The leaves of *Spondias mombin* in addition to contain alkaloids, flavonoids, tannins, saponins and phenols, are very rich in vitamin C, vitamin B1, vitamin B2 and vitamin B3 (Njoku et al, 2007). Two caffeoyl esters that are allohyroycritique 2-0-caffeoyl-acid, chlorogenic acid and the butyl ester were isolated from *Spondias mombin* (Corthout et al, 1992). The roots are considered antipyretic (Kerharo and Bouquet, 1950) and are also used to treat spasms (Hodouto. 1990). The leaves relieve stomach cramps (Morton, 1987) and reduce childbirth pain (Kramer et al, 2006). The young leaves are used in Ivory Coast as anti-abortive (Adjanohoun and Ake-assi, 1979). A tea made from the flowers and leaves is taken to relieve stomach ache, urethritis, cystitis and throat inflammations (Morton, 1987). A decoction of the root is used as purgative (Villegas et al, 1997; Nworu et al, 2011). In Belize, the decoction of fresh leaves is used against dysenteria (Rodrigues and Hasse, 2000; Sierra and Buchelli, 1986).

This study aims to provide the scientific evidence to support the use of *Spondias mombin* in traditional medicine as an analgesic.

## 2. Materials and Methods

### 2.1 Collection of plant material

Fresh leaves of *Spondias mombin* was collected in July from Banco forest (Abidjan, Ivory Coast) and authenticated at the national floristic center of the University of Felix Houphouet Boigny (Abidjan, Ivory Coast). A voucher specimen of the plant was deposited and compared with the already deposited voucher specimen number 15778. The leaves were dried at room temperature (30 °C) for 12 days, milled into powder and stored at 4 °C.

### 2.2 Preparation of extract

50 g of the plant powder was macerated in 500 ml of methanol for 24 hours. the liquid extract obtained after filtration through hydrophilic Cotton followed by Whatman n°1 filter paper was concentrated by rotary evaporator at 70 °C. The concentrate was dried at 50 °C for 4 days to obtain powder and stored at 4 °C.

### 2.3 Experimental animals

In this study, forty (40) Swiss albino mice, *Mus musculus*, weighing between 18 and 24 g of both sexes were used. All the animals were obtained from the animal house of the faculty of pharmaceutical sciences, University of Felix Houphouet Boigny (Abidjan, Ivory Coast). The mice were divided into five (5) groups of four (4) animals for each test and were kept fasting for 18 h prior to administration of treatment.

### 2.4 Drugs and chemicals

Dimethyl sulfoxide (Prolabo Batch no: 23486); Morphine chlorhydrate (Cooper batch no: 092338); Paracetamol (Perfalgan®) infusion solution (Brystol myers squibb / Batch no. 9D46314).

### 2.5 Analgesic activity assessment

The analgesic activity of the extract of *Spondias mombin* was assessed using the tail flick test and the writhing test.

**Tail flick test**

The method used was that described by D'Amour and Smith (1941) and modified by Gray et al (1972). The test of D'Amour and Smith aimed at evaluating the ability of animals to endure pain.

The mice were divided into 5 groups of 4 animals each. The methanolic extract of *Spondias mombin* and morphine were separately diluted in DMSO 2% (Dimethyl sulfoxide). Morphine dosed at 10 mg/kg b.wt was injected through intraperitoneal route to group of mice**.** 30 minutes prior to mice tails irradiation, the methanolic extract of *Spondias mombin* was administered orally at dose range. The control group ($group_1$) received the vehicle (DMSO 2%). Group (2) was treated with morphine dosed at 10mg/kg b.wt. Groups (3; 4 and 5) were respectively treated with the methanolic extract of *Spondias mombin* at dose of (420 mg/Kg; 210 mg/Kg and 105 mg/Kg b.wt). The experimental apparatus used to generate heat was the tail flick model DS emitting radiant heat at 55-60°c. A timer was triggered off as well as the radiant heat source and automatically stopped when the tail was withdrawn from the heat source. On the onset of each experiment test, the mouse was put in a restrainer box, the tail of the animal was exposed halfway along the heat path. To assess the antinociceptive effects, three tests were performed at 30 minutes intervals (0-30 min; 30-60 min; 60-90 min). Within each test, three measurements were made at one (1) minute interval. After administration of extracts, the maximum irradiation time was 15 seconds to avoid tissue damages. The tail flick (latency) mean time and its standard of deviation for each group of mice were calculated. The increasing percentage of the latency mean time was calculated by the following formula:

$$PL\ (\%) = \frac{(M_{\text{control group}} - M_{\text{treated group}})}{M_{\text{control group}}} \times 100$$

*M: Tail latency mean time*
*PL: percentage of tail latency mean time*

**Writhing test**

The method used was the one described by Koster et al (1959) and modified by collier et al (1968). A solution of acetic acid was intraperitoneally injected to a mouse; the animal twisted his body by contractions of abdomen and extension of hind legs. On experimental animals, it was the dose that inhibited the writhing pain that was sought.

Swiss mice of both sexes were divided into five (5) groups each containing 4 animals. The methanolic extract of *Spondias mombin* and paracetamol were separately dissolved in DMSO (2%). Different doses of *Spondias mombin* (420 mg; 210 mg; 105 mg/kg b.wt. were orally administered to three (3) groups (group 3, 4 and 5) of mice at the rate of 0.2ml/20g b.wt.

Paracetamol at a dose of 100 mg/kg was injected through intraperitoneal route to a group (group 2) of four (4) mice. A control group (group1) of four (4) mice was orally treated with DMSO (2%) at the rate of 0.2ml/20g b.wt. The different groups of mice were treated 30 minutes prior to the intraperitoneal injection of acetic acid (1%). After the intraperitoneal injection of the acetic acid (1%), the number of writhes was recorded for 20 minutes. The pain was manifested by hind legs stretching and the contraction of dorsal abdominal muscles. We calculated for each group the mean of writhes performed and the standard of deviation. The percentage of pain inhibition was calculated by the following formula:

$$PI = \frac{(M_{\text{control group}} - M_{\text{treated group}})}{M_{\text{control group}}} \times 100$$

*M: mean of writhes performed*
*PI: percentage of inhibition*

### 2.6 Statistical analysis

The mean value ± was calculated. The statistical analyses were carried out using ANOVA and Student's T-test. The results were significant at $P< 0.05$.

## 3. Results

### Tail flick test

The effects of the methanolic leaves extract of *Spondias mombin* and morphine on the tail withdrawal latencies from the radiant heat source and the reaction time percentage were tabulated in **Table 1**. The tail withdrawal latency of the control group was 4, 8± 0,37s. At dose of 420mg/kg, the methanolic extract of *Spondias mombin* showed no significant difference compared to the control group. However, morphine dosed at 10mg/kg showed an increase of this latency time up to 14,16± 0,54 seconds with an uprising of the reaction time percentage to 190,75%. The differences observed between the extract doses and the control groups were not statistically significant. Therefore, the methanolic extract of *Spondias mombin* did not show any central analgesic effect at the doses used.

### Writhing test

**Table 2** below showed the effects of paracetamol and *Spondias mombin* extract on the number of abdominal cramps caused by acetic acid in mice. Intraperitoneal injection of acetic acid in control group of mice caused 64.25 abdominal writhes. Paracetamol (100mg/Kg b.wt) exhibited writhing inhibition percentage of 57.59%. The methanolic extract of *Spondias mombin* at doses of (420 mg/Kg; 210 mg/Kg and 105 mg/Kg b.wt) respectively showed writhing inhibition percentage of (65.75%; 50.97% and 33.07%) compared to control group. These results showed that the analgesic activity of the methanolic leaves extract of *Spondias mombin* on mice writhing may be dose dependent and peripheral.

## 4. Discussion

In this study, the analgesic activity was investigated using two major tests. The test of D'amour and Smith using a painful radiant heat stimulus (Tail Flick test) and the second one using a noxious chemical stimulus (Writhing test). The writhing test was used because of its sensitivity, although it was not specific to the study of analgesic activity (Gupta and Verma, 1991). The methanolic extract of *Spondias mombin* at a range dose of (420 mg/Kg b.wt; 210 mg/Kg b.wt; 105 mg/Kg b.wt) decreased the number of abdominal writhes after injection of acetic acid for writhing test. The studies of Collier et al (1968) showed that the acetic acid acted indirectly by releasing mediators that stimulate endogenous nociceptive neurons. The acetic acid entails writhing of abdominal muscles by provoking visceral pain which induces the release of arachidonic acid via cyclo-oxygenase (Gupta et al, 2005). The other role of the acetic acid is to increase the intraperitoneal fluid substances such as prostaglandin (PGE2 and PGEα), serotonin, histamine, bradykinin which stimulate the receptors pain located in the peritoneum (Benthley et al, 1983). Acetic acid is used to evaluate peripheral analgesic activity (Deraeld et al, 1980; Benthley et al, 1983; Gene et al, 1998).

**Table 1**: Effect of morphine and methanolic extract of *Spondias mombin* on the withdrawal latency of mice tail (tail Flick Test)

| Group | Treatment | Dose | Reaction time in seconds | | |
|---|---|---|---|---|---|
| | | | 30 min | 60 min | 90 min |
| Control group (1) | DMSO 2% | ---------- | 4.87 ± 0.3 | 4.9 ± 0.55 | 5.16 ± 0.48 |
| Group 2 | Morphine | 10mg / Kg b.wt | 14.16 ± 0.16[a] | 11.4 ± 0.93[a] | 9.05 ± 0.65[a] |
| Group 3 | MESM1 | 420mg / Kg b.wt | 4.78 ± 0.28 | 4.43 ± 0.16 | 4.7 ± 0.17 |
| Group 4 | MESM2 | 210mg / Kg b.wt | 4.62 ± 0.42 | 4.88 ± 0.53 | 4.83 ± 0.65 |
| Group 5 | MESM3 | 105mg / Kg b.wt | 6.2 ± 0.24 | 6.4 ± 0.39 | 5.96 ± 0.58 |

*Data are expressed as mean ± SEM, n=4 in each group, and was significant at [a]P< 0.05 by student's test*
*MESM: Methanolic extract of* Spondias mombin

**Table 2**: Effects of paracetamol and methanolic extract of *Spondias mombin* on the number of abdominal cramps caused by acetic acid in mice (Writhing test)

| Group | Treatment | Dose | Number of writhes in 20 minutes (Mean ±SEM) | Inhibition (%) |
|---|---|---|---|---|
| Control group (1) | DMSO 2% | ---------- | 64.25 ± 8.75 | ---------- |
| Group (2) | Paracetamol | 100mg / Kg b.wt | 27.25 ± 2.38 | 57.59 ± 0.73[a] |
| Group (3) | MESM 1 | 420mg / Kg b.wt | 22 ± 8 | 65.75 ± 0.08[a] |
| Group (4) | MESM 2 | 210mg / Kg b.wt | 31.5± 8 | 50.97 ± 0.08 |
| Group (5) | MESM 3 | 105mg / Kg b.wt | 43 ± 5 | 33.07 ± 0.34 |

*Data are expressed as mean ± SEM, n=4 in each group, and was significant at [a]P< 0.05 by student's test*
*MESM: Methanolic extract of* Spondias mombin

In this study, the methanolic leaves extract of *Spondias mombin* at the doses of (420 mg/Kg b.wt; 210 mg/Kg b.wt; 105 mg/Kg b.wt) caused a dose dependent inhibition of pain to (65.75%; 50.97% and 33.07%). The methanolic extract of *Spondias mombin* might inhibit the release of arachidonic acid, prostaglandins and other pain mediators that depend on acetic acid. Paracetamol which is a peripheral analgesic, tested at dose of 100mg/Kg causes a pain inhibition of 57.59% which is similar to the extract at a dose of 420 mg/Kg b.wt.

Regarding the results with the tail flick test, morphine which is endowed with central analgesic activity administered at a dose of 10 mg/kg b.wt to group of mice inhibits the pain effect induced by the radiant heat. However the extract at the dose of 420 mg/Kg b.wt did not have any. The painful thermal stimuli are only selectively inhibited by central analgesic (Chang and lewis, 1989; Chau, 1989; Sayyad et al 1994). The analgesic activity of the methanolic extract of *Spondias mombin* might be peripheral. The leaves of *Spondias mombin* contain bioactive compounds such as flavonoids, tannins and saponins (Njoku et al, 2007). Plants that contain such compounds have peripheral analgesic activity (Hashemi et al, 2000). Flavonoids and saponins are inhibitors of prostaglandins (Alui et al,1998). The inhibition of cyclooxygenase and lipooxygenase alliviates peripheral pain (Griswold et al, 1991). The peripheral analgesic effect of the methanolic extract of *Spondias mombin* could be related to such enzymes.

## 5. Conclusion

This study was carried out to assess the analgesic activity of the methanolic leaves extract of *Spondias mombin*. At a dose of 420 mg/kg b.wt the extract caused pain inhibition of 65.75% using the Writhing test. Paracetamol (100 mg/kg b.wt) caused pain inhibition of 57.59%. This study allowed us to demonstrate that the methanol leaves extract of *Spondias mombin* might possess peripheral analgesic properties. This partially supports its traditional use as an analgesic for stomach cramps and childbirth pains.

More investigations are required on *Spondias mombin* to identify molecules responsible for its antinociceptive activity and explain their mechanisms.

## Conflict of Interest declaration

The authors declare no conflict of interest.

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