International Journal of Pharmacological Research
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Research Article**

Analgesic activity and phytochemical studies of an aqueous extract of leaves of *Afrormosia laxiflora* (Fabaceae) in an animal model

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Abstract

Introduction: *Afrormosia laxiflora* is a plant widely used in traditional African medicine. The leaves are used for various therapeutic purposes, particularly in the treatment of painful manifestations, fever, and inflammatory diseases. However, few scientific studies seem to have studied these properties. In order to help promote traditional African medicine, our study aimed to evaluate the analgesic activity and the phytochemical composition of an aqueous extract of *Afrormosia laxiflora* leaves in an animal model.
Materials and methods: The analgesic activity of the leaves of *Afrormosia laxiflora* was evaluated against a thermal stimulus, by the immersion test of the mouse tail in warm water, and against a chemical stimulus, by the irritation test of the paw rat with formaldehyde. A phytochemical sorting was carried out on the extract to identify the chemical groups that could be responsible for this activity.
Results: The aqueous leaf extract of *Afrormosia laxiflora* was analgesic at doses of 625 mg/kg, 1250 mg/kg and 2500 mg/kg with a dose-dependent activity. This activity remains less than that of morphine but better than that of paracetamol. The phytochemical study of the aqueous leaf extract of *Afrormosia laxiflora* showed the presence of polyphenols including flavonoids and tannins, alkaloids, saponosides and terpenes, phytochemical groups that could be responsible for the analgesic properties of this extract.
Conclusion: The aqueous leaf extract of *Afrormosia laxiflora* has analgesic activity and anti-inflammatory potential which could be further evaluated for its traditional use against pain.
Keywords: Analgesic, *Afrormosia laxiflora*, aqueous extract, leaves.

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***Article History:**
Received: 12/02/2018
Revised: 14/03/2019
Accepted: 17/03/2019
DOI: https://doi.org/10.7439/ijpr.v9i3.5122

QR Code

How to cite: Etienne E, Ismael D, Amandine L, Génévième I-N, Gièle K-S. Analgesic activity and phytochemical studies of an aqueous extract of leaves of *Afrormosia laxiflora* (Fabaceae) in an animal model. *International Journal of Pharmacological Research* 2019; 09(03): e5122. Doi: 10.7439/ijpr.v09i3.5122 Available from: https://ssjournals.com/index.php/ijpr/article/view/5122

1. Introduction

In African countries, plants are one of the main medications for treatment [1]. The therapeutic secret of these plants has been held by traditional healers for several generations [2]. One of the most common uses of some of these plants is he use against pain. Indeed, pain is increasingly a major health problem both in terms of the number of people who suffer from it, the forms in which it manifests itself and the challenges faced by therapists.

Some of these pains are resistant to conventional medicines of the available therapeutic arsenal. The research for new, more effective drugs is essential to relieving the sick. Traditional African medicine seems to overflow many plants with interesting analgesic properties and could, therefore, be an alternative.

The work of Dénou *et al.* (2017)[3] have shown that various plants are used in traditional African medicine for their analgesic properties. *Afrormosia laxiflora*

(Fabaceae) belongs to the family of plants which are more used as an analgesic [3]. The leaves of *Afrormosia laxiflora* are used to treat various diseases including pain and fever [4], as an anticonvulsant [5], and against inflammatory diseases [6]. However, few scientific studies seem to have studied these properties. Knowing that WHO recommends the upgrading of natural resources, and that herbal products are used in complementary and alternative medicine in the treatment of pain [7] and in order to contribute to the value of traditional African medicine, our study aimed to evaluate the analgesic activity and the phytochemical composition of an aqueous extract of *Afrormosia laxiflora* leaves in an animal model.

2. Material and methods

2.1. Material

2.1.1. Plant material

The study was carried out on *Afrormosia laxiflora* leaves purchased at the medicinal plant's markets in Abidjan. These leaves have been identified by the National Center of Floristry of Abidjan affiliated to Félix Houphouët-Boigny University (Abidjan). Voucher samples (AL 2012) are kept in the Pharmacology laboratory. They were subsequently washed, dried and pulverized.

2.1.2. Animal

Male and female *Mus musculus* mice, weighing between 18 and 33 g, were used for the immersion test of the mouse tail in lukewarm water; and adult rats, *Rattus norvegicus*, Wistar strain of both sexes weighing between 120 and 250g for the formaldehyde test. These animals were purchased at the Vivarium of ENS Abidjan and then maintained in the Pharmacology laboratory of the UFR Pharmaceutical and Biological Sciences of the University Felix Houphouët-Boigny of Abidjan. The animals were caged and kept under standard temperature conditions (26 ± 1 ◦C), 12 h light / dark cycle, fed, with free access to drinking water and food at least one week before experimentation. Before the beginning of the experiment, they were subjected to fasting for 12 hours with free access to water for the rats, and 4 hours for the mice.

2.1.3. Solvent and chemicals

2.1.3.1. For immersion test of the tail of the mouse in warm water

- Physiological serum
- Morphine hydrochloride 10 mg / ml (Cooper)

2.1.3.2. For the formaldehyde test

- Physiological serum
- 35% formaldehyde (Cooper)
- Doliprane 100 mg powder (Aventis Pharma)

2.1.3.3. For phytochemical sorting

- An alcoholic solution of ferric chloride at 2%, hydrochloric alcohol,
- Magnesium chips, Isoamyl alcohol, Stiasny reagent,
- Sodium acetate, Dragendorff reagent, Burchardat reagent,
- Folin-Ciocalteu reagent, sodium bicarbonate, distilled water,
- 5% Sodium Nitrite Aluminum Trichloride, Sodium Hydroxide.

2.2. Methods

2.2.1. Preparation of the aqueous extract of *Afrormosia laxiflora* leaves

To obtain the aqueous extract of *A. laxiflora* leaves, 100 g of fine powder of dry leaves were boiled in 1 liter of water for 30 min. After cooling, the decoction was filtered by passage over hydrophilic cotton and then on Wattman filters paper and subsequently lyophilized. From the lyophilizate, we prepared the stock solution at 250 mg/ml. From the stock solution, a ½ dilution range was performed to obtain 2 concentrated solutions at 125 mg/ml and 62.5 mg/ml, respectively.

2.2.2. Methods of studying analgesic activity

2.2.2.1. Immersion test of the tail of the mouse

The method used is similar to that described by Janssen *et al* (1963)[8]. Mice were divided into 5 groups of 6 mice each and received different preparations at 10 ml/kg, as follows: group 1 representing the positive control group received saline by gavage; groups 2, 3 and 4 were gavaged with aqueous extract of *A. laxiflora* at 625 mg/kg, 1250 mg / kg and 2500 mg / kg, respectively; group 5 representing the negative control lot (reference lot) was intraperitoneally injected with morphine at 10 mg/kg.

Thirty (30) minutes after the administration of these different preparations, the mouse's tail was immersed in warm water (55 ± 5°C) and the withdrawal time characterized by the sudden deflection of the tail, has been noted by not exceeding an immersion time of more than 15 seconds to avoid a burn of the tail of the mouse.

The percentage inhibition of pain by the aqueous extract of *A. laxiflora* was calculated according to the following formula: $P = \frac{(\text{Duration of the treated group} - \text{Duration of positive control group})}{(\text{Tmax} - \text{Duration of positive control group})} \times 100$

2.2.2.2. Irritation test of the rat's paw with formaldehyde

The method used was described by Dubuisson and Dennis (1978)[9] and modified by Tjolsen *et al.* (1992)[10]. 36 rats were homogeneously distributed in 6 groups and received different preparations at 10 ml/kg, as follows: group 1 representing the positive control group received saline by gavage; groups 2, 3 and 4 were gavaged with aqueous extract of *A. laxiflora* at 625 mg/kg, 1250 mg / kg and 2500 mg / kg, respectively; groups 5 and 6 representing the negative control groups (reference groups) were respectively intraperitoneally administered either morphine at 10 mg/kg or paracetamol at 100 mg/kg. Thirty minutes later, 50 µL of 2.5% formalin was injected into the plantar aponeurosis of the left hind paw of each rat.

The animals were kept under observation in a transparent plexiglass cage (20 × 20 × 30 cm). The cage was equipped with mirrors on three sides and inclined up to 45 degrees on the ground allowing a better observation nociceptive behavior. The intensity of the pain was recorded by measuring the licking time of the treated paw during the first phase (0-5 min) and during the second phase (15-30 min). The percentage of pain inhibition was calculated as follows equation:

$$P = \frac{(\text{Control group licking time} - \text{Treated group licking time})}{(\text{Control group licking time})} \times 100$$

2.2.3. Phytochemical screening

The different chemical groups of aqueous extract of *Afrormosia laxiflora* leaves were characterized by referring to the techniques described by Ronchetti and Russo (1971)[11], Hegnauer (1973)[12], Wagner (1983)[13], Békro *et al*. (2007)[14].

2.3. Statistical analysis

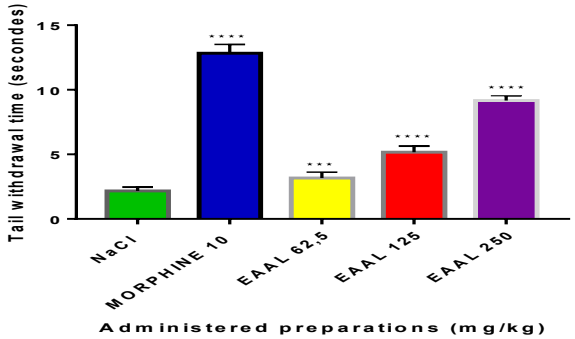
The results obtained were expressed as mean ± SD. The statistical analysis of the results was carried out by the software SPSS v 18.0. The graphical representation of the data was performed from the Graph Pad Prism 7.00 software. The comparison of averages was done using Tukey's non-parametric test. The difference was considered significant if $p < 0.05$.

3. Results

3.1. Results of the immersion test of the tail of the mouse

The mean withdrawal times of the mouse's tail of warm water as a function of the preparations received are shown in Figure 1. The aqueous extract of leaves of *A. laxiflora* at 625 mg/kg, at 1250 mg/kg and at 2500 mg/kg, prolonged the residence time of the mice tail in warm water compared to mice treated with saline ($p < 0.0001$). However, *A. laxiflora* analgesic activity was weak compared to that of the morphine ($p < 0.0001$).

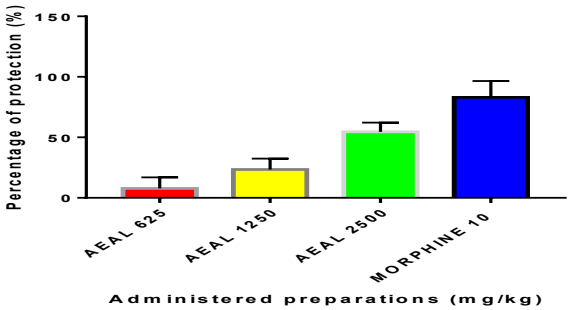
Figure 1: Mean withdrawal time of the tail of the mouse which received the aqueous extract of leaves of *A. laxiflora*



AEAL: Aqueous extract of leaves of *A. laxiflora;* ****: Tukey's test, $p < 0.0001$; *** : Tukey's test, $p < 0.001$

The percentages of inhibition pain were calculated and shown in Figure 2. The mice were protected at 7.79 ± 6.92%, 23.37 ± 6.92% and 54.54 ± 6.49%respectively with the aqueous leaf extract of *A. laxiflora* at 625 mg / kg, 1250 mg / kg and 2500 mg / kg, while morphine provided 83.11 ± 9.52% of protection. The aqueous leaf extract of *A. laxiflora* has dose-dependent analgesic activity.

Figure 2: Percentage of protection of the mouse by the aqueous leaf extract of *A. laxiflora*



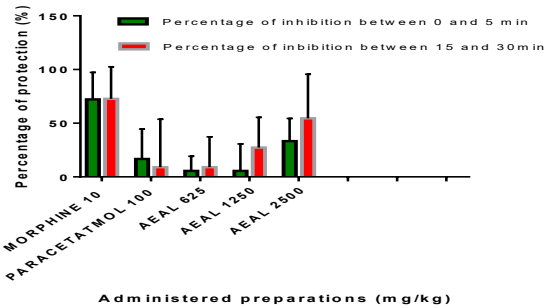
AEAL: Aqueous extract of leaves of *A. laxiflora*

3.2. Results of the formaldehyde test

Figure 3 shows the percentages of inhibition of formaldehyde-induced pain at different observation slices. The aqueous leaf extract of *A. laxiflora* at 625 mg/kg gave protection of 5.55% and 9.10%, respectively, between 0 and 5 minutes and between 15 and 30 minutes after the injection of formaldehyde. At 1250 mg/kg, this protection was 5.55% and 27.27% respectively in the same hours when it reached 33.33% and 54.55% for the dose 2500 mg/kg respectively at the same hours. The analgesic activity of the aqueous leaf extract of *A. laxiflora* is dose-dependent. The aqueous leaf extract of *A. laxiflora* at 2500 mg/kg had a better effect than paracetamol at 100mg/kg which conferred protection of 16.67% and 9.10%, respectively from 0 to 5 min. and 15 to 30 minutes after the injection of formaldehyde. The dose of 1250 mg/kg was as good as paracetamol at 100 mg/kg in the second observation slice when the effect at 625 mg/kg was equivalent to that of paracetamol at the second observation slice.

However, the analgesic effect of the aqueous leaf extract of *A. laxiflora* remains lower than that of morphine which induced at both observation slices 72.73% protection against the pain generated by formaldehyde.

Figure 3: Percentage of inhibition of pain induced by formaldehyde by aqueous leaf extract of *A. laxiflora*



AEAL: Aqueous extract of leaves of *A. laxiflora*

3.3. Results of phytochemical screening

The results of the phytochemical characterization of the aqueous extract of *A. laxiflora* are shown in Table I below. It contains polyphenols including flavonoids and tannins, alkaloids, saponosides, and terpenes.

Table I: Phytochemical Characterization of aqueous extract of *A. laxiflora* leaves

Phytochimique group	Result
Polyphenols	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponosides	+
Quinones	-
Terpenes	+

+: presence; - : absence

4. Discussion

Our study aimed to evaluate the analgesic activity of an aqueous extract of *Afrormosia laxiflora* leaves, a plant used by African populations, particularly Ivorians against various pains. Our study investigated the inhibitory effect of acute pain of *Afrormosia laxiflora* in animal models commonly used to evaluate the analgesic activity of plant extracts.

The immersion test of the mouse tail in warm water is a specific method of evaluating the analgesic activity of morphine type [15]. In fact, thermal stimuli are selectively inhibited by morphine analgesics and not by non-morphine analgesics [16]. In this study, the aqueous extract of leaves of *Afrormosia laxiflora* increased the residence time of the mice's tails in warm water compared to mice in the group that received only NaCl. The aqueous leaf extract of *Afrormosia laxiflora* had a dose-dependent effect with a pain inhibition ranging from 7% for the lowest dose used, to 54% for the 2500 mg/kg dose, the highest dose. The aqueous leaf extract of *Afrormosia laxiflora*, therefore, possesses a morphine analgesic activity. This activity is low compared to that of morphine which conferred 83% protection against pain in this study.

Refinement of the analgesic activity of the aqueous extract of *Afrormosia laxiflora* leaves was performed by performing the rat paw irritation test with formaldehyde. This test characterizes both phases of the pain. The first phase or neurogenic phase of this test is linked to direct chemical stimulation of the nociceptors, while the second depends on peripheral inflammation [10]. The second phase or inflammatory phase is mediated by the release of prostaglandins PGE2, nitric oxide, tachykinin, histamine, sympathomimetic amines, tumor necrosis factor and interleukins [17-20]. The anti-inflammatory analgesics more substantially inhibit the second phase [21].

The results on licking inhibition showed that the aqueous extract of *Afrormosia laxiflora* leaves inhibited both phases with a dose-dependent activity. Inhibition of licking was more marked in the second phase corresponding to the inflammatory phase. Thus, the aqueous extract of *Afrormosia laxiflora* leaves could possess analgesic activity coupled with an anti-inflammatory effect.

This analgesic activity of the aqueous extract of *Afrormosia laxiflora* leaves may be due in part to the presence of the flavonoids [22] contained in the extract. Indeed, flavonoids are inhibitors of prostaglandin and leukotriene production, mediating pain and inflammation [23]. Terpenes and saponosides found there may also enhance the anti-inflammatory activity of this extract [24].

5. Conclusion

Our study has shown that the aqueous extract of *Afrormosia laxiflora* leaves has analgesic activity coupled with considerable anti-inflammatory potential in animal experiments. This plant could be the subject of further evaluation that could lead to its introduction into the therapeutic arsenal analgesic and anti-inflammatory.

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