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Analgesic Activity of Aqueous Extract of *Daniellia Oliveri* Leaves (Rolfe, Hutch Et Dalz) (Fabaceae)

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ABSTRACT

The pharmacological study of the aqueous extract of *Daniellia oliveri* (Fabaceae) leaves revealed analgesic properties similar to those of Aceclofenac. The results obtained indicate that the aqueous extract of *Daniellia oliveri* leaves causes a decrease in the number of abdominal cramps in the writhing test and pain inhibition in the second phase of the formaldehyde test. However, it should be noted that the aqueous extract of *Daniellia oliveri* leaves has no inhibitory effect on pain in the tail-flick test and the first phase of the formaldehyde test. In contrast to morphine, the aqueous leaf extract of *Daniellia oliveri* is therefore an essentially peripheral analgesic. Qualitative phytochemical screening shows that the aqueous leaf extract of *Daniellia oliveri* contains polyphenols, flavonoids, saponosides, quinone substances, alkaloids, catechin, and gallic tannins, sterols, polyterpenes, and cardiotonic heterosides. The oral LD_{50} of the aqueous extract of *Daniellia oliveri* leaves conducted according to OECD guideline 423 (OECD, 2001) [1], is greater than 5000 mg/kg B.W, making this plant a substance of low toxicity, thus justifying its traditional use in painful ailments.

Keywords: *Daniellia oliveri* (Rolfe, Hutch et Dalz); Writhing; Tail-flick; Analgesic; Flavonoids.

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INTRODUCTION

Daniellia oliveri (Fabaceae) is a plant described by Rolfe R.A., Hutchison J., and Dalziel J.M. in 1954 [2] (Figure 1). It is the most widespread plant in wooded savannahs, but in the Sudano-Guinean zone it is dominant in dry forests. It is a large tree reaching 25 meters in height with a conical crown and is generally in spindle shape. The trunk is straight with light grey bark and the foliage is paripinnate. The secondary-veined leaflets connect before the margin, with a long bud often containing the young, rolled leaf, which is reddish-brown in color, as is the young foliage. Its leathery, flattened pods carry a seed that remains attached to the wing of the pod by a small filament. In Côte d'Ivoire, it is called slim, sanan respectively in Senoufo and Malinke [2, 3]

This plant is used in traditional medicine for various treatments:

- The roots are used against tuberculosis.
- The leaves are used to treat burns, headaches, glaucoma, toothache, and gastrointestinal disorders, and to treat epilepsy in Mali [4]
- The leafy branches treat angina and liver failure.

Our work proposes to highlight the pharmacological properties of the aqueous extract of *Daniellia oliveri* to provide a scientific basis for the traditional use of this plant. The present study aims to justify the use of *Daniellia oliveri* as an analgesic.

A 4m **B 6cm**

Figure 1: *Daniellia oliveri* (Fabaceae)

A: Photograph of *Daniellia oliveri* (Fabaceae)

B: Photograph of a leafy branch with flowers and flower buds of *Daniellia oliveri*

(*Http: www .ethnopharmacologia.org/recherche-dans-prelude/?-id=4640#lightbox [4640]/1)*

MATERIALS AND METHOD

Materials

Vegetal material

Young leaves of *Daniellia olive*ri (Fabaceae) were collected in March 2017 during the dry season, in the region of Korhogo (Ivory Coast). This species was described by Rolfe RA., Hutchison J. and Dalziel JM. in 1954 [2]. The leaves were dried at room temperature about 30 ± 2 °C in the shade. Finally, the dried leaves were ground into a powder from which the aqueous extract was made.

This plant has been identified and authenticated at the National Floristic Center (CNF) of the University of Félix Houphouët-Boigny (UFHB), in comparison with the herbarium found there under the number: UCJ 009291.

Animal material

It consists of mice and rats for the study of analgesic activity and acute toxicity. The mice are taken from Mus musculus species, and the Swiss strain, weighing between 25 and 30 g, aged between 55 and 70 days. The rats belong to the species Rattus norvegicus (Murideae). They are of the Wistar strain, weighing between 150 g and 180 g, and aged from 60 to 70 days. These animals were obtained from the animal facility of Ecole Normale Supérieure (ENS) (Abidjan, Ivory Coast).

The average temperature of the animal facility was 28° ± 3°C with a relative humidity of 70%. The photoperiod was 12/24.

The animals have free access to water and food. All the experimental protocols are conducted following the European directive of November 24, 1986 (86/609/EEC) and the decree of April 19, 1988[5], relating to the use of experimental animals in research.

Chemical products

The chemical products used in our study were

- Morphine: MORFIVER (France)
- Aceclofenac: ACENLAND (India)
- Acetic acid: LABOMODERNE (Belgium)
- Formaldehyde: MERCK (Germany).

Methods

Preparation of the aqueous extract of *Daniellia oliveri* (Fabaceae) leaves

The aqueous extract of *Daniellia oliveri* leaves is obtained from 250 g of *Daniellia oliveri* leaf powder mixed with 3 liters of distilled water. This mixture is boiled for 30 minutes to obtain

about 2 liters of decoction. The solution is then filtered through cotton wool and Wattman paper (1mm). The filtrate is freeze-dried. At the end of this operation, we obtain about 40 g of dark brown lyophilic substance with a yield of 16.36%.

Methods for studying analgesic activity

In this study, we considered both peripheral and central components.

Peripheral analgesic activity: writhing test.

The method used is similar to the one described by Koster and *al*., (1959)[6] and modified by Collier and *al*., (1968)[7] whose principle is the following:

Mice are divided into 5 batches of 7. In each batch, there are males and females. Aqueous extract of *Daniellia oliveri*, aceclofenac, and morphine diluted in a 9‰ isotonic NaCl solution, is injected intraperitoneally to the mice 30 minutes before the injection of acetic acid at a rate of 0,1 ml/10g Body Weight (B.W). Control mice receive physiological NaCl solution. Animals of the same batch receive the same solution at a given concentration. The 1.2% acetic acid is then injected intraperitoneally at a rate of 0.15 ml per 20 g Body Weight. The pain syndrome is characterized by stretching movements of the hind legs and twisting of the dorso ventral musculature. Ten minutes after the injection of acetic acid, we count the twists for 10 minutes.

Central analgesic activity: tail-flick test

The method used is that described by D'Amour and Smith (1941)[8] and modified by Gray and *al*., (1970)[9]**.**

Vigil rats are divided into 6 batches of 7. Aqueous extract of *Daniellia oliveri* and morphine are diluted in a 9‰ isotonic NaCl solution. The resulting solutions are injected intraperitoneally 30 minutes before tail irradiation at a rate of 1 ml/100g body weight (B.W). Rats of the same batch receive the same solution at a given concentration. A batch of 7 rats receives the physiological fluid as control rats.

The experimental device used to produce the heat is the tail-flick (UGO BASILE 7360 Italy). At the beginning of each test, the rat is immobilized in a Plexiglas cage, the tail is positioned at its mid-length in the light path, and rests on the photoelectric orifice located in the same path. The counting of the tail withdrawal latency and the emission of the radiant heat are simultaneously triggered. The heat emission and the timer are automatically stopped as soon as the tail undergoes an abrupt deflection to come out of the light path. For the determination of the nociceptive thresholds, three trials are successively carried out at 15 minute intervals, within each trial, three measurements are made.

The first measurement (maximum 9 seconds) is used as a habituation measurement, the average calculated over the last two measurements of the three trials is used to determine the nociceptive threshold. The distance of the tail to the lamp and the intensity of the irradiation (in our case, the focus is set at four) are adjusted to obtain the withdrawal of the tail for a time between 4 and 6 seconds in the control tests carried out before the injection of the substance under study.

After administration of the product, the maximum irradiation time will be 12 seconds to avoid tail burns, which would make subsequent tests random. The seconds taken by the animal to remove its tail from the caloric beam emitted by the apparatus is a function of the central analgesic effect of the test substance.

Peripheral and central analgesic activity: formaldehyde test

The method used was the one of Dubuisson and Dennis (1977)[10] and modified by Tjolsen et *al.*, (1992)[11].

We divided the vigil rats into 5 batches of 5 rats of different sexes. Aqueous extract of *Daniellia oliveri*, aceclofenac, and morphine are diluted in a 9 ‰ isotonic NaCl solution. They are injected intraperitoneally into the rats, 30 minutes before the injection of formaldehyde at a rate of 1 ml/100g Body Weight. Rats in the same batch receive the same solution at a given dose. A batch of 5 rats received the physiological solution as control rats.

Thirty minutes after this treatment, 50 µl of a 2.5% formaldehyde solution was injected under the footpad of the right hind leg of the rats, and the rats were then placed under observation for 1 hour.

The classification of the pain response is based on the following scale:

- 0: rats walk or lean firmly on the treated paw and feel no pain
- 1: the treated paw is partially raised.
- 2: the treated paw is firmly raised and painful.
- 3: the rat licks, chews, or shakes the treated paw and appears to be in pain. The rats are placed in an enclosure that allows the treated paw to be observed, the anti- nociceptive effect is determined in 2 phases. The first phase lasts from 0 to 5 minutes, and the second from 15 to 30 minutes with an intermediate period of 10 minutes.

Phytochemical screening

This qualitative study allowed us to determine the groups of chemical constituents of pharmacological interest present in the aqueous extract of *Daniellia oliveri* (Fabaceae) leaves, namely sterols, polyterpenes, polyphenols, flavonoids, tannins, quinone compounds, saponosides, alkaloids, and cardiotonic compounds.

- The search for sterols and polyterpenes was carried out by the reaction of Liberman
- Polyphenols are revealed by the ferric chloride reaction
- Flavonoids were detected by the cyanidin reaction
- The search for tannins was done by the Stiasny reagent
- Alkaloids were characterized by two reagents: Dragendorff (potassium iodobismuthate reagent) and the one of Bouchardat (iodine reagent).Saponosides are detected by the foam test.
- Borntraeger reagent is used to detect quinone substances.
- The search for cardiotonic heterosides is made by the Baljet test.[12]

Acute toxicity study

We determined the acute toxicity by the intraperitoneal route and by the oral route.

Study of acute toxicity through the intraperitoneal route

The mice were divided into 5 batches of 10 vigil mice. Each batch contained as many males as females. The different concentrations of the aqueous extracts of *Daniellia oliveri* are prepared from a 60 mg/ml stock solution and injected intraperitoneally at a rate of 0.1 ml per 10 g Body Weight. Mice of the same batch are injected intraperitoneally with a solution of a given concentration.

During this experiment, the general behavior of the mice in the cage changes in the rhythm of respiratory movements, and the appearance of any death is observed for 24 hours. The LD_{50} is determined by the graphical method of Miller and Taintter (1944)[13] and the method through the calculation of Dragsted and Lang (1959)[14].

Study of acute toxicity through the oral route

The acute oral toxicity test was carried out following Organization for Economic Co-operation and Development's (OECD) Guideline 423 (OECD, 2001) [1].

Fasting vigil mice weighing between 20 g and 25 g were randomly divided into 3 groups of 10. Each mouse received 1 ml of a single dose, assessed as mg/kg Body Weight (mg/kg B.W) of the aqueous extract of *Daniellia oliveri*.

A batch of mice receiving each 1ml of distilled water is examined in parallel as control rats. For the initial dose, one of the four levels is chosen: 5, 50, 300, and 2000 mg/kg B.W. The level chosen is the one at which mortality is expected to occur in some of the treated animals. The dose of 2000 mg/kg B.W is the one chosen from these predefined doses. Exceptionally, an additional maximum pre-determined dose of 5000 mg/kg B.W was used.

Animals were observed for the first 4 hours after treatment to record immediate deaths and once daily for 14 days.

Statistical and graphical analyzes

Statistical analyzes of values and graphical representations of data are performed using *GraphPad Prism7* software (San Diego, California, USA). The statistical difference between the results was carried out using the analysis of variance (ANOVA), followed by the Tukey- Kramer multiple comparison test, with a significance level of $P < 0.05$. The results are expressed as the mean ± error of mean (ESM).

RESULTS AND DISCUSSION

Analgesic activity

Peripheral analgesic activity: writhing test

Table 1 shows the effects of aqueous extract of *Daniellia oliveri*, aceclofenac, and morphine on the number of abdominal cramps observed after the acetic acid injection into mice. After the injection of 1.2 % acetic acid into mice, 33.66 ± 2.50 abdominal cramps were recorded after ten minutes in the control group. After treatment with morphine at 10 mg/kg of B.W, aqueous extract of *Daniellia oliveri* at 100 mg/kg of B.W, and Aceclofenac at 100 mg/kg of B.W, the number of abdominal cramps decreased significantly ($P < 0.05$) in the same time interval. The number of abdominal cramps decreased to 0; 10.5 ± 1.1 and 15 ± 1.6 respectively, which corresponds to a percentage decrease of 100 %, 61.30 % and 52.60 % compared to the control rats. This decrease in the number of abdominal cramps increases with the dose of morphine, aqueous extract of *Daniellia oliveri* and Aceclofenac.

Table 1: Dose-response effects of morphine, aqueous extract of *Daniellia oliveri* (Fabaceae) leaves an aceclofenac on the number of abdominal cramps provoked by acetic acid in mice.

Group	Control	Morphine (mg/kg B.W)			*Daniellia oliveri* (mg/kg B.W)			Aceclofenac (mg/kg B.W)		
		2.5	5	10	25	50	100	25	50	100
Number of abdominal cramps	33.66±2.5	14.1±1,6**	7±1.3***	0***	21.13±2.7**	16.13±1.5***	10.5 ±1.1***	19.30±2.1**	18±1.1**	15±1.6***
Inhibition of abdominal Cramps (%)		56.40	79.70	100	41.12	53.31	61.30	20.31	43.25	52.60

Values represent average ± ESM; n = 7 for each group *p < 0.05; **p < 0.01;* p < 0.001 compared to control rats. The aqueous extract of *Daniellia oliveri leaves*, morphine and aceclofenac decrease dose-response effects on the number of abdominal cramps provoked by acetic acid**

Central analgesic activity: the tail-flick test.

The effects of aqueous extract of *Daniellia oliveri* and morphine on the latency of rat tail withdrawal from the caloric light beam are shown in Figure 2**.** The tail withdrawal latency of control rats from the light beam is equal to 6.31 ± 0.59s. When comparing the control lot to the different lots that received 100 mg/kg of B.W of the aqueous extract of *Daniellia oliveri*, morphine at doses ranging from 2.5 to 5 mg/kg of B.W, it can be seen that the latency time does not vary significantly. At 7.5 mg/kg of BW of morphine, the latency time increased to 9.12 ± 0. 35. At 10 mg/kg of B.W, the animal no longer withdraws its tail from the path of the calorific light beam.

Peripheral and central analgesic action: formaldehyde test

Table 2 shows the dose-response effect of aqueous extract of *Daniellia oliveri* leaves, aceclofenac and morphine on the pain intensity observed in rats after injection of 2,5 % formaldehyde (n = 7).

After injection of 2.5 % formaldehyde into the control rat batch, a pain intensity of 2,80 ± 0,20 in the first phase and 2.8 ± 0.1 in the second phase were recorded.

In the presence of the aqueous extract of *Daniellia oliveri* leaves and aceclofenac, the pain intensity does not vary during the first phase, it is equal to 2.8 ± 0.1. On the other hand, it decreases during the second phase, to 1 ± 0.1 and 0,77 ± 0.1 respectively for the aqueous extract of *Daniellia oliveri* leaves, and to 1.5 ± 0.2 and 1.12 ± 0.1 for aceclofenac for doses of 50 and 100 mg/kg BW. This corresponds to a percentage of inhibition respectively of the pain of 66.66 % and 73 %; 50.20 % and 60.59 % in comparison with the control.

In the presence of morphine at doses between 1 and 10 mg/kg of BW, the pain intensity decreases equally for both phases. It decreases to 0.75 ± 0.1 and 0 respectively for doses of 5 to 10 mg/kg of B.W. This corresponds to a percentage pain inhibition of 75 % and 100 % compared to the control rats. This decrease in pain is increased with the dose of aqueous extract of *Daniellia oliveri* leaves, aceclofenac, and morphine.

Phytochemical study

The phytochemical screening shows that the chemical groups identified in the leaves of *Daniellia oliveri* is water-extractable. The main chemical constituents identified are listed in Table 3. They are polyphenols, flavonoids of the flavone type, saponosides, alkaloids, quinone substances, polyterpenes, cardiodic heterosides and catechins and gallic tannins.

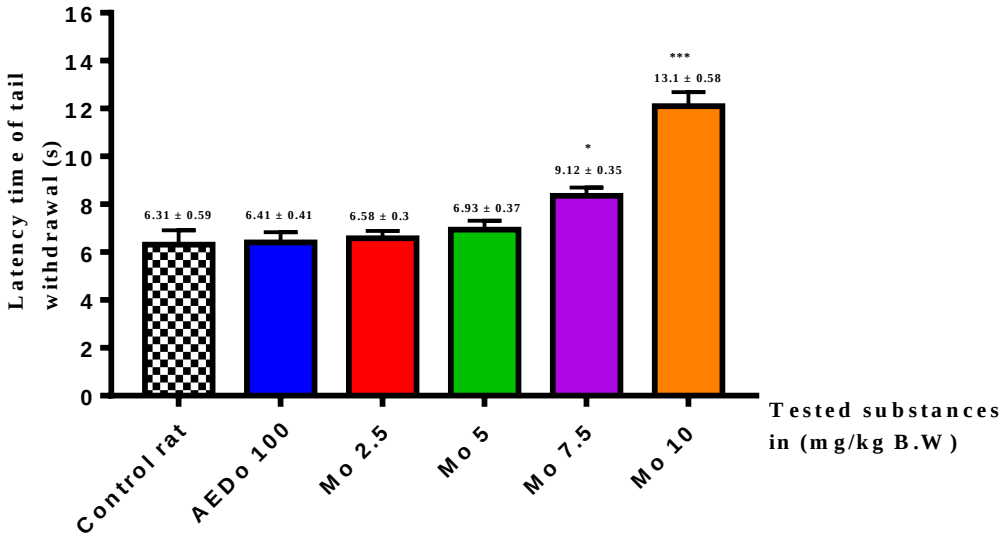

Figure 2: Comparative study of the effects of aqueous leaves extract of *Daniellia oliveri* (AEDo) and of morphine on the latency of withdrawal of the rat tail from the bundle path bright

The figure shows the withdrawal latency of the rat tail from the light beam path in the absence and presence of aqueous extract of *Daniellia oliveri* and Morphine. The aqueous extract of *Daniellia oliveri* leaves has no effect on the latency of tail withdrawal, unlike Morphine. Values represent average ± ESM; n = 7 for each group *p < 0.05; **p < 0.01;***p < 0.001 compared to control rats.

Table 2: Dose response effect of aqueous extract of *Daniellia oliveri* leaves, aceclofenac and morphine on pain intensity induced by formaldehyde formaldehyde into rats

	1ère phase		2ème phase	
	Intensity of pain	**Inhibition of pain (%)**	**Intensity of pain**	**Inhibition of pain (%)**
Control (NaCl à 9‰)	2.8 ± 0.2	-	2.8 ± 0.1	-
Daniellia oliveri				
50 mg/kg B.W.	2.8 ± 0.1	0	1 ± 0.1***	66.66
100mg/kg B.W.	2.8 ± 0.1	0	0.77 ± 0.1***	73
Aceclofenac				
50mg/kg B.W.	2.8± 0.2	0	1.5 ± 0.2**	50.20
100mg/kg B.W.	2.8± 0.2	0	1.12 ± 0.1***	60.59
Morphine				
5mg/kg B.W.	0.75 ± 0.1***	75	0.75 ± 0.1***	75
10mg/kg P.C.	0***	100	0***	100

Values represent average ± ESM; n = 7 for each group *p <0.05;p < 0.01;***p < 0.001 compared to control group.**

The aqueous extract of *Daniellia oliveri* leaves, aceclofenac and morphine decreases the pain intensity provoked by formaldehyde.

Table 3 : Phytochemical screening of aqueous extract of *Daniellia oliveri* leaves

Flavonoids		+
Saponosides		+
Polyphenol		+
	Dragendorff	+
Alkaloids	Bouchardat	+
	Valsen-Mayer	+
Coumarins		-
Quinonic compound		+
Cardiotonic heterosides		+
Sterols and polyterpenes		+
	Gallic	+
Tanins	Catechins	+

The sign (+) means that the reaction is positive.

The sign (-) means that the reaction is negative.

Acute toxicity study

The acute toxicity study of the aqueous extract of *Daniellia oliveri* leaves administered intraperitoneally yielded an LD_{50} value of 436.51 mg/kg B.W by the graphical method of Miller and Tainter (1994)[13] (Figure 3). The method of calculation of Dragsted and Lang (1957)[14] resulted in an LD_{50} value of 437.5 mg/kg B.W. The oral acute toxicity study, conducted according to OECD guideline 423 (OECD,2001)[1], showed that administration of the aqueous extract of *Daniellia oliveri* leaves through oral route has an LD_{50} superior to 5000 mg/kg B.W.

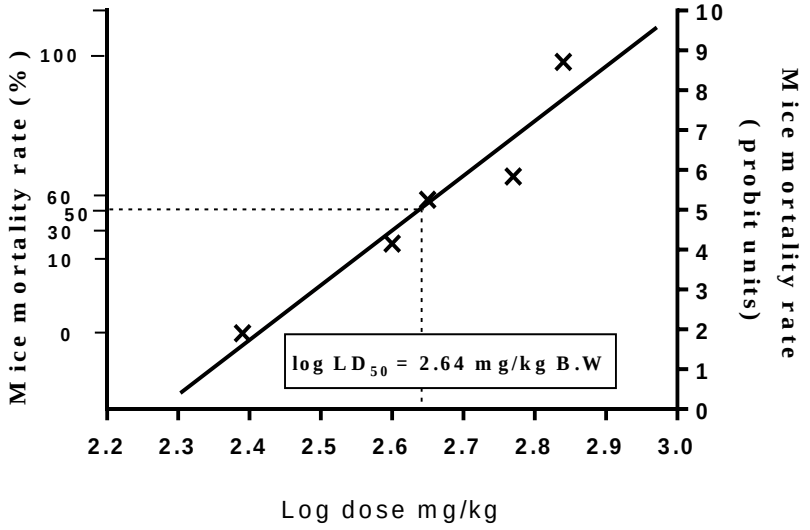

Figure 3: Toxicity curve of aqueous extract of *Daniellia oliveri* leaves with mice, by the graphic method of Miller and Tainter (1994)

The LD_{50} of the aqueous extract of *Daniellia oliveri* is equal to 437.5 mg/kg B.W.

The aqueous extract of *Daniellia oliveri* leaves administered intraperitoneally is toxic in mice.

Aqueous extract of *Daniellia oliveri* at doses ranging from 10 to 100 mg/kg of B.W, inhibits abdominal cramps due to acetic acid injection. *Daniellia oliveri* has analgesic effects similar to those of aceclofenac. At 100 mg/kg of BW, aceclofenac caused 52.60 ± 2% pain inhibition, whereas *Daniellia oliveri* caused 61.30 ± 2% inhibition at the same concentration. In the tail-flick test, the central analgesic morphine, administered at a dose of 10 mg/kg of B.W., inhibits the effect of pain induced by the light beam focused on the tail of rats. According to Sayyah[15], thermal stimuli are only inhibited by central analgesics.

The aqueous extract of *Daniellia oliveri* at a dose of 100 mg/kg of B.W does not affect this pain. It is inferred that *Daniellia oliveri* has a mainly peripheral action similar to *Ximenia americana*[16], *Saba senegalensis*, *Salvia Officinalis*[17]. Unlike central analgesics such as morphine which inhibits at both phases of the formaldehyde test [17, 18], the aqueous extract of *Daniellia oliveri* leaves has significant inhibitory effects only in the second phase of the pain induced by formaldehyde. *Daniellia oliveri* would inhibit formaldehyde-induced inflammation of the second pain phase [19].

The phytochemical screening (Table 3) showed that the aqueous extract of *Daniellia oliveri* leaves contains among other chemicals, flavonoids and saponins which are inhibitors of cyclo-oxygenases responsible for the synthesis of endogenous nociceptive mediators, such as prostaglandins, and cytokines (TNF-α, IL-1β, IL-8)[20]. Therefore, inhibition of cyclo- oxygenase and/or lipo-oxygenase leads to a decrease in peripheral pain The LD_{50} of the aqueous extract of *Daniellia oliveri* is equal to 437.5 mg/kg of B.W.

According to the classification of Diezi (1989) [21], the aqueous extract of *Daniellia oliveri* is toxic in mice.

The LD_{50} obtained in this study was lower than the one obtained by Adaku and Okwesili [22] on another aqueous extract of *Daniellia oliveri* which obtained an LD_{50} greater than 5000 mg/kg of B.W. *Daniellia oliveri* is also less toxic than the extract of *Ximenia Americana* [17]stem bark, whose LD_{50} is equal to 219 mg /kg of body weight.

The oral acute toxicity study, conducted according to OECD guideline 423 (OECD, 2001), showed that administration of the aqueous extract of *Daniellia oliveri* leaves through the oral route has an LD_{50} superior to 5000 mg/kg of B.W. According to OECD guideline 423 (OECD, 2001)[1], the aqueous extract of *Daniellia oliveri* leaves through the oral route is not toxic in mice.

CONCLUSION

The pharmacological study of the aqueous extract of *Daniellia oliveri* leaves revealed analgesic properties that could partly justify the traditional use of this plant. The presence of saponins and flavonoids determined in the phytochemical screening would be at the origin of the analgesic effects of *Daniellia oliveri*. The intraperitoneal administration of high doses of the aqueous extract of *Daniellia oliveri* leaves to mice is toxic. However, the plant is only slightly toxic by the oral route.

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