INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 6, p. 366-377, 2022

RESEARCH PAPER OPEN ACCESS

Study of the analgesic activity of the aqueous extract of *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) trunk bark

Soro Tianga Yaya*, Coulibaly Sirabana, Mian Jean Claude, Ehoussou N'douffou Célestin

Laboratory of Biology and Health, Faculty of Biosciences University Félix Houphouet-Boigny, Abidjan, Côte d'Ivoire

Key words: *Pseudocedrela kotschyi,* Analgesic, Writhing, Tail flick, Formaldehyde

http://dx.doi.org/10.12692/ijb/21.6.366-377 Article published on December 08, 2022

Abstract

Pseudocedrela kotschyi is a traditional medicine plant used by people in sub-Saharan regions to cure several diseases. The main objective of this study is to evaluate the analgesic activity of the aqueous extract of *Pseudocedrela kotschyi* trunk bark. Phytochemical screening revealed the presence of alkaloids, flavonoids, catechic tannins, saponosides, polyphenols, quinones, terpenoids, oses and holosides whereas coumarins and gallic tannins are absent. In the acute toxicity study, oral administration of the aqueous extract of *Pseudocedrela kotschyi* trunk bark did not cause any death among the rats. According to the organisation for economic cooperation and development (OECD) 423 classification system (OECD, 2001), the lethal dose 50 (LD50) of such an extract is therefore greater than 5000mg/kg body weight. On the other hand, this aqueous extract administered intraperitoneally provoked the death of certain mice. Thus an LD50 of 230.08mg/kg body weight was calculated. According to the classification of Diezi (1989), the aqueous extract of the trunk bark of this *Pseudocedrela kotschyi* is toxic by the intraperitoneal administration. Evaluation of the analgesic activity shows that the aqueous extract of this plant induces a decrease in the number of abdominal cramps in the writhing test and an inhibition of pain in the second phase of the formaldehyde test. On the other hand, the extract has no inhibiting effect on pain in the tail flick test and in the first phase of the formaldehyde test. These results justify the traditional use of *Pseudocedrela kotschyi* bark against pain.

* **Corresponding Author:** Soro Tianga Yaya ✉ tiangaso@yahoo.fr

Introduction

The therapeutic use of plants (herbal medicine) is currently experiencing a resurgence of interest among the population. Herbal medicinal products are easily accessible (Thomford *et al.*, 2015; Atchou *et al.*, 2021). About 80% of the world's population uses herbal medicine (Balekundri and Mannur, 2020). In rural areas, the majority of people use plants for treatment (Azzazi *et al.*, 2015) and in emerging countries, between 70 and 95% of the population rely mainly on medicinal plants for their primary care (Ito *et al.*, 2012), due to poverty, lack of access to modern medicine and also because plants are effective. Medicinal plants are therefore an ideal alternative to chemical drugs that are too expensive to manufacture or purchase for developing countries. It is estimated that 25% of the world's prescribed medicines are of plant origin and rely on approximately 121 active ingredients (Ratnesh, 2019).

Pain according to the International Association for the Study of Pain (IASP) is an unpleasant, sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Raja *et al.,* 2020). It can also be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain (Mishra *et al.*, 2011).

Pain is one of the most important health problems because of its prevalence and the disability it can induce. Along with fever, it is one of the most complex pathological phenomena, involving the immune system, neurobiological processes and local and regional humoral systems (D'Amour and Smith, 1941). As it is implied in virtually all human and animal diseases, pain have become the major focus of global scientific research (Ibrahim *et al.*, 2012).

Current treatment of pain involves non-steroidal anti-inflammatory drugs such as aspirin, but their undesirable side effects on gastric mucosa, kidney, bronchus and cardiovascular system are well known (Wallace and Vong, 2008), and have limited their use (Burke *et al.*, 2006). The current trend of research is the investigation of medicines of plant origin because of their afford ability and accessibility with mini side effects (Ibrahim *et al.*, 2012).

Medicinal plants are widely used by people all over the world and are a source of new active components, especially for pain and inflammatory processes (Nguelefack *et al.*, 2010). *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) or dry zone cedar (Alhassan *et al.*, 2021) is a long tree that can reach a height of 20 m with a greyish, cracked bark (Traore, 2006). It is one of the biggest trees found in tropical and subtropical countries of Africa.

The leaves are alternate and clustered at the tips of the branches. They are paripinnate with 8-18 leaflets. These alternate leaflets are almost opposite. The flowers are hermaphrodite and whitish. They have a 2-4 mm long pedicel. The calyx is lobed at the base and about 1.5 mm long. The free petals are 3.5-5 mm long. The stamens are fused into an ureolate tube about 3 mm long. The fruit is a narrow obovoid capsule. It is 7-14.5 cm long and brown. The 5-valved dehiscent capsule contains many seeds. People in sub-Saharan regions use it to cure several diseases (Kpodar *et al.,* 2016; Elufioye *et al.,* 2017; Mambou *et al.,* 2018).

Different parts of *Pseudocedrela kotschyi* are used in the traditional treatment of various diseases. The root is used in the treatment of epilepsy, dementia (Kantati *et al.*, 2016), diabetes (Salihu Shinkafi *et al.*, 2015). The stem bark is used in the treatment of cancer (Saidu *et al.,* 2015), infantile dermatitis (Erinoso *et al.,* 2016), toothache (Kayode and Sanni, 2016).

The present study aims to evaluate the analgesic *properties* of the aqueous extract of *Pseudocedrela kotschyi* in order to provide a scientific basis for its ethnopharmacological use.

Materials and methods

Material

Plant material

We used the freeze-dried infusion of *Pseudocedrela kotschyi* (Meliaceae) trunk bark. *Pseudocedrela kotschyi* trunk bark collection was carried out Dikodougou's Prefecture, in the northern part of Côte d'Ivoire.

The plant species was authenticated at the National Floristic Center (CNF) of the University Felix Houphouët-Boigny from herbarium n°8664. The barks were dried in the shade at room temperature and then ground to prepare our aqueous extract.

Animal material

The animal material used for the test is composed of rats of the species *Rattus norvegicus*, of Wistar strain and mice of the species *Mus musculus* of Swiss strain. The rats weigh between 180 and 250g, while the mice weigh between 20 and 30g. These animals were reared in the vivarium of the Ecole Normale Supérieure d'Abidjan, where the photoperiod is 12/24 hours with a relative humidity of 70% and the average temperature is 28°± 3°C. These animals have free access to water and food.

Extract, solvents and chemical reagents

The freeze-dried aqueous extract of *Pseudocedrela kotschyi* trunk bark.

- Distilled water is used for extraction.
- NaCl 9‰ solution is used to dilute the lyophilisate of *Pseudocedrela kotschyi* bark.
- Aceclofenac has been used as a reference analgesic (France).
- Morphine sulphate: SANOFI-AVENTIS (France).
- Acetic acid: MERCK (Germany).
- Formaldehyde: MERCK (Germany).

Methods

Preparation of aqueous extract of Pseudocedrela kotschyi

One hundred and fifty grams of *Pseudocedrela kotschyi* trunk bark powder was taken and put into a 5 litre beaker. Three litres of distilled water heated to 100°C are added. This mixture is stirred for 24 hours using a magnetic stirrer.

The solution was then filtered through cotton wool and Wattman paper (3mm). The filtrate obtained is freeze-dried using a freeze-dryer (type SERIAL). The lyophilisate is a light brown powder with a 20% yield.

Phytochemical screening

The phytochemical screening was carried out in order to identify the chemical constituents with pharmacological interest such as sterols, terpenoids, polyphenols, flavonoids, tannins, quinone compounds, saponosides, alkaloids, coumarins, cardiotonic heterosides, reducing compounds, oses and holosides via qualitative analysis techniques described in the literature (Bekro *et al*., 2007).

Method for study of acute oral toxicity

The acute oral toxicity study was conducted on female rats using the Organisation for Economic Co-operation and Development (OECD) protocol 423 (2001).

Method for study of acute intraperitoneal toxicity

After a 15-day adaptation period, mice were placed in cages, weighed, marked and fasted 18 hours prior to use to prevent any dietary digestive interaction and 3 hours after dosing. We used 6 batches of 10 female and male mice, to which we administered increasing doses of lyophilized total aqueous extract of *Pseudocedrela kotschyi*, diluted in a 9‰ isotonic solution of NaCl chloride, intraperitoneally (I.P). These different doses were injected at a rate of 0.1ml per 10g body weight. Each batch of mice receives a single dose. These different doses are used to determine the percentage of mortality varying between 0 and 100% during 24 hours of time.

The animals are observed and manifestations of toxicity such as increased activity, salivation, convulsions, coma and death are noted. These observations are made regularly for up to 24 hours.

Expression of results of acute intraperitoneal toxicity

The LD50 expressed inmg/kg body weight (B.W) is determined by the graphical method of Miller and Tainter (1944) and by the calculation method of Dragstedt and Lang (1957).

Graphical method or Miller and Tainter method

The percentages of dead mice in each batch are recorded and converted into probit units. The doses corresponding to these percentages are determined in mg/kg body weight. The curve expressing mouse mortality (in probit units) as a function of the logarithm of the administered dose (in mg/kg body weight) is plotted.

Linearisation of this semi-log curve allows the determination of the LD50 which is the abscissa of the point corresponding to 50% mortality.

Calculation method or Dragstedt and Lang method

This method is based on the following assumption:

- Any animal that has survived a dose given to it will survive any dose lower than that.
- Any animal that has succumbed to a dose given to it will succumb to any dose above it.

Thus, for each dose, the percentage mortality M (%) can be calculated by summing all deaths observed at lower doses and all survivors observed at higher doses.

$$M(\%) = \frac{\text{Cumulative number of deaths}}{\text{Cumulative number of living} + \text{cumulative number of deaths}} \times 100$$

The LD50 is calculated by interpolation: LD50

$$= \frac{50(X2 - X1) + (X1Y2 - X2Y1)}{Y2 - Y1}$$

X2: Upper dose limit for LD50

X1: Lower limit of the LD50

Y2: Percentage of mortality for X2

Y1: Percentage of mortality for X1

Method of studying analgesic activity

The analgesic activity will be studied according to its peripheral and central components.

Writhing test

The method used is that described by Koster *et al.* (1959) and modified by Collier *et al* (1968). The mice are divided into batches of 6 vigorous mice. In each batch there are as many males as females. The plant extract, morphine and aceclofenac are diluted in a 9‰ isotonic NaCl solution. They are injected into the mice 30 minutes before the acetic acid injection at a rate of 0.1ml/10g body weight (B.W). A batch of mice receiving a physiological 9‰ NaCl solution is examined in parallel as a control. The 1.2% acetic acid is then injected intraperitoneally at a rate of 0.15ml per 20g body weight. Ten minutes after the injection of acetic acid, the pain syndrome is characterized by stretching movements of the hind legs and twisting of the dorso-abdominal muscles. These twists are then counted over a period of 10 minutes.

Tail flick test

The method described by D'Amour and Smith (1941) and modified by Gray *et al.* is used. Rats are divided into batches of 6 vigorous rats, each batch containing male and female rats. The aqueous extract of *Pseudrocedrela kotschyi* and morphine are diluted in a 9‰ isotonic NaCl solution. These solutions are injected intraperitoneally 30 minutes before tail irradiation at a rate of 1ml/ 100g body weight. A batch of 6 rats receiving the physiological 9‰ NaCl solution is examined in parallel as a control.

The experimental device used to generate the heat is the Tail-flick (7360, Ugo basile, Comerio, Italy) which is a device composed of a bulb emitting radiant heat of 55-60°C, a timer that is triggered together with the radiant heat source, and a photocell that automatically stops the timer as soon as the animal withdraws the tail.

At the beginning of each test, the rat is immobilised in a Plexiglas cage, with the animal's tail positioned halfway along the light path, and resting on the photoelectric orifice located on 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 out of the heat path.

To determine the nociceptive thresholds, three trials were conducted successively at 15-minute intervals. Within each trial, three measurements are taken at one minute intervals.

The first measurement (maximum 9 seconds) serves as a habituation measurement. The average of the last two measurements of the three trials is used to determine the nociceptive threshold. The lamp-tail distance and the intensity of the irradiation are adjusted in order to achieve tail withdrawal after 4 to 6 seconds in the control tests performed before the injection of the test substance. After the administration of the extract, the maximum irradiation time is 15 seconds, which is the limit time to avoid damaging the tissues by a long-term exposure to the calorific light beam. The reaction time of each rat at 30, 45, 60, 75, 90 and 120 minutes is noted after administration of morphine and the concentration ranges of the extract to be studied.

Formaldehyde test

The method used is the same as that described by Dubuisson and Dennis (1977) and modified by Tjolson *et al.* (1992). The rats are divided into batches of 6 vigorous rats. Each batch contained an equal number of males and females. The plant extract, morphine and aceclofenac 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 1ml/100g body weight. A batch of 6 rats receiving the physiological 9‰ NaCl solution is examined in parallel as a control. Thirty minutes after this treatment, 50µl of a 2.5% formaldehyde solution is injected under the plantar pad of the right hind paw of the rats and then the rats are placed under observation for 1 hour.

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

0: The rats walk or lean heavily on the treated paw and feel no pain;

1: The treated paw is partially raised;

2: The treated paw is definitely raised and seems painful;

3: The rat licks, chews or shakes the treated paw and appears to be in pain.

The animals are placed in an enclosure that allows observation of the treated paw. The anti-nociceptive effect is determined in two 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.

Statistical analysis

Statistical data are expressed as mean ± standard error (M±SEM) obtained from the (n) separate experiments. Statistical differences are calculated using Student's t-test. When $p \leq 0.05$ the difference is said to be significant. Statistical analysis was performed with Graphpad prism 5.0 software.

Results

Phytochemical screening

Phytochemical screening of the aqueous extract of *Pseudocedrela kotschyi* trunk bark by qualitative characterization reactions revealed the presence of different chemical groups with chemical potentialities such as alkaloids, flavonoids, catechic tannins, saponosides, polyphenols, quinones, terpenoids, oses and holosides (Table 1).

Table 1. Chemical composition of the aqueous extract of *Pseudocedrela kotschyi* trunk bark.

Chemical compounds	Aqueous extract
Flavonoids	+
Alkaloids	+
Saponosides	+
Polyphénols	+
Coumarins	-
Terpenoids	+
Oses and holosides	+
Reducing compounds	+
Quinones	+
Catechic tannins	+
Gallic tannins	-

+= presence of compound; -= absence of compound

Acute oral toxicity

The animals were shaken a few minutes after administration of the extracts. They showed no evidence of tremors, change in respiratory rate, convulsions, salivation, diarrhoea, coma, walking backwards and self-mutilation. There were no deaths during the 14 days following administration of the extracts (Table 2).

Table 2. Percentage mortality of female rats as a function of the dose of aqueous extract of *Pseudocedrela kotschyi*

Batches	Dose administered (mg/kg B.W)	Number of rats	% deaths
1	Control (distilled water)	3	0
2	300	3	0
4	2000	3	0
5	5000	3	0

Acute intraperitoneal toxicity

Immediately after injection of the different doses between 100 and 500mg/kg B.W to the mice we observe frequent movements followed by twisting of the body, acceleration of respiration and heart rate and strong convulsions and agitation. The activity of the animals is reduced, their gait becomes sluggish and death occurs from the 15th minute to the 6th hour for doses of 150mg/kg B.W and above. After 24 hours, the surviving animals returned to normal behavior similar to that of the controls.

The acute toxicity study of the aqueous extract of *Pseudocedrela kotschyi* trunk bark by the intraperitoneal administration made it possible to calculate the LD50 value in mice. This was 230.08mg/kg bw and 240mg/kg B.W by the graphical method (Table 3, Fig. 1) and the calculation method (Fig. 2) respectively. These two values are quite close, which demonstrates the reliability of the determination methods.

Table 3. Mouse mortality rate as a function of the dose of aqueous extract of *Pseudocedrela kotschyi*.

Batches	Number of mice	Dose in mg/kg BW	Number of dead mice	Mouse mortality rate (%)	Mouse mortality rate (probit units)	Log dose mg/kg
1	10	100	0	0	1,90	2,00
2	10	150	2	20	4,15	2,18
3	10	250	5	50	5,0	2,40
4	10	300	7	70	5,52	2,48
5	10	400	9	90	6,28	2,60
6	10	500	10	100	8,71	2,70

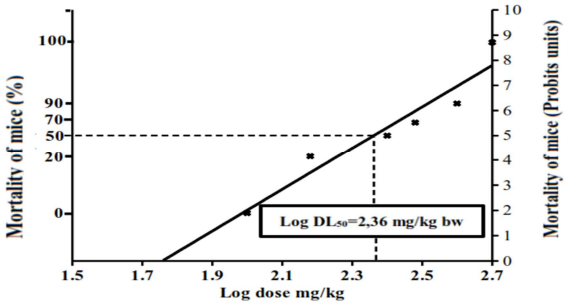

Fig. 1. Toxicity curve of aqueous extract of *Pseudocedrela kotschyi* within mice.
Log LD50=2.36mg/kg B.W.
LD50=230.08mg/kg B.W

$$M(\%) = \frac{\text{Cumulative number of deaths}}{\text{Cumulative number of living} + \text{cumulative number of dead}} \times 100$$

$$\text{The LD50 is calculated by interpolation: } LD50 = \frac{50(X2 - X1) + (X1Y2 - X2Y1)}{Y2 - Y1}$$

$$LD50 = \frac{50(300-150) + (150x70 - 300x20)}{70-20} = 240\text{mg/kg B.W.}$$

Fig. 2. Calculation method according to Dragstedt and Lang.

Analgesic activity

Writhing test

Table 4 shows the effects of aceclofenac, morphine and aqueous extract of *Pseudocedrela kotschyi* on the number of acetic acid-induced abdominal cramps within mice. After injection of acetic acid into the control batch of mice, 35 ± 0.58 abdominal cramps were recorded after 10 min. In the presence of aceclofenac with doses ranging from 50 to 200mg/kg B.W, the number of abdominal cramps gradually decreased from 28.5 ± 0.62 to 17.5 ± 0.62. This corresponds to percentages of inhibition varying from 18.53 ± 1.64% to 50.05 ± 1.14%. The number of cramps decreased from 23.33 ± 0.61 to 0 with morphine having doses ranging from 1mg/kg B.W to 10mg/ kg B.W. The percentage of inhibition for these different doses of morphine varies from 33.31 ± 1.63% to 100 ± 0%. For aqueous extracts of *Pseudocedrela kotschyi* with doses ranging from 50mg/ kg B.W to 200mg/kg B.W the number of cramps decreases from 16.5 ± 0.56 to 14 ± 0.37 corresponding to percentages of inhibition ranging from 52.75 ± 2.02% to 59.96 ± 1.12%.

Table 4. Aceclofenac, morphine and aqueous extract of *Pseudocedrela kotschyi* effects on the number of acetic acid-induced abdominal cramps within mice.

Groups	Number of abdominal cramps	Inhibition of abdominal cramps (%)
Witness	35 ± 0.58	
Aceclofenac		
50mg/kg B.W	28.5 ± 0.62	18.53 ± 1.64
100mg/kg B.W	20.5 ± 0.43**	41.35 ± 1.54
150mg/kg B.W	20 ± 0.52**	42.72 ± 2.1
200mg/kg B.W	17.5 ± 0.62****	50.05 ± 1.14
Morphine		
1mg/kg B.W	23.33 ± 0.61	33.31 ± 1.63
2,5mg/kg B.W	15.17 ± 0.31****	56.68 ± 0.32
5mg/kg B.W	6.83 ± 0.31****	80.38 ± 1.2
10mg/kg B.W	0 ± 0****	100 ± 0
Pseudrocedrela kotschyi		
50mg/kg B.W	16.5 ± 0.56****	52.75 ± 2.02
100mg/kg B.W	15.5 ± 0.5****	55.64 ± 1.65
150mg/kg B.W	15 ± 0.82****	57.2 ± 2.04
200mg/kg B.W	14 ± 0.37****	59.96 ± 1.12

Values represent mean ± SEM; n=6 for each group *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 compared to control. Aceclofenac, morphine and aqueous extract of Pseudocedrela kotschyi dose-dependently decrease the number of acetic acid-induced cramps.

Tail-flick test

The effects of aqueous extract of *Pseudocedrela kotschyi* and morphine on the latency of rat tail withdrawal from the caloric light beam are shown in Table 5.

The tail withdrawal latency of control rats from the light beam is equal to 6.08 ± 0.33 s. Aqueous extract of *Pseudocedrela kotschyi* at a dose of 200mg/kg B.W and morphine at a dose of 2.5mg/kg B.W resulted in a very small increase in this latency time. Morphine with doses of 5mg/kg B.W., 7.5mg/kg B.W. and 10mg/kg B.W. significantly increased the latency of tail withdrawal to the nociceptive thermal stimulus by 7.41 ± 0.13 s, 8.4 ± 0.18 s and 12.17 ± 0.26 s respectively. This corresponds to percentage increases in latency of 21.96±5.61%; 31.55±10.01% and 100±0% respectively compared to the control.

Table 5. Effects of morphine and aqueous extract of *Pseudocedrela kotschyi* on rat tail removal latency from the heat beam path.

Groups	Tail withdrawal latency (s)	Latency increase (%)
Witness	6.08 ± 0.33	
Morphine		
2.5mg/kg B.W	6.86 ± 0.21	13.68 ± 2.5
5mg/kg B.W	7.41 ± 0.13*	21.96±5.61
7.5mg/kg B.W	8.4 ± 0.18*	31.55±10.01
10mg/kg B.W	12.17 ± 0.26***	100 ± 0
Pseudrocedrela kotschyi		
200mg/kg B.W	6.33 ± 0.31	3.41 ± 1.05

The values represent the average ± SEM; n=6 for each group *p<0.05; **p<0.01; p<0.001; p<0.0001 compared to the control group.

The aqueous extract of *Pseudocedrela kotschyi* results in a very small increase in tail withdrawal latency. On the other hand, morphine causes at doses ranging from 5mg/kg to 10mg/kg P.C causes, a significant increase in tail withdrawal latency.

Formaldehyde test

Table 6 shows the effects of morphine, aceclofenac and aqueous extract of *Pseudocedrela kotschyi* on formaldehyde-induced pain on the rat paw. After injection of the 2.5% formaldehyde into the control rat batch, an identical pain intensity of about 2.8 is recorded in the first and second phase.

It was found that aceclofenac, aqueous extract of *Pseudocedrela kotschyi* at the same doses ranging from 50 to 200mg/kg B.W did not induce any variation in pain intensity during the first phase. Over the course of the second phase, however, they induce a decrease in pain intensity. The aqueous extract of *Pseudocedrela kotschyi* at the doses of 150 and 200mg/kg B.W and aceclofenac at the dose of 150mg/kg B.W. lead to a significant decrease in pain intensity of 0.65 ± 0.24**, 0.75 ± 0.14** and 0.9 ± 0.1** respectively. This corresponds to pain inhibition percentages of 73.86 ± 5.77%, 77.35 ± 8.78% and 68.64 ± 3.87% respectively. Morphine at doses of 1mg/kg B.W to 10mg/kg B.W resulted in a significant decrease in pain to total inhibition in both phases.

Table 6. Effects of morphine from aceclofenac and aqueous extract of *Pseudocedrela kotschyi* on formaldehyde-induced pain on rat leg.

Groups	1st phase		2nd phase	
	Pain intensity	Inhibition (%)	Pain intensity	Inhibition (%)
Witness	2.88 ± 0,13		2.87 ± 0.12	
Morphine				
1mg/kg B.W.	1.88 ± 0.13	34.72 ± 6.14	1.90± 0.12	33.79 ± 6.14
2.5mg/kg B.W.	1.53 ± 0.06*	46.87 ± 1.83	1.60 ± 0.13*	44.25 ± 1.83
5mg/kg B.W.	0.75 ± 0.14***	74.95 ± 4.38	0.76 ± 0.06***	73.51 ± 6.14
10mg/kg B.W.	0 ± 0***	100 ± 0	0± 0***	100 ± 0
Acéclofénac				
50mg/kg B.W.	2.88 ± 0,1	0	2.63 ± 0.24	8.36 ± 4.82
100mg/kg B.W.	2.87 ± 0.09	0	2.08 ± 0.15	27.52 ± 3.53
150mg/kg B.W.	2.88 ± 0.08	0	1.95 ± 0.21	32.05 ± 5.11
200mg/kg B.W.	2.87 ± 0.13	0	0.9 ± 0.1**	68.64 ± 3.87
Pseudocedrela kotschyi				
50mg/kg B.W.	2.88 ± 0.1	0	2.5 ± 0.29	12.89 ± 7.23
100mg/kg B.W.	2.88 ± 0.09	0	1.95 ± 0.21	32.05 ± 6.18
150mg/kg B.W.	2.88 ± 0.14	0	0.75 ± 0.14**	73.86 ± 5.77
200mg/kg B.W.	2.87 ± 0.14	0	0.65 ± 0.24**	77.35 ± 8.78

The values represent the average ± SEM; n=6 for each group *p<0.05; **p<0.01; p<0.001; p<0.0001 compared to the control group.

The different doses of the aqueous extract of *Pseudocedrela kotschyi* and those of aceclofenac induce a decrease in pain intensity only during the second phase while the different doses of morphine cause a decrease in pain intensity during both phases.

Discussion

Phytochemical screening of the aqueous extract of *Pseudocedrela kotschyi* revealed the following compounds: alkaloids, flavonoids, catechol tannins,

saponosides, polyphenols, quinones, terpenoids, reducing compounds, oses and holosides. These same chemical compounds were revealed by Adeniyi *et al.* (2010); Alhassan *et al.* (2014) with methanolic extract of stem bark and aqueous extract of *Pseudocedrela kotschyi* roots respectively.

In the acute oral toxicity study, the aqueous extract of *Pseudocedrela kotschyi* did not result in death at 5000mg/kg B.W. According to the OECD 423 Globally Harmonised System of Classification (GHS) (OECD, 2001), any non-lethal substance administered at this dose has an LD50 greater than 5000mg/kg B.W. The aqueous extract of *Pseudocedrela kotschyi* therefore has an LD50 greater than 5000mg/kg B.W. Under this classification system, the aqueous trunk bark extract of *Pseudocedrela kotschyi* is classified in Category 5, a category of relatively low acute toxicity.

This LD50 is identical to that obtained by Essiet *et al.* (2016) with the ethanolic extract from the leaves of *Pseudocedrela kotschyi*. It is, however, higher than that determined by Daniel (2018). The latter determined an LD50 of 1225mg/kg B.W with the aqueous extract of stem bark of *Pseudocedrela kotschyi.*

The acute toxicity study of the aqueous extract of *Pseudocedrela kotschyi* intraperitoneally calculated LD50 values in mice. This is 230,08mg/kg B.C and 240mg/kg B.W respectively by the graphical method and the calculation method. According to the classification of Diezi (1989), a pharmacological substance with an LD50 value of less than 5mg/kg B.W is said to be very toxic. Those with an LD50 between 5 and 50mg/kg B.W are extremely toxic, those with an LD50 in the range of 50 and 500mg/kg B.W are considered toxic.

The substance with an LD50 between 500 and 5000mg/kg B.W is of low toxicity and the substance with an LD50 greater than 5000 is non-toxic. According to this classification, the aqueous extract of *Pseudocedrela kotschyi* administered intraperitoneally is toxic.

This result is different from that of Akuodor *et al.* (2013) who determined an LD50 of 775mg/kg B.W intraperitoneally of aqueous extracts of *Pseudocedrela kotschyi.*

This toxicity of the aqueous extract of *Pseudocedrela kotschyi* determined intraperitoneally is similar to that of the aqueous extract of the stem bark of *Ximenia americana*, *Tamarindus indica* which have respective LD50 values of 237.5mg/kg B.W by Soro *et al.* (2009) and 377±27mg/kg B.W by Souza (2005).

This difference in toxicity results would be due either to the mode of administration, to the type of solvent or to the geographical location from which the sample comes. This plant therefore deserves to be used with caution with humans.

The evaluation of the analgesic power of the aqueous extract of stem bark of *Pseudocedrela kotschyi* by the abdominal cramping method (torsion test) revealed that they possess analgesic power. This analgesic power is superior to that of aceclofenac.

Generally, acetic acid test is recommended for preliminary assessment of anti-nociceptive activity (Ganeshpurkar and Rai, 2013). It can demonstrate analgesic effects of low intensities, but this analgesic potential is non-specific (Vogel et Vogel, 1997).

It is not possible to indicate whether this potential results from peripheral or central action (Asongalem *et al.*, 2004). As a result, it is only useful for an initial selection of substances with analgesic action (Le Bars *et al.*, 2001).

The central analgesic activity of *Pseudocedrela kotschyi* extracts was evaluated by testing their effect on heat-induced pain focused on the tail of rats (Tail-flick test). It is recognized that thermal stimuli are selectively inhibited by central analgesics and not by peripheral analgesics (Sayyah *et al.*, 2004).

Morphine, which is a reference central analgesic, caused at different doses a decrease in heat-induced pain focused on the tail of rats, however, aqueous extracts of *Pseudocedrela kotschyi* had no effect on heat induced pain focused on the tail.

The aqueous extract of *Pseudocedrela kotschyi* therefore has a peripheral analgesic effect.

In order to confirm these results, a comparative study of the effects of morphine, aceclofenac and the aqueous extract of *Pseudocedrela kotschyi* on formaldehyde-induced pain was carried out. In this test exist two phases of pain (Deuis *et al.,* 2017). In the second phase many hyperalgesic and inflammatory mediators like prostaglandins, serotonin and histamine are released (Mbiantcha *et al.,* 2011).

The aqueous extract of *Pseudocedrela kotschyi* only reduced pain during the second phase unlike morphine which reduced pain during both phases. Only central analgesics inhibit formaldehyde-induced pain during both phases (Vasudevan *et al.*, 2007).

The aqueous extract of *Pseudocedrela kotschyi* therefore has a peripheral analgesic effect. Medicinal plants are widely used worldwide by populations and are a source of new active components, especially treating pain and inflammatory processes (Calixto *et al.*, 2004). The analgesic activity of the aqueous extract of bark from the trunk of *Pseudocedrela kotschyi* would therefore be related to the presence of some of its active components.

Some of these compounds such as phenolic compounds, saponosides, flavonoids and alkaloids are indeed known to have analgesic properties in other medicinal plants (Shchérazade *et al.,* 2021).

The ability of the aqueous trunk bark extract of *Pseudocedrela kotschyi* to reduce the number of abdominal cramps induced by acetic acid suggests that it inhibits the activity of lipooxygenases (LOX) or cyclooxygenases (COX), the production of prostanoids (PGE2, PGF2α) and inflammatory mediators (Ojewole, 2006; Chavan *et al.*, 2010).
Indeed, abdominal contractions caused by intraperitoneal injection of acetic acid in rats are due to the production and release of algogenic mediators via cyclooxygenases and prostaglandin biosynthesis (Karrat *et al.,* 2022).

These released mediators sensitize peritoneal cholinergic and histamine nociceptors and induce increased vascular permeability. This results in a painful sensation (Frederico *et al.*, 2009; Wantana *et al.*, 2009).

The aqueous extract of *Pseudocedrela kotschyi* may have the same mechanism of action as aceclofenac which is a peripheral analgesic.

Conclusion

The results of this study show that the aqueous extract of bark from the trunk of *Pseudocedrela kotschyi* has analgesic properties. These analgesic properties are probably due to the presence of phytochemicals such as alkaloids, flavonoids, phenolic compounds and saponosides highlighted in this aqueous extract.

Acknowledgement

Authors wish to thank to people of Kapreme and National Floristic Center (CNF) of the University Felix Houphouët-Boigny for providing valuable information about plant and its identification.

References

Adeniyi CBA, Odumosu BT, Aiyelaagbe OO, Kolude B. 2010. *In-vitro* Antimicrobial Activities of Methanol Extracts of *Zanthoxylum xanthoxyloides* and *Pseudocedrela kotschyi*. Africa Journal Biomedecine **13,** 61-68. https://hdl.handle.net

Akuodor GC, Essien AD, Essiet GA, David-Oku JL, Akpan FV. 2013. Evaluation of Antipyretic Potential of *Pseudocedrela kotschyi* Schweint. Harms (Meliaceae). European Journal of Medicinal Plants **3(1)**, 105-113. https://doi.org/10.9734/ EJMP/ 2013/

Alhassan AM, Ahmed QU, Malami I, Zakaria ZA. 2021. *Pseudocedrela kotschyi*: A review of ethnomedicinal uses, pharmacology and phytochemistry. Pharmaceutical Biology **59(1),** 953-961.

Alhassan MA, Ibrahim M, Musa IA. 2014. Phytochemical screening and antimicrobial evaluation of stem bark of *Pseudcedrela kotschyi* (Schweinf.) Herms. British Journal of pharmaceutical Research **4,**1934-1944.

Asongalem EA, Foyet HS, Folefoc GN, Dimo T, Ngongang J, Kamtchouing P. 2004. Analgesic and anti-inflammatory activities of *Erigeron floribundus*. Journal of Ethnopharmacology **91(2-3),** 301-308.

Atchou K, Dakey KA, Etroubeka-Tsono F, Agban A, Karou SD, Ameyapoh Y., De Souza C, Gbeassor M. 2021. The effect of *Pseudocedrela kotschyi* (Schweinf.) Harms extracts against *Staphylococcus aureus* growth. Bacterial Empire **4(1),** 11-15. https://doi.org/10.36547/be.2021.4.1.11-15.

Azzazi MF, Shahin, SI, Nofal AM, Abd El. Rahman AF. 2015. A Preliminary Study on Antimicrobial Activities of Essential Oils and Egyptian Pollen Extract Against some phytopathogenic Fungi and Bacteria. Egyptian Journal of Botany **2**, 363-374.

Balekundri A, Mannur V. 2020.Controle de la qualité des herbes traditionnelles et des produits à base de plantes: une revue. Future Journal of Pharmaceutical Sciences, **6(2020),** 67.

Bekro YA, Bekro JAM, Boua BB, Tra BFH, Ehile EE. 2007. Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) (Caesalpiniaceae). Revue. Sciences &. Nature **4(2),** 217-225. DOI: 10.4314/scinat.v4i2.42146

Burke A, Smyth E, Fitzgerald GA. 2006. Analgesic-antipyretic Agents; Pharmacotherapy of Gout. Goodman and Gilman's the Pharmacological Basis of Therapeutics, 11th ed. McGraw-Hill, New York, pp. 671-716.

Calixto JB, Campos MM, Otuki MF, Santos AR. 2004. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Medica **70,** 93-103.

Chavan MJ, Wakte PS, Shinde DB. 2010. Analgesic and antiinflammatory activity of Caryophyllene oxide from *Annona squamosa* L. bark. Phytomedicine **17,** 149-51.

Collier HOJ, Dinneen L C, Johnson CA, Scheider C. 1968. The abdominal contraction response and its suppression by antinociceptive drug in the mouse. British Journal of Pharmacology and Chemotherapy **32(2),** 295-310.

D'amour FE, Smith DL. 1941. A method for determining loss of pain sensation. Journal of pharmacology and Experimental therapeutics **72(1),** 74-79.

Daniel A. 2018. Effects of *Pseudocedrela kotschyi* stem bark on blood chemistry and histology of some organs in rats. Journal of Clinical and experimental toxicology **2(1),** 25-31.

Deuis JR, Dvorakova LS, Vetter I. 2017. Methods Used to Evaluate Pain Behaviors in Rodents. Frontiers in Molecular Neuroscience **10,** 284. DOI: 10.3389/fnmol.2017.00284.

Diezi J. 1989. Toxicologie: principes de base et répercussions cliniques. In: Pharmacologie: des principes fondamentaux aux applications thérapeutiques. Ed Slatkine, Genèvre, PP.33-34.

Dragsted A, Lang B. 1957. Etude de la toxicité par administration unique d'un nouveau médicament. Annales Pharmaceutiques Françaises 11.

Dubuisson D, Dennis SG. 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4(2), 161-174. DOI: 10.1016/0304-3959(77)90130-0.

Elufioye TO, Abdul AA, Moody JO. 2017. Cytotoxicity Studies of the extracts, fractions, and isolated Compound of *Pseudocedrela kotschyi* on Cervical Cancer (HeLa), Breast Cancer (MCF-7) and Skeletal Muscle Cancer (RD) Cells. Pharmacognosy Research **9(1),** 46-50. DOI: 10.4103/0974-8490.199776.

Erinoso SM, Fawibe OO, Oyelakin AS, Ajiboye AA, Agboola DA. 2016. Herbal recipes used for the traditional management of infantile dermatitis in Odeda, southwestern Nigeria. African Journal of Traditional, Complementary and Alternative Medicines **13(3),** 33-43. http://dx.doi.org/10.4314.

Essiet GA, Akuodor GC, Aja DO, Megwas AU, Ekenjoku JA, Nworie EM. 2016. Antidiarrhoeal and antioxidant properties of ethanol leaf extract of *Pseudocedrela kotschyi*. Journal of Applied Pharmaceutical Science **6 (03),** 107-110.

Frederico AV, Higor FL, Elson AC. 2009. Evaluation of the antinociceptive and anti-inflammatory effects of the acetone extract from *Anacardium occidentale* L. Brazilian. Journal of Pharmaceutical Sciences **3(45),** 437-442.

Ganeshpurkar A, Rai G. 2013. Experimental evaluation of analgesic and anti-inflammatory potential of *Oyster mushroom* Pleurotus florida. Indian Journal of Pharmacology **45(1),** 66-70.

Gray WD, Osterberg AC, Scut JT. 1970. Mesurement of the analgesic efficacy and potency of pentaezocine by the D'amour and Smith method. Journal of Pharmacology and Experimental Therapeutics **172(1),** 154-162.

Ibrahim B, Sowemimo A, Rooyen AV, Venter MV. 2012. Anti-inflammatory, analgesic and antioxidant activities of *Cyathula prostrate* (Linn.) Blume (Amaranthaceae). Journal of Ethnopharmacology **141,** 282-289.

Ito A, Munakata K, Imazu Y, Watanabe K. 2012. First nationwide attitude survey of Japanese physicians on the use of traditional Japanese medicine (Kampo) in cancer treatment. Evidence-Based Complementary and Alternative Medicine *2012***(13),** 1-8.

Kantati YT, Kodjo KM, Dogbeavou KS, Vaudry D, Leprince J, Gbeassor M. 2016. Ethnopharmacological survey of plant species used in folk medicine against central nervous system disorders in Togo. Journal of Pharmacology **181,** 214-220.

Karrat L, Abajy MY, Nayal R. 2022. Investigating the anti-inflammatory and analgesic properties of leaves ethanolic extracts of *Cedruslibani* and *Pinusbrutia*. Heliyon **8(4),** e09254.

Kayode J, Sanni P. 2016. Survey of barks used for medicine in the central zone of Lagos State. Nigeria. Journal of Botanical Papers **1(1),** 1-7.

Koster R, Anderson M, De-Beer EJ. 1959. Acetic acid analgesic screen. Federation proceedings **18,** 412-417.

Le Bars D, Gozariu M, Cadden SW. 2001. Animal models of nociception. Pharmacological Reviews **53(4),** 597-652.

Mambou CS, Nono RN, Chouna JR, Nkeng-Efouet-Alango P, Sewald N. 2018. Antibacterial secotirucallane triterpenes from the stem bark of *Pseudocedrela kotschyi*. Zeitschrift für Naturforschung C **73(5-6),** 241-246.

Mbiantcha M, Kamanyi A, Teponno RB, Tapondjou AL, Watcho P, Nguelefack TB. 2011. Analgesic and Anti-Inflammatory Properties of Extracts from the Bulbils of *Dioscorea bulbifera* L. var sativa (Dioscoreaceae) in Mice and Rats. Evidence-Based Complement Alternative Medicine **2011,** 912935. DOI: 10.1155/2011/912935.

Miller LC, Tainterml. 1944. Estimation of LD50 and its error by mean of logarithmic. Probit paper. Proceedings of the Society for Experimental Biology and Medecine **57,** 261-264.

Mishra D, Ghosh G, Kumar PS, Panda PK. 2011. An experimental study of analgesic activity of selective Cox-2 inhibitor with conventional NSAIDs. Asian Journal of Pharmaceutical and Clinical Research **4 (1),** 0974–2441.

Nguelefack TB, Dutra RC, Paszcuk AF, Andrade EF, Tapondjou LA, Calixto JB. 2010. Antinociceptive activities of the methanol extract of the bulbs of Dioscorea bulbifera L.Var sativa in mice is dependent of NO-cGMP-ATP-sensitive-k+ channel activation. Journal of Ethnopharmacology **128,** 567-74.

OECD. 2001. Test guidline 423, OECD guidline for chemicals. Acute Oral Toxicity-Acute Toxic class Method 14 p.

Ojewole JAO. 2006. Antinociceptiv, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* fisch. and C.A. Mey. (Hypoxidaceae) corn (African potato) aqueous extract in mice and rats. Journal of Ethnopharmacology **103,** 126-34.

Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, Keefe FJ, Mogil JS, Ringkamp M, Sluka KA, Song XJ, Stevens B, Sullivan MD, Tutelman PR, Ushida T, Vader K. 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain **161(9),** 1976-1982.

Ratnesh KR. 2019. Innovation of herbals and traditional medicine: global challenges and opportunity for India**.** Indian Journal of Agriculture and Allied Sciences **5(4),** 87-112.

Saidu IN, Umar KS, Isa MH. 2015. Ethnobotanical survey of anticancer plants in Askira/Uba local government area of Borno State, Nigeria. African Journal of Pharmacy and Pharmacology **9(5),** 123-130.

Salihu Shinkafi T, Bello L, Wara Hassan S, Ali S. 2015. An ethnobotanical survey of antidiabetic plants used by Hausa-Fulani tribes in Sokoto, Northwest Nigeria. Journal of Ethnopharmacology **172(1),** 91-99. DOI: 10.1016/j.jep.2015.06.014.

Sayyah MA, Hadidi NB, Kamalinejad MB. 2004. Analgesic and anti-inflammatory activity of *Lactuca sativa* seed extract in rats. Journal of Ethnopharmacology 92325-329.

Shchérazade OS, Pétronille AZ, Joseph F, Georges A. 2021. Study of the analgesic effect of the aqueous extract of the leaves of *Citrus aurantifolia* (Rutaceae) in mice. GSC Biological and Pharmaceutical Sciences **14(03),** 207-214.

Soro TY, Traore F, Sakande J. 2009. Activité analgésique de l'extrait aqueux de *Ximenia americana* (Linné) (Olacaceae). Comptes Rendus Biologies **332(4),** 371-377.

Souza. 2005. Contribution à la mise en évidence des mécanismes d'action pharmaco-physiologiques de ABS, une recette médicale anti-hémorroïdaire de la pharmacopée africaine, sur le système cardiovasculaire et le muscle intestinal lisse de mammifères. Thèse de Physiologie Animale, Université d'Abidjan Cocody, Côte d'Ivoire, 154p.

Thomford NE, Dzobo K, Chopera D, Wonkam A, Skelton M, Blackhurst D, Chirikure S, Dandara C. 2015. Pharmacognogenomics Implications of Using Herbal Medinal Plants on African Populations in Health Trasition. Pharmaceuticals **8(3),** 637-663.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. 1992. The formalin test: an evaluation of the method. Pain **51(1),** 5-17.

Traore MC. 2006. Etude de la phytochimie et des activités biologiques de quelques plantes utilisées dans le traitement traditionnel de la dysménorrhée au mali. Thèse de Pharmacie, Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie, Université de Bamako, Mali 176p.

Vasudevan M, Gunnam KK, Parle M. 2007. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract, Journal of. Ethnopharmacology **109,** 264-270.

Vogel GH, Vogel WH. 1997 Analgesic, anti-inflammatory and antipyretic activities. In Drug discovery and evaluation. Pharmacological assays, Berlin (Allemagne) **1,** 360-418.

Wallace JL, Vong L. 2008. NSAID-induced gastrointestinal damage and the design of GI-sparing NSAIDs. Current Opinion in Investigational Drugs **9,** 1151-1156.

Wantana R, Tassanee N, Subhadhirasakul S. 2009. Antinociceptive, antipyretic, and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. Journal Natural Medicines, **63(6)**, 290-2966.