https://doi.org/10.33472/AFJBS.2.3.2020.72-80 ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Evaluation of anti-inflammatory and healing activities of dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum*

Kassim Dosso[1*], Dago Serge David Attemene[2], Abiba Ouattara Gboko[3], Benoit Banga N'guessan[4] and Angoue Paul Yapo[5]

[1]Laboratory of Physiology and Pharmacology, Faculty of Environment, University of Jean Lorougnon Guede of Daloa, 02 P.O. Box 150 Daloa 02, Côte d'Ivoire. E-mail: kassdoss02@yahoo.fr

[2]Laboratory of Physiology and Pharmacology, Faculty of Environment, University of Jean Lorougnon Guede of Daloa, 02 P.O. Box 150 Daloa 02, Côte d'Ivoire. E-mail: sergedavid@hotmail.com

[3]Laboratory of Biotechnology in Agro – Resources Valorization and Natural Substances, Department of Biological Sciences, University of Korhogo, P.O BOX 1328 Korhogo, Korhogo, Côte d'Ivoire. E-mail: gbokoabiba@yahoo.fr

[4]Department of Pharmacology and Toxicology, School of Pharmacy, College of Health Sciences, University of Ghana, P.O Box KB 52, Korle-Bu, Accra, Ghana. E-mail: benoitnguessan@yahoo.fr

[5]Laboratory of Physiology and Pharmacology, UFR Sciences of Nature, University of Nangui Abrogoua, 01 P.O. Box 801 Abidjan 01, Côte d'Ivoire. E-mail: angoue_yapo@yahoo.fr

Article Info

Volume 2, Issue 3, July 2020
Received : 28 January 2020
Accepted : 17 May 2020
Published : 09 July 2020
doi: 10.33472/AFJBS.2.3.2020.72-80

Abstract

The objective of this study was to evaluate the anti-inflammatory activity of the dichloromethane fraction of an ethanol extract of *Piliostigma reticulatum,* a medicinal plant from Côte d'Ivoire and widely used by traditional healers against inflammatory diseases. Inflammation is characterized by redness, pain and swelling. Anti-inflammatory drugs are agents that reduce inflammation. *In vivo* studies of reduction of wound diameter and the test of carrageenan 1% were used for this work. For reducing wound diameter, fraction effects were tested in wound induced rats. The percentage of wound reduction was determined. Furthermore, fraction effect on acute inflammation induced by carrageenan 1% was studied. The dichloromethane fraction at a dose of 200 mg/kg body weight reduces significantly the wounds surfaces at 61.88% of inhibition at 15 day and 94.33% of inhibition at 30 day. This action was similar to the one provoked by betadine with a wound surface of 97.63% of inhibition at the thirtieth day. The dichloromethane fraction like the diclofenac reduced significantly the volume of edema from 0.7 ± 0.2 to 0.2 ± 0.1* ml (71.42% of inhibition) for 600 mg/kg body weight, the high dose. The study of acute toxicity showed that the dichloromethane fraction at high doses, could reduced mobility in mice and provoked death. The observation of anti-inflammatory and healing activities suggest that the use of *Piliostigma reticulatum* in traditional medicine to treat inflammatory diseases could be justified. Phytochemical screening showed that the most active fraction contains: flavonoids, tannins, reducing sugars and polyphenols.

Keywords: *Piliostigma reticulatum, Anti-inflammatory, Healing, Carrageenan, Côte d'Ivoire*

* *Corresponding author: Kassim Dosso, Laboratory of Physiology and Pharmacology, Faculty of Environment, University of Jean Lorougnon Guede of Daloa, 02 BP 150 Daloa 02, Côte d'Ivoire. E-mail: kassdoss02@yahoo.fr*

1. Introduction

Inflammation may happen in response to processes such as tissues injury, cell death, cancer, ischemia and degeneration (Azab *et al*., 2016). When there is injury to any part of human body, the arterioles in the encircling tissue dilate. There is a raised blood circulation towards the area (Burke *et al*., 2005; and Apu *et al*., 2012). These reasons are causing swelling and pain (Apu *et al*., 2012). It is also a biological response to protect the organism for elimination and to start the recovery (Middleton *et al*., 2000; and Etame Loé *et al*., 2018). Inflammation is in other way, an important source of radical oxygen produced directly by phagocyte cells (Etame Loé *et al*., 2018; and Luczkiewicz *et al*., 2001). The excess of radical in organism are the causes of certain diseases such as cancer, asthma, hepatic troubles and cardiovascular diseases (Ademiluyi and Oboh, 2008). The use of anti-inflammatory drugs provokes side effects. The study of plants anti-inflammatory plants followed by phytochemical screening to determine the constituents can be without side effects (Etame Loé *et al*., 2018). In the case, the study of the can necessary to have efficacies products without second effects.

Piliostigma reticulatum is traditionally used to treat many affections. According to Yelemou *et al*. (2007), this plant is used against diarrhea and tuberculosis. For Betti *et al*. (2011), *Piliostigma reticulatum* showed its efficacy against rheumatism. Betti and Mabere (2010) are reported that this plant had antidiarrheal and anti-fever activities. Also, Salawu *et al*. (2009) noticed an important utilization of *Piliostigma reticulatum* to treat wounds. Scientifically, previous studies of this plant showed its antidiarrheal activity (Dosso *et al*., 2012), anti-secretory activity (Dosso *et al*., 2012), anti-bacterial activity (N'guessan *et al*., 2015) and its toxicity (Dosso *et al*., 2014). The anti-inflammatory and healing activities of *Piliostigma reticulatum* can be necessary to scientifically confirm the activities of this plant against gastrointestinal disorders binding to inflammation diseases.

The aim of this current investigation was to determine the anti-inflammatory and healing activities of the dichloromethane fraction of a crude ethanol extract of the stem bark of *Piliostigma reticulatum* in rats.

2. Materials and methods

2.1. Plant collection

Stem barks of *Piliostigma reticulatum* (DC.) Horscht (Ceasalpiniaceae) were collected in Abidjan (South region of Côte d'Ivoire) in October 2018. The plant was identified and authenticated by the National Centre of Floristic of University of Cocody-Abidjan. A voucher specimen (N° 18033) of the plant was deposited in the herbarium of this centre.

2.2. Animal material

Swiss albinos mice (male and female) weighing between 20 and 30 g and the young adult albinos rats *Wistar* (weighing 170-220 g) of both sexes were used. These animals, provided by the faculty of Biosciences (University of Felix Houphouet Boigny, Côte d'Ivoire) were housed in standard metal cages. They were kept under standard laboratory temperature conditions one week before experiments for acclimation. Animals were fed with food pellets (Ivograin ®, Abidjan, Côte d'Ivoire) and were given water *ad libitum*. They were deprived from food for at least 24 h prior to experiments but allowed free access to drinking water (anti-inflammatory activity). The equipment usage handling and sacrificing of the animals were performed in accordance with the European Council legislation 87 / 609 / EEC for the protection of experimental animals (Mitjans *et al*., 2008).

2.3. Preparation of dichloromethane fraction

Stem barks of *Piliostigma reticulatum* were washed with distilled water, cleaned, cut into small pieces and kept at room temperature for two weeks. Then they were ground into a fine powder. The powder (100 g) was extracted with two liters of ethanol (96%) / water (80:20) for 24 h under constant stirring (this operation was repeated twice). The extract was filtered twice through cotton wool, then through Whattman filter paper (N° 1). The filtrate was evaporated to dryness using a rotavapor (Buchi R110 / NKE6540 / 2) at 45 °C, and dried under reduced pressure. Percentage yield was found to be 14.2%.

After successive liquid–liquid fractionations, five fractions (heptane, dichloromethane, ethyl acetate, butanol and water) were obtained from the crude ethanol extract (Harborn, 1984; and Samsam-Shariat, 1992).

2.3.1. Effects of dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum in wounds induced rats

The solutions were been administrated by oral route. The dose of 200 mg / kg body weight of this fraction was used after obtaining the powder of plant. According to the protocol described by Sagliyan *et al*. (2010), a circular wound of 2 centimeter (cm) of diameter was realized on the thoracolumbar shoulder blade region of each rat anesthetized with ether. Three groups of five rats were put in individual cage. Groups received respectively normal saline (0.9%) at 10 ml / kg (group 1, control group), 2 ml of betadine (group 2, reference group) and 200 mg / kg body weight of the fraction (group 3, test group). The study (treatment and diameter measures of wounds) was realized each three days for 30 days. Measures were realized with a ruler to calculate wound surfaces. During experiment, animals received food and water and rat skin wounds were disinfected by alcohol 95 °C. The percentage of reduction of wounds was obtained by the following formula:

$$Percentage\ of\ reduction = \frac{Surface\ of\ initial\ wound - Surface\ of\ wound\ treated}{Surface\ of\ initial\ wound} \times 100$$

2.3.2. Effects of dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum on acute inflammation induced by carrageenan 1% in rats

Solutions were given by oral route. The solutions were been administrated by oral route by gavage. The method described by Elion Etou *et al*. (2014) and Charles *et al*. (2015), was used. Thus, 25 rats deprived of food and water 24 h before experimentation were divided into 5 groups of five rats. The group 1 and 2 received respectively NaCl 0.9% at 10 ml / kg (control group) and diclofenac at a dose of 5 mg / kg body weight (reference group). The groups 3, 4 and 5 received respectively the fraction at the doses of 200, 400 and 600 mg / kg body weight. One hour after treatment, the carrageenan (1%) was injected in the hind paw (foot pad) at 0.1 ml. The anti-inflammatory effect of the fraction was evaluated by measuring the volume of edema of the hind paw which received the carrageenan 1% carrageenan (1%) at 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h using a plethismometre Ugo Basile 7140 (Elion Etou *et al*., 2014).

2.3.3. Acute toxicity effects of dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum

Forty eight mice were divided into seven groups of six animals. The dichloromethane *fraction* was orally given at doses of 500; 800; 1,000; 2,000; 3,000; 5,000 and 7,000 mg / kg body weight, to animal groups (one dose per group). Simultaneously, the control group received 1 ml of normal saline (0.9%). General signs of weakness, symptoms of toxicity and mortality were noticed for a period of 48 h and then 14 days (Lorke, 1983). The LD_5, LD_{50} and LD_{95} of the fraction were calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

2.4. Phytochemical analysis of the dichloromethane fraction

The dichloromethane fraction was screened for the presence of tannins, flavonoids, alkaloids, sterols, saponins, polyphenols, polyterpenes and anthraquinones. Detection of these components was performed to the method of Bekro *et al*. (2007).

3. Data analysis

Graph Pad Prism Version 7.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany. Comparisons between means were done used ANOVA (one way) followed by Turkey's multiple comparison. $p < 0.05$ was considered statistically significant in all analysis.

4. Results

4.1. Effects of betadine and dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum in wounds induced rats

Table 1 shows the delay of healing of wounds healing for 30 days. In control group, wounds surfaces diminish slowly and pass of 263 ± 2 to 127 ± 11 mm^2 at day 15 with 57.71% as percentage of inhibition. At day 30, this percentage was 77.95%. Wound surfaces in rats treated with betadine were quickly and significantly reduced and 62.99% of inhibition at day 15 was observed. Also, this surface fell to 6 ± 4.9 mm^2 with 97.63% of inhibition at day 30. The fraction at a dose of 200 mg / kg body weight significantly reduced wound surfaces at 61.88% of inhibition at day 15. This surface remained 15 ± 5.1 mm^2 with 94.33% of inhibition at day 30.

Table 1: Effects of betadine and dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum* on the wounds induced in rats

Groups	Wound Surfaces (mm^2) and percentage of reduction										
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30
Control (Normal Saline)	263 ± 2	255 ± 1 (3.04%)	202 ± 5 (23.19%)	143 ± 0.3 (45.62%)	135 ± 4.2 (48.67%)	127 ± 11 (57.71%)	105 ± 3.5 (60.07%)	80 ± 5 (69.58%)	69 ± 3.5 (73.76%)	65 ± 9.1 (75.26%)	58 ± 4.5 (77.95%)
Reference (Betadine)	254 ± 3.6	189 ± 2.7 (25.59%)	167 ± 4.2 (34.25%)	143 ± 2.8 (43.7%)	134 ± 8.1 (47.24%)	94 ± 4.3* (62.99%)	67 ± 6.7* (73.62%)	54 ± 2.4* (78.74%)	36 ± 4.9** (85.82%)	15 ± 2.5*** (94.1%)	6 ± 4.9*** (97.63%)
Fraction (200 mg / kg)	265 ± 4.3	254 ± 7.2 (4.1%)	222 ± 6.7 (16.22%)	151 ± 4.6 (43.02%)	132 ± 3.8 (50.19%)	101 ± 9.1* (61.88%)	78 ± 3.6* (70.56%)	47 ± 5.5* (82.26%)	28 ± 4.7* (89.43%)	19 ± 4.8** (92.83%)	15 ± 5.1** (94.33%)

Note: Data are mean ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle treated group (one-way ANOVA followed by Turkey's multiple comparison test).

4.2. Effects of diclofenac and dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum on the volume of edema induced in rats by carrageenan 1%

The volume of edema in control group increased regularly from 0.3 ± 0.2 to 1.9 ± 0.1 ml the sixth hour. The percentage was zero. For the group treated by diclofenac, the volume of edema was significantly reduced from 0.4 ± 0.1 to 0.1 ± 0.1** ml with 75% of inhibition. The fraction significantly reduces the volume of edema respectively from 0.8 ± 0.3 to 0.7 ± 0.3 ml (12.5% of inhibition) for 200 mg / kg body weight, from 0.8 ± 0.4 to 0.5 ± 0.1ml (37,5% of inhibition) for 400 mg / kg body weight and from 0.7 ± 0.2 to 0.2 ± 0.1* ml (71.42% of inhibition) for 600 mg / kg body weight (Figure 1).

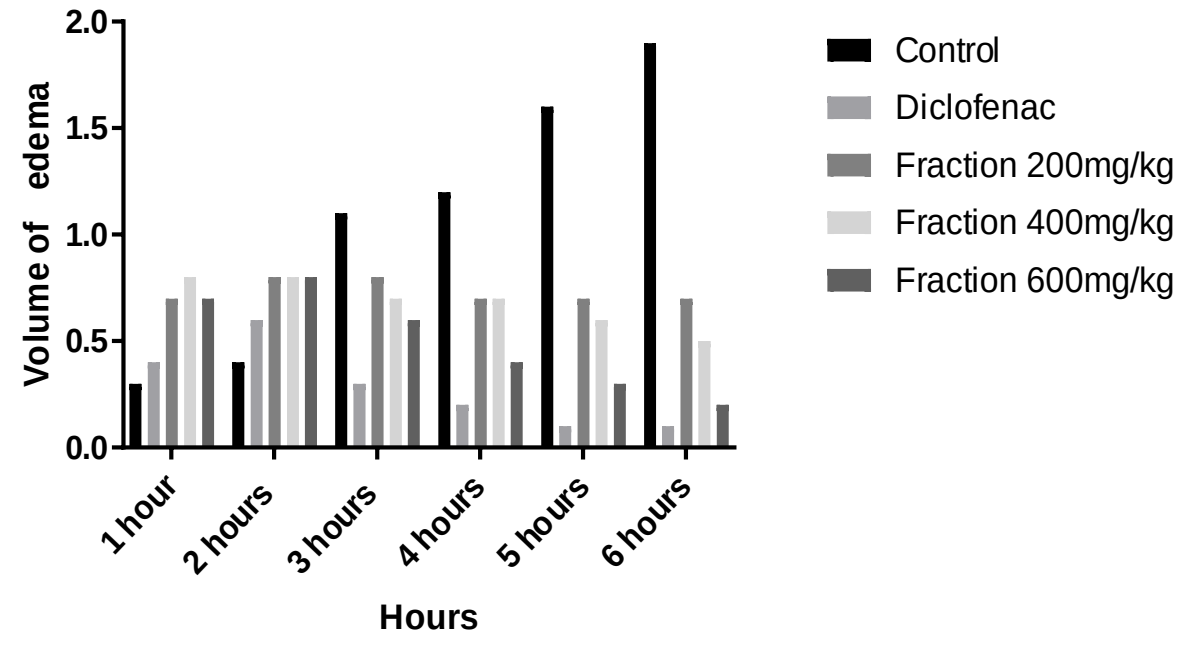

Figure 1: Effects of diclofenac and dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum* on the volume of edema induced in rats by carrageenan

Note: One-way ANOVA followed by a Turkey's multiple comparison test.

4.3. Acute toxicity effects of dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum

After treatment, mobility was observed in mice at a dose of 1,000 mg / kg body weight. However, death in animals intervened at a dose of 2,000 mg / kg body weight (Table 2). At a dose of 3,000 mg / kg body weight, 50%of death in mice was observed (Table 3). The DL50 was therefore 3,000 mg / kg body weight. The DL5 and the DL95 were respectively 1,217 mg / kg body weight and 6,543 mg / kg body weight. The quotient DL5 / DL95 was 0.18. This result is far from 1.

Table 2: Acute toxicity signs induced by fraction at different doses

Groups	Acute toxicity signs	
	Mobility	Death
Control	+	–
Fraction 500 mg / kg	+	–
Fraction 800 mg / kg	+	–
Fraction 1000 mg / kg	–	–
Fraction 2000 mg / kg	–	+
Fraction 3000 mg / kg	–	+
Fraction 5000 mg / kg	–	+

Table 3: Percentage of mortality in rats treated by fractions at different doses

Groups	Number of animals	Doses	Number of death	Mortality (%)
1	6	Control	0	0
2	6	Fraction 500 mg / kg	0	0
3	6	Fraction 800 mg / kg	0	0
4	6	Fraction 1000 mg / kg	0	0
5	6	Fraction 2000 mg / kg	2	33.33
6	6	Fraction 3000 mg / kg	3	50
7	6	Fraction 5000 mg / kg	5	83.33
8	6	Fraction 5000 mg / kg	6	100

4.4. Phytochemical analysis of dichloromethane fraction of an ethanol extract of stem bark of Piliostigma reticulatum

Phytochemical screening tests of dichloromethane fraction revealed the presence of major components such as tannins and flavonoids. Polyphenols and reducing sugars were present, and anthraquinones, alkaloids, coumarins, polyterpenes and sterols were absent (Table 4).

Table 4: Phytochemical screening of dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum*

Chemical components	Dichloromethane fraction
Anthraquinones	_
Alkaloids	_

Table 4 (Cont.)

Chemical constituents	Dichloromethane fraction
Polyphenols	+
Tannins	++
Coumarins	–
Reducing sugars	+
Polyterpenes	–
Flavonoids	++
Sterols	–
Note: (–) absent, (+) present, and (++) major chemical constituents	

5. Discussion

The healing properties were investigated using model of wounds skin. The surface of wound is a parameter often used to evaluate scar on skin (Suntar *et al*., 2011; and Ghashghaii *et al*., 2017). Scar is a complex phenomenon generates by the organism to reply to damage putting back the functions of tissues disturbed and can be sum up in three phases: phase of inflammation (0-3 days), phase of multiplication (3-12 days) and phase of reconstitution (3-6 months) (Priya *et al*., 2002; and Oliveira *et al*., 2016). Scar provokes also the formation of the fibrinogen and the integrin protein (Suriyamoorthy *et al*., 2014).

The dichloromethane fraction induced a significantly reduction of wounds surfaces. This healing action was intense in rats treated with the betadine used as a reference drug. Many healing medicines like the cicatrol arise from plants extracts. The healing effect of plants extracts could be attributed to the presence of flavonoids and tannins which have ability to accelerate the tissue regeneration (Priya *et al*., 2002; and Suriyamoorthy *et al*., 2014). The screening of the dichloromethane fraction showed a significant presence of flavonoids and tannins. These flavonoids and tannins could be responsible of healing activity observed in our study.

The results of this study showed that the dichloromethane fraction reduces significantly the volume of edema induced by carrageenan 1% in rats. Carrageenan is a mucopolysaccharide which inducing maximum edema three hour after following its injection (Elion Etou *et al*., 2014; and Charles *et al*., 2015). The molecular mechanism of carrageenan is the release of many mediators provoking inflammation (Ouedraogo *et al*., 2012).

The anti-inflammatory effects of the dichloromethane fraction were therefore illustrated by the diminution of the volume of edema. Previous studies showed the anti-inflammatory effects of some plant extracts like the essential oil of *Lippia multiflora* (Verbenaceae) by Abena *et al*. (2003); the extract of *Treculia africana* (Moraceae) by Ayoola *et al*. (2011) and the ethanol extract of stem bark of *Buchholzia coriacea* (Capparidaceae) by Charles *et al*. (2015). The anti-inflammatory effects are often attributed to phenolic components (Chika *et al*., 2012; Duru *et al*., 2013; and Enechi and Nwodo, 2014). Thus, flavonoids, tannins and phenols have anti-inflammatory properties (Mota *et al*., 1985; Capiralla *et al*., 2012; Quinone *et al*., 2013; and Alinejhad *et al*., 2016). Indeed, some components of flavonoids like quercetin and myricetin have a strong inhibition on the COX and LOX (Kim *et al*., 1993). Also, other flavonoids like flavonol and flavone have an anti-inflammatory activity (Lee *et al*., 1993). The presence of tannins, flavonoids and polyphenols during the phytochemical screening in fraction could be responsible of its anti-inflammatory effects.

The acute toxicity study of dichloromethane fraction showed that the LD_{50} was 3,000 mg / kg body weight. Animals showed toxicity signs in a dose-dependent manner. At a dose of 7,000 mg / kg body weight there were no survivors. These results are consistent with those of Diallo and Diouf (2002), and Dosso *et al*. (2014). The quotient DL_5 / DL_{95} is far from 1. These results indicate that the use of this fraction is not safe in its therapeutic use with the understanding that the therapeutic dose is not distinct from its toxic dose (Tamboura *et al*., 2005). The active compounds observed in this fraction could be explained by the toxicity signs observed in rats. Thus, when in excess in the body, some chemical compounds may exceed their therapeutics activities; incite some malfunctions or lethal disorders (Lohoues *et al*., 2006). For instance, astringent tannins have a role in

reducing foods in animals (Agaie *et al*., 2007). This causes weakness in animals leading to immobility. Polyphenols are endowed with surfactant and hemolytic properties (Lohoues *et al*., 2006). Thus, according to the same authors, compounds that are swallowed or even inhaled, are known to cause among others, digestive tract burns, cyanosis, hypoxia and seizures. Schultz and Riggin (1985) stated in this regard that the phenol poisoning lead to death by acute respiratory failure. These explications explanations could have caused immobility and death of animals in our study.

6. Conclusion

Dichloromethane fraction of limit an ethanol extract of stem bar its k of *Piliostigma reticulatum* possesses anti-inflammatory and scaring activities. These results showed that the fraction contains biological active compounds such as tannins and flavonoids which according to scientific studies, have anti-inflammatory and healing properties. These results justify the use of *Piliostigma reticulatum* against inflammation diseases and wounds by traditional healers. However, bioassay-guided fractionation studies are necessary to highlight the active principles and their mechanism of action. Moreover, the use of aerial parts of the plant could be a solution than its roots, therefore the biodiversity degradation.

Acknowledgment

The authors are grateful to late Professor Ake ASSi Laurent of the National Centre of Floristic, University Felix Houphouet Boigny of Cocody, Abidjan for botanical identification of the plant. We are also thank to Professor Boua Boua Benson of Nangui Abrogoua University, Abidjan for his help in phytochemical screenings. We express our gratitude to the responsible of the laboratory of pharmacology of Felix Houphouet Boigny University.

References

Abena, A. A., Diatewa, M., Gakosso, G., Gbeassor, M., Hondi-Assah, T. H. and Ouamba, J. M. (2003). Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. *Fitoterapia*, 74, 231-236.

Ademiluyi, A.O. and Oboh, G. (2008). Antioxydant properties of methanol extracts of mistletoes (*Viscum album*) from cocoa and cashew tree in Nigeria. *African Journal of Biotechnology.* 7(17), 3138-3142.

Agaie, B. M., Onyeyili, P. A., Muhammad, B. Y. and Ladan, M. J. (2007). Acute toxicity effects of the aqueous leaf extract of *Anogeissus leiocarpus* in rats. *Afr. J. Biotech*. 6(7), 886-889.

Alinejhad, D., Asayesh, M. A. and Mariyam, A. (2016). Determination of the anti-inflammatory property of tannins from the rind of Calamansi (*Citrus microcarpa*, Rutaceae). *J Int Oral Health*, 8, 546-553.

Aliu, Y. O. and Nwude, N. (1982). Phytochemistry and preliminary toxicity studies of methanol extract of stem bark of Garnicia Kola. *Vet Pharmacol toxicol Exp*. 104-110.

Apu, A. S., Bhuyan, S. H., Prova, S. S. and Muhit, A. M. D. (2012). Anti-inflammatory activity of medicinal plants native to Bangladesh: A review. *J. Appl. Pharm. Sci*. 2(2), 7-10.

Ayoola, M. D., Gbolade, A. A., Ibitoye, S. F. and Moody, J. O. (2011). In vivi inflammaotory methanol extract of *Treculla Africana* and *Buchholzia coriaceae* Engl. *Journal Pharm. Sci*. 10, 27-31.

Azab, A., Nassar, A. and Azab, A.N. (2016). Anti-inflammatory activity of natural products. *Molecules,* 21, 1321.

Bekro, Y., Bekro, J., Boua, B. B., Tra Bi, F. and Ehile, E. E. (2007). Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend et Zarrucchi (Caesalpiniaceae). *Sci. Nat*. 4(2), 217-225.

Betti, J. L. and Mebere, S. R. Y. (2010). An ethnobotanical study of medicinal plants used in the Kalamaloué National Park, Cameroon. *J. Med. Plants Res*. 5(8), 1447-1458.

Betti, J. L., Mebere, S. R. Y. and Nchembi, T. F. (2011). Contribution to the knowledge of non-wood forest products of the far north region of Cameroon: Medicinal plants sold in the Kousséri market. *J. Ecol. Nat. Environ*. 3(7), 241-254.

Burke, A., Smyth, E. and FitzGerald, G. A. (2005). Analgesic-antipyretic agents; pharmacotherapy of gout. In Brunton L. B., Lazo J. S. and Parker K. L. (Eds.), Goodman and Gilman's: the pharmacological basis of therapeutics (2nd ed., pp. 617-715), New York: McGraw-Hill.

Capiralla, H., Vingtdeux, V., Zhao, H., Sankowski, R., Al-Abed, Y., Davies, P. and Marambaud, P. (2012). Resveratrol mitigates lipopolysaccharide and Abeta-mediated microglial inflammation by inhibiting the TLR4 / NF-kappaB / STAT signaling cascade. *J. Neurochem*. 120, 461-472.

Charles, E., Elion Itou, R. D. G., Etou Ossibi, A. W., Attibayeba Ongoka, P. R. and Abena, A. A. (2015). Effet anti-inflammatoire et cicatrisant des extraits aqueux et ethanolique des écorces du tronc de *Bucholzia coriacea* Engl. (Capparidaceae). *J. Appl. Biosci*. 94, 8858-8868.

Chika, E., Moses, I., Chigozie, U., Olisa, A., Ifeanyichkwu, I., Michael, A. and Charles, E. (2012). Evaluation of antibacterial activity of the leave extracts of *Buchholzia coriaceae*. *Sci. Pharm. Biol. Res*. 2(4), 204-208.

Diallo, B. and Diouf, A. (2002). Odonto-Stomatologie. *Trop*. 92, 5-11.

Dosso, K., N'guessan, B. B., Bidie, A. P., Gnangoran, B. N., Méité, S., N'guessan, D., Yapo, A. P. and Ehilé, E. E. (2012). Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigma reticulatum* (Caesalpiniaceae) in rats. *Afr. J. of Tradit Complement and Altern Med*. 9(2), 242-249.

Dosso, K., N'guessan, B. B., Amoateng, P. and Gnangoran, B. N. (2012). Anti-secretory effects of a dichloromethane fraction of stem bark of *Piliostigma reticulatum* (Cesalpiniaceae). *J. Med. Biomed. Sci*. 1(3), 13-20.

Dosso, K., N'guessan, B. B., Gnangoran, B. N. and Yapo, A. P. (2014). Acute toxicity effects of dichloromethane fraction of ethanol extract of stem bark of *Piliostigma reticulatum* on rats. *Afr. J. Plant Sci*. 8(8), 405-409.

Duru, M., Ugbogu, A., Amadi, B., Odika, P. O., Chima-Ezika, O., Anudike, J. and Osuocha, K. (2013). Chemical constituents of *Buchholzia coriacea* seed. *Acadmia Edu*. 70, 39-41.

Elion Itou, R. D. G., Sanogo, R., Nsonde Ntandou, F. G., Ondele, R., Peneme, B. M., Okiemy Andissa, N., Diallo, D., Ouamba, J. M. and Abena, A. A. (2014). Anti-inflammatory and analgesic effects of aqueous extract of stem bark of *Ceiba pentadra*. Gaertn. *Pharmacol. Pharm*. 5, 1113-1118.

Enechi, O. C. and Nwodo, O. F. C. (2014). Anti-ulcer and gastric, anti-secretory activities of seed extract of *Buchholzia coriacea* in *Wistar* albinos rats. *Afr. J. Biotech*. 13, 2755-2761.

Etame Loe, G. T., Ngaba, G. P., Kamdom, M., Mpondo Mpondo, E. and Dibong, S. D. (2018). Evaluation des activités anti-inflammatoire et antiradicalaire de l'extrait au vin de palme des feuilles de *Phragmanthera capitata* recoltées sur *Psidium guajava* au cameroun. *International J. Biol. Chem. Sci*. 12(1), 233-243.

Ghashghaii, A., Hashemnia, M., Nikousefat, Z., Zangeneh, M. M. and Zangeneh, A. (2017). Wound healing potential of methanolic extract of *Scrophularia striata* in rats. *Pharm. Sci.* 23, 256-263.

Harborn, J. B. (1984). Phytochemical methods In: a guide to modern techniques of plant analysis (2nd ed., pp. 4-7). London: Chapman and Hall.

Kim, H. K., Namgoong, S. Y. and Kim, H. P. (1993). Anti-inflammatory activity of flavonoids: Mouse ear edema inhibition. *Arch. Pharm. Res*. 16, 18-24.

Lee, S. J., Kun, H. S., Hyeun, W. C., Lae, C. D., Keun, Y. J., Sam, S. K. and Hyun, P. K. (1993). Anti-inflammatory activity of naturally occurring flavone and flavonol glycosides. *Arch. Pharm. Res*. 16(1), 25-28.

Lohoues, E. E., Tiarou, G. G., Monde, A. A., Djinhi, J. and Sess, E. D. (2006). Proriétés toxicologiques aigues du latex de *Calotropis procera* chez le rat. *Pharm. Med. Trad. Afr*. 14, 187-199.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol*. 54, 275-287.

Luczkiewicz, M., Cisowski, W., Kaiser, P., Ochocki, R. and PIOtrowski, A. (2001). Comparative analysis of phenolic acids in mistletoe plants from various hosts. *Acta Pol. Pharm*. 58, 373-379.

Middleton, E., Kandaswami, C. and Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol. Rev*. 52, 673-839.

Mitjans, M., Garcia, L., Marrero, E. and Vinardell, M. P. (2008). Study of ligmed-A, an antidiarrheal drug based on liguin, on rat small intestine enzyme activity and morphometry. *J. Vet. Pharmacol. Ther*. 24, 349-351.

Mota, M. L., Thomas, G. and Barbosa Filho, J. L. (1985). Anti-inflammatory actions of tannins isolated from the bark of *Anacardium occidentale* L. *J. Ethnopharmacol*. 13(3), 289-300.

N'guessan, B. B., Dosso, K., Gnangoran, B. N., Amoateng, B., Asiedu-Gyekye, I. J. and Yapo, A. P. (2015). Antibacterial and antispasmodic activities of a dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum*. *J. Pharm. BioAll. Sci*. 7(2), 128-135.

Oliveira Gonzalez, A. C., Costa, T. F., Arojo Andrade, Z. and Piexoto Modrado, A. R. A. (2016). Wound healing: A literature review. *An Brass Dermatol*. 91(5), 614-620.

Ouedraogo, N., Lompo, M., Sawadogo, R. W., Tibiri, A., Hay, A. E., Koudou, J., Dijoux, M. C. and Guissou, I. P. (2012). Etude des activités anti-inflammatoire, analgesique et antipyrétique des décoctés aqueux des feuilles et des raciness de *Pterocarpus erinaceus* Poir. (Fabaceae). *Phytother.* 10, 286-292.

Priya, K. S., Gnanamani, A., Radhakrishman, N. and Babu, M. (2002). Healing potential of *Datura alba* on burn wounds in albino rats. *Ethnopharmacol*. 83, 193-199.

Quinone, M., Miguel, M. and Aleixandre, A. (2013). Benificial effects of polyphenols on cardiovascular disease. *Pharmacol. Res*. 68(1), 125-131.

Sagliyan, A., Ceribasi, A. O., Gunay, C., Han, M. C., Benzer, F. and Kandemir, M. F. (2010). Effects of dietary supplementation with whey proteins on surgical wound healing in rats. *Rev. Med*. 161(10), 455-462.

Salawu, O. A., Tijani, A. Y., Obidike, I. C., Rafindadi, H.A. and Emeje, M. (2009). Anti-ulcerogenic properties of methanolic root extract of *Piliostigma reticulatum* (DC) Hoechst (Syn. Bauhinia reticulate DC) Leguminosae in rats. *Afr. J. Pharm. Pharmacol.* 3(5), 252-258.

Samsam-Shariat, S. H. (1992). Qualitative and quantitative evaluation of the active constituents and control methods for medical plants (3rd ed., pp. 23-30). Mani Publications. Isfahan (in Persian).

Schultz, T. W. and Riggin, G. W. (1985). Predictive correlations for the toxicity of alkyl and halogen substituted phenols. *Toxicol. Lett*. 25, 47-54.

Suntar, I., Baldemir, A., Coskun, N., Keles, H. and Akkol, E. K. (2011). Wound healing acceleration effect of endemic ononis species growing in Turkey. *Ethnopharmacol*. 135, 63-70.

Suriyamoorthy, S., Subramaniam, K., Raj-Durai, S.J., Wahaab, F. and Chitraselvi, R. P. E. (2014). Evaluation of wound healing acitivity of *Acacia caesia* in rats. *Wound Med*. 7, 1-7.

Tamboura, H. H., Bayala, B., Lompo, M., Guissou, P. and Sawadogo, L. (2005). Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of Holarrhena floribunda (G. Don) Durand & Schinz, Leptadenia hastata (Pers.) Decne and Cassia sieberiana (DC) used by veterinary healers in Burkina Faso, *Afr. J. Tradit Complement Altern. Med*. 2(1), 13-24.

Yelemou, B., Bationo, B., Yameogo, G. and Millogo-Rasolodimby, J. (2007). Woods and forests of the tropics. *Coden*. 291, 55-66.

Cite this article as: Kassim Dosso, Dago Serge David Attemene, Abiba Ouattara Gboko, Benoit Banga N'guessan and Angoue Paul Yapo (2020). Evaluation of anti-inflammatory and healing activities of dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum*. *African Journal of Biological Sciences.* 2(3), 72-80.