In Vivo and in Vitro Anti-Inflammatory Activities of Extracts of Pandiaka angustifolia (Vahl.) Hepper (Amaranthaceae) Used in Traditional Medicine in Burkina Faso

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Abstract

Background: Pandiaka Angustifolia Valh Hepper (Amaranthaceae) whole plant is used in folk Burkina’s medicine to treat ailments with an inflammatory component. Previous studies revealed the antioxidant capacity, xanthine oxidase, and lipoxygenase inhibitory activities of the plant, but to the best of our knowledge, its anti-inflammatory activities were not reported before. Therefore, this study was designed to evaluate the anti-inflammatory and analgesic activity of P. angustifolia hexane and aqueous extracts using in vitro enzymatic methods and in vivo methods and verify the best anti-inflammatory extract implication in KATP pathways. Experiments: acute toxicity of the plant was conducted under OECD 423 guidelines. Phospholipase and cyclooxygenases were pro-inflammatory enzymes used to evaluate in vitro anti-inflammatory effects of plant extracts while carrageenan induced edema method was used to evaluate the anti-edematous activity and acetic acid inducing writhing method to evaluate the non-morphine analgesic effect of herbal mixture. ATP sensitive K\textsuperscript+ channel assay was performed in vivo using the glibenclamide as ATP-sensitive potassium channel (K\textsubscript{ATP}) blocker. Results: enzymatic inhibition assays revealed that both hexane and aqueous extracts of P. angustifolia were good inhibitors against sPLA\textsubscript{2} activity with IC\textsubscript{50} values of 14.23 ± 0.72 µg/mL and 11.56 ± 0.11 µg/mL, respectively. Aqueous extract presented the best inhibition for COX-1 (IC\textsubscript{50} = 24.76±0.51 µg/mL) while hexane extract concentration that inhibit 50% of COX-2 was lesser than that of aqueous extract. P. angustifolia aqueous extract orally administrated to NMRI mice caused no death at the dose of 3000 mg/kg b.w indicating that the plant toxicity is low. While hexane extract was unable to reduce Carrageenan-induced edema, ethanolic extract were significantly active when extract was orally administrated. Non-morphine analgesic activity evaluation revealed that ethanolic extract was more efficient on writhing reduction than hexane extract. Nociception effect of the plant is linked with its effects on K\textsuperscript+ ATP sensitive channels. Conclusion: Results indicate that the anti-inflammatory potential of P. angustifolia may be due to its polar phytoconstituents and observed pharmacological activities provide the scientific basis for the medicinal use of the plant in the treatment of ailment associated with inflammation.

Keywords: Pandiaka angustifolia; Enzymatic activity; Anti-inflammatory activity; Analgesic activity.

1. Introduction

Human beings are constantly exposed to noxious and pathogens, that are responsible of different ailments [1, 2]. Inflammation, a biological response of vascularized tissues to injuries (infections and damaged tissues) is characterized by increased blood flow to the tissue causing increased temperature, redness, swelling and pain. It is considered as a secret killer and has been linked with diseases like asthma, heart attacks, cancers, Alzheimer’s and other diseases [3].

The inflammatory reaction is a succession of coordinated biological events that aims to circumcise and eliminate pathogens and noxious that injure every single component of the body [4]. It implicates several mediators and effectors sourced from inflammatory enzymatic activation [5]. Phospholipase (sPLA\textsubscript{2}) and cyclooxygenases (COX-1 and COX-2) are among the majors inflammatory enzymes that generate respectively arachidonic acid (A.A.) through sPAL\textsubscript{2} activation and prostaglandins (PGs) generated through COXs activity [6]. Mediators of inflammation like PGs are implicated in the apparition of cardinal signs of inflammation and they have a direct link with the opening of

[Image 38x733 to 564x766]

[Image 442x563 to 476x575]

[Image 477x793 to 506x824]
2. Materials and Methods

2.1. Plant Material

The whole plant of *P. angustifolia* was harvested in January 2016 from natural habitat at Yaagma catholic sanctuary (30 km northern periphery of Ouagadougou, Burkina Faso). Botanical identity was assessed in the laboratory of biology and vegetal ecology (University Joseph KI-ZERBO, Ouagadougou, Burkina Faso) and a voucher specimen (CI: 16889) was deposited. Plant material was dried at room temperature, pulverized and defatted with petroleum ether in the proportion 1:10 (w/v) and residual marc were conserved for future uses.

2.2. Chemicals

Chemical were from analytical grade. Different solvents, acetylsalicylic acid, carrageenan, dimethylsulfoxide (DMSO), hydrocortisone, quercetin, acetaminophen, acetic acid, sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, Carrageenan and acetylsalicylic acid were supplied by Sanofi Winthrop Industry (France). 1,2-dihexanoylthio-glycerophosphocholine (1,2dHGPC), secretory phospholipase A2 (sPLA2) from bee venom and 5,5'-dithiobis-2- nitrobenzoic acid (DTNB) were obtained from Cayman Chemical Co. (MI, USA). For the colorimetric inhibition of COX-1 and human COX-2, Screening Kit (Item No. 560131) manufactured by Cayman Chemical, USA was used.

2.3. Experimental Animals and Housing

Naval medical research institute (NMRI) female mice (7 to 8 weeks old, 25 to 35 g body weight) provided by the animal housing facility of the University Joseph KI-ZERBO, were used. Mice were kept in an environmentally controlled breeding room (20-25 °C, humidity, 12 h photoperiod), fed with standard laboratory food and water *ad libitum*. Mice were fasted 17 h before experiments. Animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Burkina Faso (Ethics committee acceptance CE-UOI-2018-05) and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC) [12]. All evaluations were performed between 9 a.m. and 16 p.m.

3. Experimental Methods

3.1. Preparation and Extraction of Plant Material

The extraction consisted of steeping a mass 50 g of defatted plant material in 500 mL of hexane. After mechanical stirring for 48 hours, the mixture was filtered using Whatman N°1 filter paper and concentrated in a rotatory evaporator (Buchi Rotavapor RE-3, Buchi Labortechnic AG, Switzerland) at an approximative temperature of 35 °C, concentrated filtrate was stored at 4°C until further use, while the residual marc was reused after a drying period of 8 h for a new extraction using water as solvent.

3.2. Enzymatic Inhibition

3.2.1. Phospholipase A2 (sPLA2) Inhibition Assay

sPLA2 inhibition activity was assayed using the method described by D’Almeida, et al. [13], with slight modifications. Briefly, test samples (enzyme activity in presence of an inhibitor) were prepared after mixing 50 µL of buffer Tris-HCl (10 mM, pH 8) with 10 µL of enzyme sPLA2 (1µg/mL) and extracts of plant dissolved in DMSO was used at different concentration to determine the concentration that inhibit 50 % of enzyme activity, while positive control sample (enzyme activity without inhibitor) was prepared using the same reagent except plant extract which was replaced by hexane or water . The reaction was initiated by the addition of 150 µL of 1,2-dihexanoylthioglycerophosphocholine (1,2-dHGPC) (1.66 Mm). After 15 min of incubation in a 96 wells microplate, 10 µL of DTNB (10 mM) were added then the mixture absorbance was recorded using a microplate reader (Epoch, BioTeck
Involvement of ATP-Sensitive K+ Channel Pathway

The non-morphine analgesic activity involving the K⁺ channels was evaluated using the procedure described by Perimal [18], with slight modifications. Four groups of 7 mice were divided as follows: the control group receives only the vehicle (saline), the second group receives glibenclamide (an ATP sensitive K⁺ channel inhibitor) and the last two groups receive respectively the hexane and aqueous extracts at a dose of 400 mg / kg of body weight. All mice except the first were pretreated with glibenclamide (10 mg / kg) 15 min before administration of saline or both extracts. Mice were injected with acetic acid, 1 hour after treatment. Five minutes after the acetic acid injection the analgesic effect was evaluated according to the number of abdominal contortions induced by the intraperitoneal injection of acetic acid (0.6%) as described by Winter and Risley [15]. The anti-edematous activity was evaluated as a percentage of reduction of the edema in treated mice compared to negative control using the following formula:

\[
\text{inhibition} \% = \frac{A - B}{A} \times 100
\]

A represents the average difference paw edema volume in the negative control group and B represents the average difference of paw edema in treated group mice.

3.3.3. Non-morphine Analgesic Activity: Involvement of ATP-Sensitive K+ Channel Pathway

The non-morphine analgesic activity involving the K⁺ channels was evaluated using the procedure described by Perimal [18], with slight modifications. Four groups of 7 mice were divided as follows: the control group receives only the vehicle (saline), the second group receives glibenclamide (an ATP sensitive K⁺ channel inhibitor) and the last two groups receive respectively the hexane and aqueous extracts at a dose of 400 mg / kg of body weight. All mice except the first were pretreated with glibenclamide (10 mg / kg) 15 min before administration of saline or both extracts. Mice were injected with acetic acid, 1 hour after treatment. Five minutes after the acetic acid injection the analgesic effect was evaluated according to the following formula:

\[
\text{inhibition} \% = \frac{N_b \cdot N_t}{N_b} \times 100
\]

Nb is the average of the number of contortions of the mice of the blank control group and Nt is the average of the number of contorted mice of the batch treated.
number of contortions was recorded for 15 min. The analgesic effect was evaluated according to the following formula

\[
\text{Inhibition} \% = \frac{N_b - N_t}{N_b} \times 100
\]

### 3.4. Statistical Analysis

The statistical analysis was performed with GraphPad Prism 5.03 for Windows (Graph Pad Software, Inc., California USA), using One-way of variance (ANOVA). All results were expressed as the mean ± S.E.M. Dunnett’s test for comparisons with control group were used for analysis of differences between tested samples/groups and controls. Differences were considered significant when the p value was less than 0.05 (p < 0.05).

### 4. Results and Discussion

#### 4.1. Acute Toxicity Effects of *P. Angustifolia*

The mice body weight and relative organ weight were significantly changed (Table 1). In the acute toxicity assessment, no animal deaths were observed, no changes in the appearance of internal organs both in groups receiving the extract at the dose of 2400 mg/kg b.w. and of 3000 mg/kg b.w. as compared to control group. However, about 30 min after administration, all mice in the two extract treated groups presented behavioral changes, they were prostate, and presented limit interest in feeding. About 45 minutes after drugs administration animals regained their usual behaves. Therefore, the LD<sub>50</sub> of aqueous extract is greater than 3000 mg/kg b.w. in mice based on OCDE 425/2008 guideline [19]. Hence, the results suggest that the extract is safe.

Extract effects on organs revealed that liver, lung, heart and brain were not affected by drug administration over the testing period. Their weight was statistically the same in treated group as in the control one. Therefore, the spleen and the kidney weight were found reduced in the tested group. Changes in the weight of internal organs were considered as an indicator of chemical exposure. Spleen play a capital role in immunity while kidney is the main blood filter very susceptible to toxicants because high volume of blood flows through it and it filter large amounts of toxins which can concentrate in its tubules. Results observed may indicate that in the aqueous extract of *P. angustifolia* exist compounds that affect their functions.

#### 4.2. In Vitro Pro Inflammatory Enzyme Inhibition by Extracts of *P. Angustifolia* Secretary Phospholipase A2 (sPLA2) and COX-1 and COX-2 Inhibitor Activity Assay

Phospholipases are family of enzyme responsible for hydrolyzing the sn-2 fatty acids of membrane phospholipids generating free fatty acids and lysophospholipids [6], COXs and LOX further catalyze the metabolism of free arachidonic acids in different eicosanoids that are powerful mediators in the inflammatory process [20]. Numerous studies have established the physiopathology of many diseases such as cerebral illnesses [21], cardiovascular disorders [22], cancers [23], asthma, respiratory distress syndrome [24] and progression of tonsillitis disease [25] in the elevation of inflammatory enzymes levels. Thus, the activity reduction of the sPLA2 and COXs is a beneficial process towards the limitation of the inflammation process.

The IC<sub>50</sub> results obtained for sPLA2 inhibitor activity assay and COX-1 and COX-2 assay are expressed in Table 2. Both hexane and aqueous extracts of *P. angustifolia* showed significant inhibition against sPLA2 with respectively IC<sub>50</sub> values of 14.23 ± 0.72 µg/mL and 11.56 ± 0.11 µg/mL. Their activities were respectively 4.56 and 5.62 times greater than the activity of acetylsalicylic acid used as control. In the case of cyclooxygenases inhibition, the results obtained revealed that the aqueous extract (IC<sub>50</sub>=34.76 ± 0.51) was more inhibitory of COX-1 than the hexane extract. This was no longer the case for the inhibition of isoform 2 of cyclooxygenase where hexane extract was 3 time more inhibitor than aqueous extract. Our previous study on the phytochemistry of the plant revealed that the *P. angustifolia* content a large amount of phenolic compounds. Pharmacological effect of the plant may be due to the presence of phenolic compounds and compounds like in polar extracts.

#### 4.3. In Vivo Anti-Edematous and Analgesic Effects of *P. Angustifolia*

##### 4.3.1. Anti-Edematous Activity

The effects of aqueous extract on carrageenan induced -paw edema in mice have been shown in table 3. The different doses administrated orally inhibited significantly the edema induced after carrageenan injection. Oral administration of plant extract reduced edema rate dose dependently. The paw edema induced by carrageenan is an experiment animal model used to evaluate the acute anti-inflammatory activity of natural substances and drugs. The carrageenan injection provokes the activation of mast cells and the release of chemical mediators [26]. Mediators release after inflammation induction initiation occurs in three successive phases; first phase lasted for 1 hour that mediated by histamine, serotonin and the second phase was due to release of bradykinin during 2-3 hours; prostaglandins were produced during the last phase (4-6 h) [27]. The aqueous extract inhibited preponderantly the inflammatory response during the fist and the third phases of edema. At different doses, the oral administration of aqueous extract caused an important inhibition of paw edema induced by carrageenan injection. The maximal inhibition was noted at fifth hour after the oral administration (78.37% at 600 mg/kg). It suggests that the oral administration could more inhibit mediators of inflammation produced during the first phase (histamine and serotonin) and the prostaglandins biosynthesis during the last phase.
4.3.2. Analgesic Activity

The writhing induced by acetic acid injection is an experimental animal model used to evaluate the analgesic potential of pharmacological substances like NSAIDs. Substance P, histamine, serotonin and prostaglandins are among the mediators stimulating the nociceptive neurons after the injection of acetic acid in mice [28]. Results obtained showed that the oral administration of aqueous extract of *P. angustifolia* was able to inhibit significantly the writhing induced by acetic acid. Elevation of administrative doses causes less writhing inhibition indicating that the drug analgesic action was dose-dependent. The analgesic effect of aqueous extract of *P. angustifolia* may be due to the inhibition of the release of cytokines and pro-inflammatory mediators such as prostaglandins. Writhing number counted after the oral administration of the extract were reported in Table 4. Paracetamol, the reference product caused an inhibition more important than the aqueous extract at the same dose.

4.3.3. Contribution of K\textsubscript{ATP} Channel Pathway to the Analgesic Effect of Aqueous Extract

The alteration in peripheral analgesic effect of 400 mg/kg aqueous extract with pre-treatment of K\textsubscript{ATP} channel blocker glibenclamide at the dose of 10 mg/kg (i.p) is demonstrated in figure 1. Glibenclamide did not affect the number of writhes in acetic acid-induced writhing test when administrated alone. Glibenclamide pre-treatment followed by aqueous extract significantly (*p*<0.05) reversed the decrease in the number of writhing induced by aqueous extract of *P. angustifolia*. Regarding the observed results, we can conclude to the possibility that aqueous extract of *P. angustifolia* may have peripheral analgesic effects because of its capacity to reduce the amount of substances that trigger nociception or their activities after the acid acetic injection.

5. Conclusion

The outcomes of this study reveal that aqueous extract of *P. angustifolia* has an anti-inflammatory potential that may be explained by its ability to inhibit pro-inflammatory enzymes phospholipase and cyclooxygenase. Anti-edematous activity of the plant and its analgesic activity may be due to its ability to inhibit the production of inflammatory mediators like histamine, serotonin and prostaglandins, additionally, the analgesic effect of the extract may be due to the activation of K\textsubscript{ATP} channels involved in nociception.

In the light of these findings and considering that *Pandiaka angustifolia* is a natural source of pharmacological actives compounds whose toxicity is low, could be used after the identification and withdrawal of compounds implicated in spleen and kidney possible disfunction in the management of inflammation. Results credit the medicinal use of this plant, but also reveal it possible effect on spleen and kidney. Further investigations will be initiate to identify and isolate pharmacological active compounds of this species.

*Figure-1.* The effect of 10 mg/kg glibenclamide pre-treatment on the analgesia induced by 400 mg/kg of aqueous extract of *P. angustifolia* in acetic acid writhing test. AEpa: Aqueous extract of *P. angustifolia*, GB, Glibenclamide***p*<0.001; significant and differences based on the control group, & *p*<0.05; significant differences based on 400 mg/kg aqueous extract of *P. angustifolia*. One-way analysis of variance (ANOVA) followed by Tukey’s HSD multiple comparison test was performed. Values expressed as mean ± S.E.M. (*n* = 8)
Table 1. Weekly weight (g) and relative organs weight (%) after two weeks

| Parameters | Aqueous extract doses. | Control group |
|------------|------------------------|---------------|
|            | 2400 mg/kg b. w. | 3000 mg/kg b. w. | |
| Body weight | | | |
| 1st day | 25.78 ± 0.34 | 26.51 ± 0.37 | 29.10 ± 0.21 |
| 7th day | 29.33 ± 0.28 | 30.81 ± 0.41 | 30.98 ± 0.34 |
| 14th day | 29.67 ± 0.19 | 32.02 ± 0.11 | 33.17 ± 0.10 |
| Organs weight | | | |
| Liver | 4.27 ± 0.26 | 4.42 ± 0.21 | 4.70 ± 0.09 |
| Spleen | 0.38 ± 0.08 | 0.36 ± 0.07 | 0.44 ± 0.07 |
| Kidney | 0.92 ± 0.05 | 0.84 ± 0.09 | 1.09 ± 0.05 |
| Lung | 0.72 ± 0.09 | 0.71 ± 0.09 | 0.73 ± 0.07 |
| Heart | 0.45 ± 0.02 | 0.47 ± 0.05 | 0.42 ± 0.03 |
| Brain | 1.2 ± 0.13 | 1.25 ± 0.07 | 1.22 ± 0.06 |

Table 2. IC₅₀ results of sPLA₂, COX-1 and COX-2 by hexane and aqueous extracts of P. angustifolia

| Samples | sPLA₂ IC₅₀ (µg/mL) | COX-1 IC₅₀ (µg/mL) | COX-2 IC₅₀ (µg/mL) |
|---------|--------------------|--------------------|--------------------|
| Hexane extract | 14.23 ± 0.72c | 85.9 ± 1.01d | 61.59 ± 2.23b |
| Aqueous extract | 11.56 ± 0.11b | 34.76 ± 0.51b | 185.41 ± 1.41c |
| Quercetin | 0.95 ± 0.04a | 15.89 ± 2.27b | 9.23 ± 0.42a |
| Indomethacin | nd | 0.597 ± 0.17a | 8.80 ± 0.92a |
| Acetylsalicylic acid | 65 ± 1d | nd | nd |

Results are expressed as the mean values. Values showing the same letter are not significantly different (p < 0.05) from another in the same column. nd: not determined

Table 3. Effect of administration of aqueous extract of P. angustifolia on carrageenan induced hind paw edema

| Samples | Doses (mg/kg b.w) | Increase in paw volume (mL) | Edema inhibition (%) |
|---------|------------------|-----------------------------|----------------------|
|         |                  | 1 h | 3 h | 5 h | 1 h | 3 h | 5 h |
| Control | - | 0.26 ± 0.03 | 0.31 ± 0.04 | 0.37 ± 0.05 | - | - | - |
| AEpa   | 200 | 0.19 ± 0.05* | 0.22 ± 0.04** | 0.15 ± 0.04** | 26.92 | 29.03 | 59.45 |
|        | 400 | 0.14 ± 0.03*** | 0.17 ± 0.03** | 0.12 ± 0.03** | 45.15 | 45.16 | 67.56 |
|        | 600 | 0.10 ± 0.06** | 0.12 ± 0.06** | 0.08 ± 0.03** | 61.53 | 61.29 | 78.37 |
| Hdc    | 10 | 0.21 ± 0.03* | 0.29 ± 0.04 | 0.25 ± 0.03** | 20.83 | 17.65 | 32.43 |

Data are expressed as mean ± SEM (n=3), AEpa: Aqueous extract of P. angustifolia, Hdc, hydrocortisone. ** p < 0.001 significant from control (one-way ANOVA analysis followed by Dunnett’ test).

Table 4. Effect of administration of aqueous extract on writhing induced by acetic acid (mean ±SEM, n=6)

| Samples | Doses (mg/kg b.w) | Numbers of writhing |
|---------|------------------|---------------------|
| Control | - | 68.43 ± 2.00 |
| Aqueous extract | 100 | 49.24 ± 1.93** |
| | 200 | 34.22 ± 2.02** |
| | 400 | 26.19 ± 2.35** |
| Paracetamol | 100 | 37.33 ± 1.77** |

** p < 0.001 significant from control (one-way ANOVA analysis followed by Dunnett’ test).

Conflict of Interest Statement
We declare that we have no conflict of interest.

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References
[1] Mackenbach, J. P., 2006. "The origins of human disease: a short story on ‘where diseases come from.” J. Epidemiol. Community Health, vol. 60, pp. 81-86.
[2] Lindahl, J. F. and Grace, D., 2015. "The consequences of human actions on risks for infectious diseases: A review." Infect. Ecol. Epidemiology, vol. 5, pp. 1-11.
[3] Singh, S. and Sedha, S., 2018. "Medicinal plants and their pharmacological aspects.” Fpi, vol. 1, pp. 156-170.
[4] Liu, R. and Nikolajczyk, B. S., 2019. "Tissue immune cells fuel obesity-associated inflammation in adipose tissue and beyond.” Front. Immunol, vol. 10, pp. 1-17.
[5] Aziz, M., Jacob, A., Yang, W. L., Matsuda, A., and Wang, P., 2013. "Current trends in inflammatory and immunomodulatory mediators in sepsis." J. Leukoc. Biol., vol. 93, pp. 329-342.
[6] Hanna, V. S. and Hafez, E. A. A., 2018. "Synopsis of arachidonic acid metabolism: A review.” J. Adv. Res., vol. 11, pp. 23-32.
Du, X. and Gamper, N., 2013. "Potassium channels in peripheral pain pathways: Expression, function and therapeutic potential." Curr. Neuropharmacol., vol. 11, pp. 621–640.

Zhu, X., Liu, J., Gao, Y., Cao, S., and Shen, S., 2015. "ATP-sensitive potassium channels alleviate postoperative pain through JNK-dependent MCP-1 expression in spinal cord." Int. J. Mol. Med., vol. 35, pp. 1257-1265.

Quan, L. D., Thiele, G. M., Tian, J., and Wang, D., 2008. "The development of novel therapies for rheumatoid arthritis." Expert Opin. Ther. Pat., vol. 18, pp. 723-738.

Wongrakpanich, S., Wongrakpanich, A., Melhado, K., and Rangaswami, J., 2018. "A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly." Aging Dis., vol. 9, pp. 143-150.

Nacoulima/Ouedraogo, O. G., 1996. Plantes médicinales et Pratiques médicinales Traditionnelles au Burkina: cas du plateau central. Thèse de doctorat d’État, Faculté des sciences et techniques, Université de Ouagadougou, Burkina Faso, tome, p. 285.

EEC, 1986. "Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes." Official Journal of the European Communities, vol. L358, pp. 1-29.

D’Almeida, R. E., Isla, M. I., Vildoza, E. D. L., Quispe, C., Schmeda-Hirschmann, G., and Alberto, M. R., 2013. "Inhibition of arachidonic acid metabolism by the Andean crude drug Parastrephia lucida (Meyen) Cabrera." J. Ethnopharmacol., vol. 150, pp. 1080-1086.

OECD, 2001a. Guideline for the testing of chemicals. Acute oral toxicity-acute toxic class method (tg 423), adopted 22.03.96; revised method adopted: 17th december 2001. Paris: OECD.

Winter, C. A. and Risley, E. A., 1962. "Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory Drugs." Proc. Soc. Exp. Biol. Med., vol. 111, pp. 544-547.

Emmanuel, A. M., Thiombiano, N. O., Martin, K., and Antiinflammatory Drugs." Proc. Soc. Exp. Biol. Med., vol. 111, pp. 544-547.

Ouagadougou, Burkina: cas du plateau central. Thèse de doctorat d’État, Faculté des sciences et techniques, Université de Ouagadougou, Burkina Faso, tome, p. 285.

Perimal, E. K., 2011. “Zerumbone-Induced Antinociception: Involvement of the 1-Arginine-Nitric Oxide-cGMP -PKC-K+ATP Channel Pathways.” Basic Clin. Pharmacol. Toxicol., vol. 108, pp. 155-162.

Tessaro, F. H. G., Ayala, T. S., and Martins, J. O., 2015. Lipid mediators are critical in resolving inflammation: A review of the emerging roles of eicosanoids in diabetes mellitus. Biomed Res. Int.

Sun, G. Y., Jensen, M. D. X. J., and Simonyi, A., 2004. "Phospholipase A2 in the central nervous system: Implications for neurodegenerative diseases." J. Lipid Res., vol. 45, pp. 205-213.

Holmes, M. V., 2013. "Secretory phospholipase A2-IIA and cardiovascular disease: A mendelian randomization study." J. Am. Coll. Cardiol., vol. 62, pp. 1966-1976.

Cho, J. H. and Han, J., 2017. "Molecules and cells phospholipase D and its essential role in cancer." Mol. Cells., vol. 40, pp. 805-813.

Pniewska, E. and Pawlizczak, R., 2013. "The involvement of phospholipases A2 in asthma and chronic obstructive pulmonary disease." Mediators Inflamm., vol. 2013, p. 12.

Carvalho, B. M. A., 2013. "Snake venom PLA2s inhibitors isolated from brazilian plants: Synthetic and natural molecules." Biomed Res. Int., vol. ID153045, pp. 1-8.

De Araújo, I. W. F., 2016. "Analgesic and anti-inflammatory actions on bradykinin route of a polysulfated fraction from alga Ulva lactuca." Int. J. Biol. Macromol., vol. 92, pp. 820-830.

Abdulkhaled, L. A., Assi, M. A., Abdullah, R., Zami-Saaid, M., Taufiq-Yap, Y. H., and Hezme, M. N. M., 2018. "The crucial roles of inflammatory mediators in inflammation: A review." Veterinary World, vol. 11, pp. 627-635.

Amaya, F., Izumi, Y., Matsuda, M., and Sasaki, M., 2013. "Tissue injury and related mediators of pain exacerbation." Curr. Neuropharmacol., vol. 11, pp. 592-597.