Toxicity, chemical composition, anti-inflammatory and antioxidant activities of plants used for the treatment of helminth infections in the Kara and Central region of Togo.

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ABSTRACT

Objectives: Traditional healers (THs) from the Central and Kara regions of Togo use Aframomum melegueta (Alligator piper), Khaya senegalensis (Senegal mahogany) and Xylopia aethiopica (Kani pepper) for the treatment of helminths infections. We previously confirmed the anthelmintic effects of these plants. THs had little information about plants compounds, anti-inflammatory, antioxidant activities and toxicity. The present study aimed to investigate anti-inflammatory, antioxidant activities and toxicity of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica used for the treatment of helmintiasis in the Central and Kara regions of Togo.

Methodology and Results: Anti-inflammatory activity was evaluated using the inhibition method of lipoxygenase type I-B extracted from soybean. The concentrations of polyphenols and flavonoids were measured respectively by the Folin-Ciocalteu reagent reduction method and the Aluminium chloride colorimetric method. Antioxidant activity was assessed by the DPPH and ABTS assays. Acute and subchronic toxicity was performed on Wistar rats according to OECD recommendations. Khaya senegalensis and Xylopia aethiopica showed, a greater anti-inflammatory activity by inhibiting lipoxygenase activity in vitro and antioxidant activity (Aframomum melegueta; ABTS 32.79±3.79 mgEAA/100mg and Xylopia aethiopica; DPPH IC₅₀ of 2278.89±104.68 µg/mL). Khaya senegalensis contained a high concentration of flavonoids (1.39±0.07 mgEQ/100mg) and phenols (329.21±19.99 mgEGA /100mg). No toxic effects were observed for the chosen doses with these plants extract.

Conclusions and application of findings: Extracts of Khaya senegalensis and Xylopia aethiopica had anti-inflammatory activities. In addition, extracts of Khaya senegalensis and Aframomum melegueta had excellent antioxidant power and had the highest concentrations of polyphenols and flavonoids. This finding could justify the traditional use of these plant...
organ extracts for the treatment of helminth infections and provide scientific evidence to traditional healers in the central region and Kara in Togo. However, further studies are necessary to determine the molecules responsible for the pharmacological properties of these plant organ extracts and their mechanisms of action.

Key words: Aframomum melegueta, Khaya senegalensis, Xylopia aethiopica, anti-inflammatory activity, antioxidant activity.

INTRODUCTION

The use of plants for health care is a matter of culture and tradition in Africa (Jouad et al., 2001; Kokou et al., 2001). It is noted that for primary health needs, a large part of the African population uses traditional medicine, whose remedies are mainly herbal (Karou et al., 2011; Tchacondo, 2011; WHO, 2012). Medicinal plants continue to provide evidence of a very effective remedy against diseases, particularly helminthiasis (Atab et al., 2020). Aframomum meleguetaseeds are used in Africa to control diarrhoea and gastroenteritis (Jiofack et al., 2008). Xylopia aethiopica was mainly used for healing lymphatic filariasis, schistosomiasis (Oloyede and Aduramigba-Modepe, 2011). Xylopia aethiopica is commonly used in Nigeria to treat parasitic gastrointestinal helminths (Suleiman et al., 2005). Ademola et al. suggested the use of Khaya senegalensis extract in anthelminthic therapy in veterinary practice (Ademola et al., 2004). The anti-trypanosomal activity of Khaya senegalensis has also been studied by some authors (Ibrahim et al., 2013). We recently showed that Aframomum melegueta, Khaya senegalensis, Xylopia aethiopica are the main plants used by traditional healers to treat helminths infections in the Central and Kara regions of Togo and this was interestingly demonstrated and confirmed that these plants had anthelminthic effects in vitro (Atab et al., 2020). Hyperreactive onchocerciasis was associated with higher inflammatory Th17 responses (Hoerauf et al., 2003; Taylor et al., 2010; Katawa et al., 2015). Many plants have shown anti-inflammatory and antioxidant activities that could contribute to treat helminths induced disorder like skin inflammation and elephantiasis (Hoerauf, 2008; Hoerauf et al., 2011; Ritter et al., 2017). The traditional healers in the study had little information about plants bioactivities, compounds and toxicity. This study was undertaken to investigate on toxicity, compounds, anti-inflammatory and antioxidant activities of the main plants used by the traditional healers from the Central and Kara regions of Togo for the treatment of helminths infections.

MATERIALS AND METHODS

Experimental animals: Male (170g - 210 g) and female (150g - 185 g) Wistar strain albino rats *Rattus norvegicus* aged approximately 4 months were used for acute and subchronic toxicity. Females were neither pregnant nor lactating. The breeding of these animals took place in the LAMICODA animal house of the University of Lomé. The animals were subjected to natural day and night alternation corresponding to 12±1 h of day and night. They had free access to water ad libitum and feed fed with rodent standard diets. The experimental protocols were guided by the OECD guideline for the care and use of laboratory animals (OCDE, 2001, OCDE, 2009).

Plants material: Following an ethnobotanical survey, three plant organs were selected because of their use by THs for the treatment of helminths infections in the Kara and Central regions of Togo. These were trunk bark of Khaya senegalensis harvested in the forest of Tchavadé (central region of Togo), grains of Aframomum melegueta and fruits of Xylopia aethiopica bought fresh on the market (Figure 1). These extracts are washed and air-dried at laboratory temperature (18 and 25°C) and then crushed. From the powders obtained, a hydroethanolic extraction was prepared. Then different concentrations were obtained with distilled water and filtered using a 0.45μm millipore membrane.
Chemicals and reagents: Borate buffer, Lipoxygenase type I-B, Linoleic acid, quercetin, gallic acid, Folin-Ciocalteu (FCR), Sodium Carbonate Solution, Aluminium Chloride (AlCl₃), Methanol, 2, 2-diphenyl-1-picyrlyhydrazyl (DPPH), 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), Potassium persulfate and Ethanol were purchased from Sigma Aldrich (Spruce, St Louis, Germany). Tryptone Salt solution, Plate Count Agar (PCA), Crystal Violet Agar, Neutral Red Bile Lactose (NRBL) and Sabouraud + Chloramphenicol was supplied by Oxid (Basingtoke, Hampshire, England).

Microbiological quality control: In order to evaluate the microbiological quality of plant extracts, 1/10 dilution was prepared by adding 1 ml of each hydroethanolic extract in 9ml of Tryptone Salt solution. This was based on the standardized methods of the “Association Française de Normalisation” (AFNOR) and adopted within the WAEMU countries: Order of December 21, 1979 (AFNOR, 2009). The mass seeding technic was adopted for the research of mesophilic aerobic flora on PCA medium at 30°C for 72 hours (Reference Method NF V08-051 February 1999). Total coliforms were researched on NRBL at 30°C for 24 hours (reference method NF V08-050 1999); Sabouraud + Chloramphenicol medium were sown and incubated at 30°C during 72 hours (NF ISO7954 1988) for moulds and yeasts. The results were then interpreted according to the criteria of the AFNOR standards adopted for Traditional Improved Medicines (AFNOR, 2009).

**In vitro anti-inflammatory activity:** The in vitro anti-inflammatory activity of these plant extracts was evaluated using the inhibition method of lipoxygenase type I-B extracted from soybean. Enzyme solution (146µl) prepared in borate buffer (0.2M; pH 9.0) was mixed with 3.7µl of sample at the final concentration of 98.83µg/mL of extracts. The reaction was then triggered by the addition of 150µL of substrate (linoleic acid of final concentration 1.25mM) and the optical density was recorded at 234 nm with a spectrophotometer (EPOCH 251465, Biotek instruments USA Micro well plate Reader). Borate buffer and enzyme were used as negative controls, substrate and enzyme as positive controls, quercetin and gallic acid at 10µg/mL were used as reference anti-inflammatory molecules. The test was triplicate under the same conditions. The lipoxygenase inhibition percentage (%) was calculated at 1st, 5th and 10th time according to the following equation:

\[
\%I = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]

*Control OD = enzyme activity in the absence of samples; Sample OD = activity of the enzyme in the presence of samples.*

**Phytochemical screening and antioxidant activity of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica**

Phytochemical screening.

**Total phenolic contents determination:** Polyphenols were quantified according to the protocol described by Singleton et al., (Singleton et al., 1999). 25 µL of the extract (0.1 mg/ml) were incubated with 125 µL of Folin-Ciocalteu (FCR, company, country and city) solution (0.2N) in 96-well plate at room temperature for 5min. Thereafter, 100 µL of Sodium Carbonate Solution (75 g/l) were added. The absorbances were read at 760 nm with a spectrophotometer (EPOCH 251465, Biotek instruments USA Micro well plate Reader) after one hour of incubation. The concentrations of phenols in milligrams of gallic acid equivalent (EGA)/100mg extract were calculated according to the formula

\[
C = \frac{(C_1 \times V_f)}{(C_i \times V_i)} \times 100
\]

*C: concentration of the component; C_i: concentration of final dilution of standard; V_i: initial concentration of the test sample;*
Vi: initial volume of the test sample; Vf: final volume in the well containing the test sample.

Total Flavonoids Contents determination: The flavonoids of the plant extracts were determined by the colorimetric method of AlCl₃ described by Dowd and adapted by Arvouet-Grand et al., (Arvouet-Grand et al., 1994). This method is based on the absorption capacity at 415 nm of the aluminium-flavonoid complex formed during the reaction. For this purpose, 100µL of each extract diluted in 1mg/ml of methanol was added to 100µL of a 2% AlCl₃ methanolic solution in the wells of a 96-well plate. The flavonoids concentration in milligrams of quercetin equivalent (EQ)/100mg extract were calculated according to the previous formula:

\[ C = \frac{(Cl \times Vf)}{(Ci \times Vi)} \times 100 \]

Antioxidant activities by DPPH radical scavenging activity: The antioxidant activity of the extracts was assessed through their capacity of scavenging stable radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This activity was determined using the stable radical DPPH, according Velázquez et al. (2003) and Toudji et al. (2018) methods. Indeed, the DPPH test consists of the reduction of absorbance at 517 nm due to the stable free radical DPPH• in the presence of a donor of the radical H•. The assay was performed in triplicate using 96-well plates (Nalge Nunc International, NY, USA). Serial dilution aliquots were made from extracts in solution (10 mg/mL) in methanol by mixing 100µL of each sample with 200µL of DPPH (20mg/L). After 15 min of incubation in darkness at room temperature, the resultant absorbance was measured at 517 nm with a spectrophotometer (EPOCH 251465, Biotek instruments USA Micro well plate Reader) against a blank well with 100µL methanol and 200µL of DPPH. Quercetin and Gallic acid were used as reference products. The percentage of inhibition was calculated according to the equation:

\[ I% = \frac{A0 - AE}{A0} \times 100 \]

A0: Absorbance of the blank; AE: Absorbance of the sample.

ABTS radical scavenging assay: Free radical scavenging activity of plant samples was determined by 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay (Re et al., 1999). ABTS+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM Potassium persulfate (1:1), stored in the dark at room temperature for 12-16 hours before use. Discoloration is monitored by measuring absorbance at 734 nm. Then, 37.7 mg of ABTS is dissolved in 9.802 mL of distilled water to which 6.48 mg of Potassium persulfate is added. The mixture was homogenized and stored in the dark at room temperature for 12 hours. It was then diluted in ethanol to an absorbance of approximately 0.70 ± 0.02 at 734 nm. The free radical capture percentage (I%) was determined using the following formula:

\[ I% = \frac{A0 - AE}{A0} \times 100 \]

A0: Absorbance of the blank; AE: Absorbance of the sample.

In vivo evaluation of plant extracts toxicity

Acute toxicity.: Acute toxicity was conducted by intragastric tube gavage on male rats according to OECD recommendations (OCDE, 2001). For this study, rats were divided into 4 batches of 3 rats. The first three batches took respectively the hydroethanolic extract of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica in a single dose of 5000 mg/Kg bw. The control batch was treated with a saline solution (NaCl 9%). This solution was used to prepare the different concentrations of the aqueous extract. The animals were observed regularly for 3 hours for 24 hours and then every 6 hours up to 48 hours after dosing to note any changes in their behaviour (Bürger et al., 2005; Ogbonna et al., 2008). Observations focused on mobility, noise and pinch sensitivity, feeding, breathing, and faeces appearance (Lienou et al., 2007).

Subchronic toxicity: According to the OECD recommendations, the doses of 100 and 300 mg/Kg bw were considered for the achievement of subchronic toxicity. The rats were divided into 4 batches of 8 animals each (4 males and 4 females). Three batches received daily doses of 100 and 300 mg/Kg bw hydroethanolic extracts of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica for 28 days. The control lot (4th lot) was treated with NaCl 9% over the same period. The behaviour of the rats was observed on a daily basis. The animals were sacrificed at the end of treatment, according to OECD recommendations (OCDE, 2009). The blood of each animal was collected both in a tube containing an anticoagulant (EDTA) for blood formula count with the Sysmex XN550 automated system (Sysmex, Paris, France). After centrifugation of the Tubes without anticoagulant, sera obtained were used to determine blood glucose levels, ureaemia, creatininaemia, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (PAL) according to the instructions of the kits (Laklit, Eu-gen) on a DIRUI DR-7000D spectrophotometer (Dirui, Tozkoparan Mah, Gungoren, Istanbul) and as performed by Toudji et al. (Toudji et al., 2017).
**Statistical analysis:** The statistical analysis was performed using GraphPad Prism 5.2 software. The values are presented as mean ± standard error. Significance levels between treated and control batches were measured by the U test of Mann Whitney. If p<0.05, the difference between the values is considered significant.

**RESULTS**

**In vitro Anti-inflammatory activity:** The extracts of *Khaya senegalensis* and *Xylopia aethiopica* have an interesting anti-inflammatory activity showed by their capacity to inhibit lipoxygenase activities respectively of 70.28% and 65.22% compared to the reference molecules while *Aframomum melegueta* does not exceed 35% (Table 1).

| Time | Quer | Acg | AM | KS | XA |
|------|------|-----|----|----|----|
| 1st min | 52.04±2.45 | 63.66±2.67 | 34.53±0.50 | 70.28±3.55 | 65.22±1.63 |
| 5th min | 1.39±0.39 | 25.52±1.39 | 92.34±2.18 | 11.16±0.96 |
| 10th min | 23.52±0.10 | 93.93±2.84 | 11.06±0.62 |

AM: *Aframomum melegueta*; KS: *Khaya senegalensis*;XA: *Xylopia aethiopica*; Quer: Quercetin; Acg: Gallic acid. The values are expressed as a mean value ± SD.

**Photochemical screening and antioxidant activity:** the phenols and flavonoids contained in plant extracts were determined respectively, using the Folin-Ciocalteau reagent reduction method and the aluminium chloride colorimetric method. The extracts of *Khaya senegalensis* and *Aframomum melegueta* had the highest concentrations of polyphenols respectively of 329.21±19.99 mg EGA/100mg and 174.45±14.62 mg EGA/100mg and flavonoids respectively of 1.39±0.07 mg EQ/100mg and 1.02±0.05 mg EQ/100mg, in contrast to *Xylopia aethiopica*, which contained a low concentration (Table 2). Similarly, these plant extracts had the ability to complex DPPH- and ABTS⁺ free radicals. *Khaya senegalensis* had the highest antiradical activity (IC₅₀ = 6.37±0.09 µg/mL) with the DPPH test while with the ABTS test, *Aframomum melegueta* extracts had the highest inhibitory capacity (32.79±3.79 mgEAA/100mg extract) followed by *Khaya senegalensis* extracts.

| Extracts | Total phenols (mg EGA/100mg) | Total flavonoids (mg EQ/100mg) | Antioxidant activities |
|-----------|-------------------------------|-------------------------------|------------------------|
|           |                               |                               | DPPH (IC₅₀ µg/mL) | ABTS (mgEAA/100mg) |
| *K. senegalensis* | 329.21±19.99 | 1.39±0.07 | 6.37±0.09 | 5.66±0.15 |
| *A. melegueta* | 174.45±14.62 | 1.02±0.05 | 22.26±2.15 | 32.79±3.79 |
| *X. aethiopica* | 15.27±1.09 | 0.52±0.04 | 2278.89±104.68 | 1.89±0.19 |

**Acute oral toxicity:** Observations based on changes in skin, hair, salivation and behaviour, including various manifestations of tremor, convulsions, diarrhoea, sleep and coma. Apart from the diarrhoea observed in the lot that received *Aframomum melegueta* extracts, no abnormal behaviour or deaths were observed in animals treated orally at any dose, which did not allow for the determination of the LD₅₀ (Table 1). The oral LD₅₀ for the hydro ethanol extract of *Aframomum melegueta*, *Khaya senegalensis* and *Xylopia aethiopica* is therefore greater than 5000 mg/kg bw.

**Subchronic toxicity:** During the treatment period, the rats showed no abnormal behaviour. However, in male rats, the lots that was treated with *Khaya senegalensis* extracts had lost weight from 14th day onwards, unlike the one treated by *Aframomum melegueta* and *Xylopia aethiopica*. For female rats, the batches of *Aframomum melegueta* and the 300 mg/Kg b.w. dose of *Khaya senegalensis* had lost weight from to 7th day and 14th day respectively. Control lots in both sexes had gained weight (Figure 2). After sacrifice of the rats, no apparent difference was observed between the test and control lots. In addition, haematological analysis showed no significant changes in red blood cells count, haemoglobin levels, MCV, platelets, neutrophils and lymphocytes count in male rats, but the white blood cell, MCH, MCHC and monocyte counts of the 300 mg/kg dose group of *Khaya senegalensis* increased (Table 3). The same trend was observed in female rats where white blood cell and platelet counts increased in the 300 mg/kg.
b.w. dose of *Aframomum melegueta* and *Khaya senegalensis* (Table 4). The same was for monocytes with the 100 and 300 mg/Kg b.w. doses of *Aframomum melegueta*. Neutrophil and basophilic polynuclear cells count in the 300 mg/Kg b.w. dose lots of *Khaya senegalensis* extract increased (Table 4). With respect to biochemical parameters, Alanine aminotransferase was significantly increased in the batch of male rats for the 300 mg/Kg b.w. dose of *Aframomum melegueta* extract (Table 5). The same observation was made with creatinine and Alanine aminotransferase in male rats and then Aspartate aminotransferase and Alanine aminotransferase for the 300 mg/Kg b.w. dose of *Khaya senegalensis* (Tables 5 and 6). There were no significant variations between the organs of the male rats tested compared to the control except for the increase of the weight of the spleen and the kidneys for the 300 mg/Kg b.w. lot of *Aframomum melegueta* extract (Figure 3). On the other hand, the weight of livers, heart, lungs and kidneys of female rats, which had received the extracts of *Aframomum melegueta* and *Khaya senegalensis*, had significantly decreased (Figure 4).
Figure 2: Variation of body weight of male and female rats at 100 and 300 mg/kg of *Aframomum melegueta*, *Khaya senegalensis* and *Xylopia aethiopica*. Male rats (A, B and C) and female rats (D, E and F). D0, D7, D14 and D28 correspond to the 7-day interval of weight gains during the 28-day subchronic toxicity assessment. Asterisks show statistical differences (U test of Mann Whitney) between 100 and 300 mg/Kg bw doses vs Control (*p<0.05, **p<0.01, ***p<0.001).
Table 3: Effects of hydroethanolic extracts of *Aframomum melegueta* (AM), *Khaya senegalensis* (KS) and *Xylopia aethiopica* (XA) on hematological parameters of male rats

| Parameters | Control | 100 mg/Kg | 300 mg/Kg | p | 100 mg/Kg | 300 mg/Kg | p |
|------------|---------|-----------|-----------|---|-----------|-----------|---|
| WBC (10⁹/L) | 6.18±0.16 | 7.74±3.36 | 7.44±1.58 | 7.91±2.87 | 6.27±1.74 | 9.54±2.30* | 6.40±1.66 | 0.0286 |
| RBC (10¹²/L) | 9.03±0.61 | 9.23±0.49 | 9.27±0.42 | 8.83±0.41 | 9.235±0.18 | 8.91±0.26 | 9.02±0.18 |
| HB (g/dL) | 14.53±1.14 | 14.78±0.75 | 14.73±0.70 | 14.10±0.74 | 14.68±0.41 | 13.90±0.43 | 14.15±0.17 |
| HCT (%) | 50.73±4.40 | 51.58±3.63 | 52.45±2.46 | 48.60±4.93 | 52.78±0.45 | 49.98±1.13 | 49.93±0.45 |
| MCV (fL) | 56.08±1.08 | 55.85±2.10 | 56.55±1.17 | 54.90±3.14 | 57.08±0.89 | 56.08±0.42 | 55.43±1.32 |
| MCH (pg) | 16.08±0.21 | 16.00 | 15.85±0.19 | 15.95±0.25 | 15.88±0.13 | 15.60±0.08 | 15.70±0.42 |
| MCHC (g/dL) | 28.63±0.24 | 28.70±1.09 | 28.08±0.42 | 29.15±1.62 | 27.80±0.67 | 27.80±0.22* | 28.33±0.10 | 0.0284 |
| PLT (10⁹/L) | 771.0±98.86 | 800.8±140.6 | 796.3±127.6 | 890.5±170.2 | 789.3±136.2 | 804.0±79.05 | 745.8±56.57 |
| NEU (10⁹/L) | 1386±458.2 | 1568±691.3 | 1603±589.7 | 2753±2845 | 1274±40.45 | 3314±1442 | 1888±1013 |
| LYM (10⁹/L) | 5463±982.5 | 5279±1706 | 5855±722.8 | 3681±1805 | 4872±1503 | 4732±495.6 | 5173±747.7 |
| MO (10⁹/L) | 483.0±66.14 | 2397±2864* | 1033±787.3 | 2988±803* | 1309±2584* | 1254±625.7* | 633.0±527.4 | 0.0294 |
| EO (10⁹/L) | 1485±1371 | 382.5±257.3 | 1037±581.0 | 361.7±448.0 | 350.0±247.5 | 499.7±11.26 | 123.5±247.0 |
| BAS (10⁹/L) | 6342±584.3 | 2928±1670* | 2845±1432* | 3477±2487* | 2298±1257* | 2802±454.4* | 5131±735.5 | 0.0286 |

Values represent the mean ± SD (n = 4/group) of haematological parameters; *p* < 0.05 vs. Control. WBC: White blood cells; RBC: Red blood cell; HB: Haemoglobin; HCT: Haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; PLT: Platelets; NEU: Neutrophils; LYM: Lymphocytes; MO: Monocytes; EO: Eosinophils; BAS: Basophils.
Table 4: Effects of hydroethanolic extracts of *Aframomum melegueta* (AM), *Khaya senegalensis* (KS) and *Xylopia aethiopica* (XA) on haematological parameters of female rats

| Parameters | Control | 100 mg/Kg | XA | p  | 300 mg/Kg | AM | KS | XA | p  |
|------------|---------|-----------|----|----|-----------|----|----|----|----|
| WBC (10^9/L) | 4.8±0.72 | 6.31±0.67 | 6.32±0.421 | 8.54±2.15 | p | 8.93±0.19* | 10.78±1.48* | 7.01±1.14 | 10.78±1.48* |
| RBC (10^12/L) | 6.5±2.49 | 7.92±0.55 | 7.43±1.05 | 9.17±0.94 | p | 8.97±0.25 | 8.38±0.38 | 8.64±0.12 | 8.38±0.38 |
| HB (g/dL) | 12.13±1.79 | 13.03±1.07 | 12.90±1.23 | 15.00±1.68 | p | 14.73±0.53 | 13.83±0.53 | 14.08±0.40 | 13.83±0.53 |
| HCT (%) | 36.40±12.40 | 46.90±2.92 | 46.85±4.51 | 54.98±4.14 | p | 52.38±1.19 | 48.90±1.20 | 50.98±1.12 | 48.90±1.20 |
| MCV (fL) | 57.40±3.82 | 59.20±1.06 | 63.43±3.61 | 60.08±1.72 | p | 58.50±0.70 | 58.48±2.05 | 58.93±0.61 | 58.48±2.05 |
| MCH (pg) | 21.53±7.967 | 21.53±7.967 | 18.43±0.38 | 17.45±0.89 | p | 16.43±0.19 | 16.53±0.13 | 16.28±0.26 | 16.53±0.13 |
| MCHC (g/dL) | 36.83±10.94 | 27.78±0.53* | 27.55±0.26* | 29.25±1.15 | p | 28.10±0.43* | 28.28±0.82 | 27.60±0.24* | 28.28±0.82 |
| PLT (10^9/L) | 1192±166.5 | 1237±146.7 | 945.5±162.4 | 849.5±92.19 | p | 764.3±21.36* | 840.7±53.82* | 1145±105.1 | 840.7±53.82* |
| NEU (10^9/L) | 1867±579.7 | 2591±1134 | 2528±531.3 | 4055±2864 | p | 2266±522.2 | 5273±2296* | 2950±756.7 | 5273±2296* |
| LYM (10^9/L) | 3477±1631 | 3844±928.2 | 2489±357.3 | 2995±1851 | p | 4670±487.3 | 3044±166.4 | 3367±1091 | 3044±166.4 |
| MO (10^9/L) | 453.3±267.5 | 1241±435.7* | 912.5±270.6 | 2364±3261 | p | 2457±955.2* | 1262±592.2 | 1699±948.2* | 1262±592.2 |
| EO (10^9/L) | 332.0±469.5 | 152.7±215.9 | 538.0±644.0 | 131.5±263.0 | p | 170.7±241.4 | 485.7±388.9 | 501.3±403.5 | 485.7±388.9 |
| BAS (10^9/L) | 3178±1271 | 2159±980.5 | 6321±3817 | 2713±1829 | p | 1956±568.6 | 8384±1594* | 1964±277.6 | 8384±1594* |

Values represent the mean ± SD (n = 4/group); *p < 0.05 vs. Control. WBC: White blood cells; RBC: Red blood cell; HB: Haemoglobin; HCT: Haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; PLT: Platelets; NEU: Neutrophils; LYM: Lymphocytes; MO: Monocytes; EO: Eosinophils; BAS: Basophils.
Table 5: Effects of hydroethanolic extracts of *Aframomum melegueta* (AM), *Khaya senegalensis* (KS) and *Xylopia aethiopica* (XA) on biochemical parameters of male rats

| Parameters       | Control   | 100 mg/Kg | 300 mg/Kg |
|------------------|-----------|-----------|-----------|
|                  | AM        | KS        | XA        | p        | AM        | KS        | XA        | p        |
| Glucose (g/L)    | 0.97±0.14 | 1.10±0.18 | 1.15±0.13 | 1.19±0.17 | 1.05±0.26 | 1.05±0.13 | 1.01±0.37 |          |
| Urea (g/L)       | 0.29±0.07 | 0.31±0.06 | 0.31±0.15 | 0.29±0.04 | 0.31±0.08 | 0.33±0.09 | 0.32±0.09 |          |
| Creatinine (g/dL)| 0.90±0.07 | 0.89±0.03 | 0.84±0.04 | 0.74±0.09 | 0.84±0.03 | 0.77±0.05* | 0.82±0.08 | 0.0294   |
| AST (U/L)        | 110±11.53 | 119.8±10.24 | 123.8±11.09 | 121.8±13.20 | 116.8±9.74 | 131.5±9.47 | 127.5±15.02 |          |
| ALT (U/L)        | 72.25±4.57 | 88.0±4.97 | 75.5±6.24 | 86.8±33.98 | 96.00±4.32* | 92.00±8.2* | 87.75±7.41 | 0.0286   |
| ALP (U/L)        | 184.3±20.87 | 146.3±7.85 | 133.3±19.60 | 201.3±15.28 | 176.5±11.82 | 171.8±8.261 | 157.8±10.72 |          |

The values represent the mean ± SD (n = 4 / sex / group) *p < 0.05 vs. Control. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.

Table 6: Effects of hydroethanolic extracts of *Aframomum melegueta* (AM), *Khaya senegalensis* (KS) and *Xylopia aethiopica* (XA) on biochemical parameters of female rats

| Parameters       | Control   | 100 mg/Kg | 300 mg/Kg |
|------------------|-----------|-----------|-----------|
|                  | AM        | KS        | XA        | p        | AM        | KS        | XA        | p        |
| Glucose (g/L)    | 0.75±0.12 | 1.02±0.21 | 1.09±0.18 | 0.97±0.24 | 1.06±0.14 | 1.06±0.19 | 1.07±0.30 |          |
| Urea (g/L)       | 0.33±0.08 | 0.44±0.17 | 0.38±0.17 | 0.34±0.08 | 0.46±0.24 | 0.53±0.09 | 0.46±0.23 |          |
| Creatinine (g/dL)| 0.84±0.06 | 0.83±0.05 | 0.78±0.04 | 19.31±37.13 | 0.75±0.03 | 0.82±0.01 | 0.73±0.08 |          |
| AST (U/L)        | 123.0±6.05 | 126.8±3.86 | 139.5±7.50 | 126.8±12.04 | 135.3±16.03 | 147.0±7.35* | 123.3±5.74 | 0.0286   |
| ALT (U/L)        | 45.50±5.80 | 55.75±9.94 | 44.25±5.32 | 70.50±14.20 | 59±5.48 | 68.25±13.94* | 49.50±10.02 | 0.0294   |
| ALP (U/L)        | 182.3±7.37 | 191.0±23.42 | 181.3±10.81 | 201.0±12.36 | 210.0±33.85 | 193.8±18.26 | 206.8±12.53 |          |

The values represent the mean ± SD (n = 4 / sex / group) *p < 0.05 vs. Control. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.
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Figure 3: Effects of hydroethanolic extracts of Aframomum melegueta (AM), Khaya senegalensis (KS) and Xylopia aethiopica (XA) on organ weights in male rats. Asterisks show statistical differences (U test of Mann Whitney) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).
Figure 4: Effects of hydroethanolic extracts of *Aframomum melegueta* (AM), *Khaya senegalensis* (KS) and *Xylopia aethiopica* (XA) on organ weights in female rats. Asterisks show statistical differences (U test of Mann Whitney) between the groups indicated by the brackets (*p < 0.05, **p < 0.01, ***p < 0.001).
DISCUSSION

Medicinal plants are now increasingly used in the African pharmacopoeia (Karou et al., 2011; Tchacondo, 2011 WHO, 2012). These plants are a source of multitudes of molecules potentially effective against diseases (Rout et al., 2009; Veeresham, 2012Shakya, 2016). Following our recent studies, the trunk bark of Khaya senegalensis, the grains of Aframomum melegueta and the fruits of Xylopia aethiopica used by THs in the Central and Kara regions of Togo, for the treatment of helminthiases showed a real antihelminthic activity (Ataba et al., 2020). The objective of this study was to investigate the anti-inflammatory, antioxidant activity and toxicity of these main plants used for the treatment of helminthiases. The results obtained in this study confirm the anti-inflammatory potential of hydroethanol extracts of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica through the inhibition of type I-B lipooxygenase to varying. Lipooxygenases are an extensively studied class of enzymes recognized classically as drug targets for the treatment of inflammation. (Wisastra and Dekker, 2014). Then, a variety of compounds have been introduced to modulate lipooxygenase enzyme activity and ultimately to provide new drugs for inflammation (Wisastra and Dekker, 2014). Given that, helminth infections induced inflammatory conditions such as skin disorder, elephantiasis (Hoerauf et al., 2003; Hoerauf 2005, Katawa et al., 2015), use of these plants by THs to treat helminth infection is for good interest. This result is consistent with that of Jiang et al. (2013) who found a 62% inhibition of foot oedema in animals with a petroleum ether extract at 0.5 and 1 g/kg. Ademola et al., suggested the use of Khaya senegalensis extract in anthelmintic therapy in veterinary practice (Ademola et al., 2004). The anti-trypanosomal activity of Khaya senegalensis has also been studied by some authors (Ibrahim et al., 2013). While Xylopia aethiopica inhibit lipooxygenase at 65.22±1.63%, the extract of this plant is the poorest in phenolic and flavonoid compounds. Xylopia aethiopica was mainly used for healing Lymphatic Filariasis, Schistosomiasis (Oloyede and Aduramigba-Modeupe, 2011). Xylopia aethiopica is commonly used in Nigeria by THs to treat parasitic gastrointestinal helminths (Suleiman et al., 2005). In addition, the ethanolic extract of Aframomum melegueta has anti-inflammatory properties by inhibiting the activity of cyclooxygenase-2 (COX-2) (Illic et al., 2014). Aframomum melegueta seeds are also used in Africa to control diarrhoea and gastroenteritis (Jiofack et al., 2008). The pathogenicity of helminths infections such as skin disorder, elephantiasis, is the result of the inflammatory reactions to these parasites (Maizels and Lawrence, 1991, Taylor, 2003, Van Riet et al., 2007). This would justify their traditional uses in the treatment of helminths infections. Furthermore, this study found that these plants had anthelmintic effect with lethal doses values of 233 μg/mL, 265 μg/mL and 550 μg/mL, respectively for Xylopia aethiopica, Aframomum melegueta and Khaya senegalensis (Ataba et al., 2020). Phytochemical screening of the plant revealed that Aframomum melegueta, Xylopia aethiopica and Khaya senegalensis contain phenolic and flavonoid compounds. The results of this study show that among the extracts studied, it is the trunk bark of Khaya senegalensis that contains the most, i.e. 329.21 ± 19.99 mg of gallic acid equivalent per 100 mg of sample. This result is 697.6 times higher than that of Karou et al., (2005) who found a value of 47.19±0.13μg gallic acid per gram of sample taken in Ouagadougou (Karou et al., 2005). This large difference could be due to the different climatic and soil conditions on the one hand and on the other hand to the degree of maturity of the plants at the time of sample collection. Phenolic compounds not only play a physiological role, a means of plant defence (El Halawany et al., 2014) and a tool for attracting pollinating agents, but are also powerful antioxidants. Indeed, previous studies have shown that phenolic compounds have the capacity to adsorb free radicals (Koba et al., 2008; Idoh et al., 2013; Toudji et al., 2018). It is true that many synthetic molecules are currently prescribed to alleviate these physiological disorders; however, not only are plant-based antioxidants more protective than synthetic antioxidants (Akter et al., 2014), they are also accessible to all budgets and are renewable. DPPH and ABTS tests were used to show antioxidant activities of plants extracts. The present study, found the capacity of phenols to adsorb free radicals. Moreover, the difference in phenol results observed with those of Karou et al., (2005) is also reflected in the ability of both samples to complex the ABTS+ radical (Karou et al., 2005). The DPPH test is used to measure the anti-radical power of pure molecules or plant extracts (Da Porto et al., 2000; Tirzitis and Bartoz, 2010). It measures the ability of an antioxidant to reduce the chemical radical DPPH by transferring a hydrogen atom. Statistical analysis applied to the results of the antioxidant activities of these three types of extracts, measured with ABTS+ staining, shows that these results are significantly different at (P<0.05, n=3). This could mean that each extract of this plant has
a different molecular composition and therefore deserves to be studied. The results of this present work prove that these plant extracts are good natural antioxidants and that their use in phytotherapy can help the human body to deal with an imbalance between the production and regulation of free radicals. The dosage and the lack of information on the toxicity of medicinal plants are the main reasons and the brake on the development of phytotherapy (Sharma et al., 2010). Here, the acute toxicity study revealed no abnormal behaviour or deaths were observed in animals treated orally with the dose of 5000 mg/Kg of bw except for the diarrhoea observed on the lot that received the extracts of Aframomum melegueta. The oral LD₅₀ of the hydroethanolic extract of Aframomum melegueta, Khaya senegalensis and Xylopia aethiopica is thus higher than 5000 mg/Kg bw. However, female rats dosed with 300 mg/Kg of Aframomum melegueta and Khaya senegalensis extracts lost weight from 7th day and 14th day respectively while control lots in both sexes had a weight gain. After sacrifice of the rats, no apparent difference was observed between the test and control lots. This was confirmed by haematological analysis, which showed no significant changes in red blood cells, haemoglobin level, platelets, neutrophils and lymphocytes in male rats, but white blood cell count, MCH, MCHC and monocytes in the 300 mg/Kg dose lot of Khaya senegalensis were increased. Similarly, the same trend was observed in female rats where white blood cell and platelet counts were increased in the 300 mg/Kg dose of Aframomum melegueta and Khaya senegalensis. Lots of the 300 mg/Kg dose of Khaya senegalensis extracts showed increases in neutrophils and basophils. This would be the dose effect as similar studies have shown toxicity of Aframomum melegueta and Khaya senegalensis at high doses (Ilic et al., 2010; Onu et al., 2013). Alanine aminotransferase is a cytosolic enzyme secreted by liver cells and released into the bloodstream in the event of hepatic cell necrosis (Kaneko, 1995, Dufour et al., 2000). It is an important indicator of hepatotoxicity (Pratt and Kaplan, 2000). Aspartate aminotransferase is also an indicator of hepatocyte destruction. Although in addition to the liver, it is present in the heart, skeletal muscles, lungs and kidneys (Dufour et al., 2000). Levels of Alanine aminotransferase and Aspartate aminotransferase rise rapidly when the liver is damaged for a variety of reasons including hepatic cell necrosis, hepatitis, cirrhosis, and hepatotoxicity of certain drugs (Dufour et al., 2000; Pratt and Kaplan, 2000). In our study, the concentration of Alanine aminotransferase in the male batch that took the 300 mg/Kg dose of Aframomum melegueta extract was significantly increased. The same observation is made with creatinine and Alanine aminotransferase in male rats and then Aspartate aminotransferase and Alanine aminotransferase for the 300 mg/Kg dose of Khaya senegalensis. The same result has been reported by other authors with increases in Alanine aminotransferase, Aspartate aminotransferase and alkaline phosphatase in rats with higher doses of Khaya senegalensis (Yakubu et al., 2005; Onu et al., 2013). The biochemical and haematological parameters measured are indicative of the physiological state of vital organs in rats. Therefore, the variations of these parameters would explain those observed in the organs. In male rats, only an increase of spleen and kidneys weights was observed in the 300 mg/Kg lot of Aframomum melegueta extract compared to the control batch. The organs of female rats showed some variation mainly for the extracts of Aframomum melegueta and Khaya senegalensis. These were livers, heart, lungs and kidneys for the doses of 100 and 300 mg/Kg, which experienced a significant decrease in weight. On the other hand, Aframomum melegueta was found to have a hepato-protective effect on rats (Idoh et al., 2013). In addition to its hepato-protective potential, Aframomum melegueta is believed to have anti-apoptotic activity (Gbekley et al., 2017). No signs of toxicity were observed for the extract of Xylopia aethiopica but studies have demonstrated its toxicity at higher doses (Obodo et al., 2013; Obhakhan et al., 2014; Chris-Ozoko et al., 2015). There was no mortality during the experiment, so variations in biological parameters do not completely affect the safety of these extracts at low doses. Similarly, these data are broadly consistent with previous results of our team who found that Aframomum melegueta and Xylopia aethiopica were not cytotoxic at 200 μg/mL but Khaya senegalensis showed moderate toxicity with peripheral blood mononuclear cell mortality greater than 20% at the same concentration (Ataba et al., 2020).

CONCLUSION AND APPLICATION OF RESULTS
Togolese plant organ extracts have various properties that can be useful in healing diseases. In our context, extracts of Khaya senegalensis and Xylopia aethiopica have an interesting capacity to inhibit lipoxygenase. In addition, extracts of Khaya senegalensis and Aframomum melegueta have the highest concentrations of polyphenols, flavonoids and have the capacity to complex DPPH- and ABTS+ free radicals. Our data
suggest that acute and subchronic toxicity oral administration of the hydroalcoholic extract of these extracts did not produce significant toxic effects in male and female Wistar rats. Thus, this could provide assurance for the medical use of these plants in traditional medicine of Togo. Through these pharmacological actions of these plant extracts could be exploited in the development of treatments for inflammatory disorders caused by helminths. For this purpose, additional studies would be necessary to determine the molecules involved in the pharmacological properties of these plant organ extracts as well as their modes of action.

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| ABTS | 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid |
| AFNOR | Association Française de Normalisation |
| AlCl₃ | Aluminium Chloride |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| b.w. | Body weight |
| BAS | Basophils |
| COX-2 | Cyclooxygenase-2 |
| DPPH | 2, 2-diphenyl-1-picrylhydrazyl |
| EDTA | Ethylene diamine tetraacetic acid |
| EGA | Gallic acid equivalent |
| EO | Eosinophils |
| EQ | Quercetin equivalent |
| FCR | Folin-Ciocalteu |
| HB | Haemoglobin |
| HCT | Haematocrit |
| IC₅₀ | The half maximal inhibitory concentration |
| ISO | International Organization for Standardization |
| LD₅₀ | Median Lethal Dose |
| LYM | Lymphocytes |
| MCH | Mean corpuscular haemoglobin |
| MCHC | Mean corpuscular haemoglobin concentration |
| MCV | Mean corpuscular volume |
| MO | Monocytes |
| NEU | Neutrophils |
| NF | Norme française |
| NRBL | Crystal Violet Agar, Neutral Red Bile Lactose |
| OD | Optic density |
| OECD | Organisation for Economic Co-operation and Development |
| PCA | Plate Count Agar |
| PLT | Platelets |
| RBC | Red blood cell |
| Th17 | T helper 17 cell |
| THs | Traditional healers |
| WAEMU | West African Economic and Monetary Union |
| WBC | White blood cells |

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