Journal of Applied Biosciences 162: 16764 - 16794
ISSN 1997-5902

Anti-inflammatory, antibacterial and antioxidant activities of *Chenopodium ambrosioides L. (Chenopodiaceae)* extracts

Batcha Ouadja[1], Gnatoulma Katawa[1], Gerard A. Toudji[1], Laura Layland[2], Efui H. Gbekley[1], Manuel Ritter[2], Kokou Anani[1], Yaovi Ameyapoh[1], Simplice D. Karou[1]*
[1] Laboratoire de Microbiologie et de Contrôle de qualités des denrées Alimentaires (LAMICODA), Université de Lomé, Togo
[2] Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn (UKB), Bonn, Germany.
***Corresponding author:** simplicekarou@hotmail.com

Submitted on 12th April 2021. Published online at www.m.elewa.org/journals/ on 30th June 2021
https://doi.org/10.35759/JABs.162.7

ABSTRACT
Objective: Chenopodium ambrosioides is an aromatic plant widely used in the Togolese traditional medicine. However, little is known about its pharmacological properties. The present study aimed to investigate the anti-inflammatory, antibacterial and antioxidant activities of its extracts.
Methodology and results: Thus, an ethnopharmacological survey was undertaken in Kara town in the northern part of Togo to assess the main uses in the traditional medicine and Gas Chromatography coupled with Mass Spectral Analysis (GC-MS) was used to identify several compounds in the extracts. Antioxidant activity was evaluated by FRAP, ABTS, DPPH methods. Anti-inflammatory and antimicrobial activities were assessed by soybean lipoxygenase inhibition and *in vitro* broth micro dilution techniques, respectively. Propidium iodide cell death was evaluated by flow cytometry and *in vivo* toxicity was assessed using wistar rats. The ethnopharmacological survey revealed that traditional healers use *C. ambrosioides* to treat malaria, intestinal worms and inflammation in addition to healing wounds. The hydroethanolic extract had high content of total phenols (324.80±17.30 µgEAG/mg) and flavonoids (63.20±8.70 µgEQ/mg), however the highest antioxidant, antimicrobial and antiinflammatory activities were obtained with the essential oil. GC-MS analysis leads to identification of hydrocarbon monoterpenes such as 2-carene, ortho-cymene and α-terpinene as the major components of the essential oil. All the tested extracts induced cell death (14.60 ± 9.23% at 2 µL/mL, 72.64 ± 15.92% at 200µL/mL and 89.50 ± 7.16 at 100 mg/mL for essential oil, hydrosol and hydroethanolic extract, respectively).
Conclusion and application of results. The present study demonstrated various pharmacological activities of hydroethanolic extract, essential oil and hydrosol of leaves of *Chenopodium ambrosioides.* The essential oil had antioxidant, antimicrobial and antiinflammatory activities and could be the most active component of the leaves of *Chenopodium ambrosioides.* Our findings highlighted perspectives for the discovery of new medicinal molecules derived from plant extrats and confirmed certain practices of traditional healers.
Keywords: *Chenopodium ambrosioides,* extract, pharmacological activities

INTRODUCTION

Chenopodium ambrosioides, commonly called Mexican tea, is an aromatic plant belonging to *Chenopodiaceae* family (Kliks, 1985). This plant is widely used in the Togolese pharmacopoeia and is known for its virtues (Magbéfon *et al.*, 2009). The leaves are used for the treatment of abscesses, epilepsy and nausea (Alain *et al.*, 2012) and moreover, the whole plant has been used as vermifuge (Chevallier, 1996; Kuete, 2014). The laboratory screening of the plant showed that its extract exhibits antimicrobial (González *et al.*, 2010; Hameed, 2014; Peter *et al.*, 2018) and anti-parasitic activities (Aline *et al.*, 2014; Fernando *et al.*, 2008; Salifou *et al.*, 2013). Other reports on the plant mentioned its antioxidant, anti-inflammatory, antipyretic, analgesic and antidiabetic activities (Luz *et al.*, 2017; Muhammad *et al.*, 2016; Carlos *et al.*, 2017, Ibironke *et al.*, 2007,Wanderson *et al.*, 2018; Song *et al.*, 2011; Magbéfon *et al.*, 2009). The essential oil of the plant, obtained by distillation exhibited insecticidal and acaricidal properties (Chiasson *et al.*, 2004,Cloyd *et al.*, 2007). Through various techniques, it was proven that this essential oil exerts more pronounced anti-microbial (Alain *et al*., 2012; Cécile *et al*., 2006) , anti-parasitic (Chuan *et al.*, 2011; Monzote *et al.*, 2011) activities than the ethanolic extract. Mostly used in popular medicine as an anti-inflammatory, this activity of essential oil is less documented (Alain *et al.*, 2012). However, its uncontrolled use can be sources of harmful effects (Koba *et al.*, 2009,Ruth *et al.*, 2015). Chemical examination showed that the composition of this oil highly varies depending of climatic and pedological conditions of the growing environment (Alain *et al.*, 2012;Carolina *et al.*, 2008Lohani *et al.*, 2012). Few studies focused on bioactivities and adverse effects of hydroethanolic extracts, essential oil and hydrosol of leaves of *Chenopodium ambrosioides L. (Chenopodiaceae)* from Togolese biodiversity (Ouadja *et al.*, 2020). Hence, this study aimed to investigate the pharmacological activities of hydroethanolic extracts, essential oil and hydrosol of leaves of *Chenopodium ambrosioides L. (Chenopodiaceae)* from Togolese biodiversity.

MATERIAL AND METHODS

Chemicals and reagents: Folin–Ciocalteau reagent, sodium carbonate, aluminium trichlorue ($AlCl_3$), gallic acid, ascorbic acid, quercetin, methanol, 2,2-diphenyl-picryl-hydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), linoleic acid, sodium sulfate, sodium phosphate, soda and soybean lipoxygenase were purchased from SIGMA Aldrich (Spruce, St Louis, Germany); tween 80 from Sci-Tech Co. (Gangdong Guanghua, Shantou, China) ; Sabouroud chloramphenicol agar, Mueller Hinton broth, Mueller Hinton agar from OXOID (Basingstoke, HAMPSHIRE, ENGLAND). Phosphate Buffer Salin (PBS), Pancoll, Roswell Park Memorial Institute medium (RPMI 1640), Fetal Bovine Serum (FCS), Dimethylsulfoxide (DMSO) is from ThermoFisher (GIBCO, New York, USA).

Ethnopharmacological survey: The ethnopharmacological survey was conducted from August to October 2017 in Kara town in the northern part of Togo, which is situated about 400 km from Lomé at 9° to 10° North latitude and 1° to 1° 30' East longitude (figure 1). A semi-structured questionnaire sheet including the name of the plant, pathology treated, parts used, route of administration, outcomes of the treatment, origin of the knowledge was administered. The respondents were traditional herbalists.

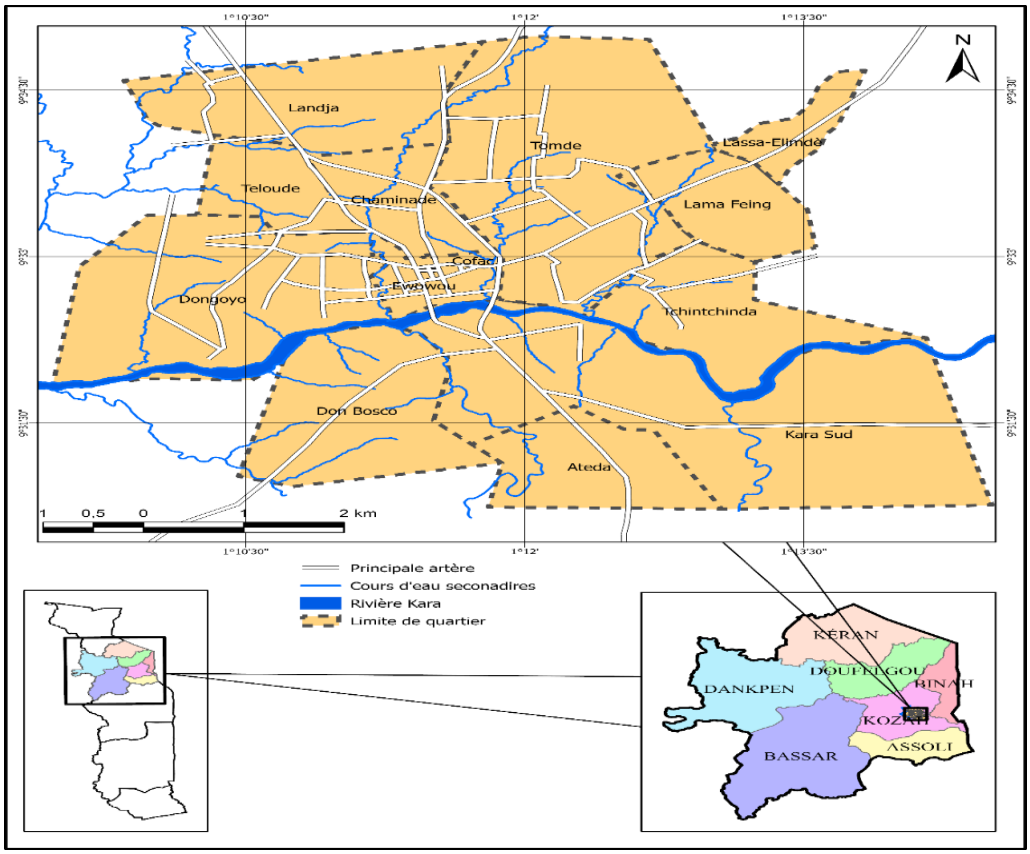


Fig.1: Map of Togo showing Kara region and the study area

Plant materials and extractions: The leaves of mature *C. ambrosioides* plants were harvested in the area of Kara between August and October 2017. The plant was botanically authenticated at the department of Botany and Plant Ecology of the University of Lomé, where voucher specimen was deposited at the herbarium under the code number «Togo 15451». The samples were dried in the laboratory at room temperature prior to ethanol extraction and hydrodistillation. The dried leaves were pulverized using a Binatone Moulinex blender. One hundred grams of the powder was percolated with one litre of ethanol 70% v/v for 48 hours. The mixture was decanted and filtered through Wattman paper. The filtrate was evaporated until dry under reduced pressure using a Heidolph Laborata 4000 type rotavapor at 60°C. The extraction rate was calculated by the following formula:

$$T = \frac{M - M0}{P} \times 100$$

T: rate, M: mass of the flask containing the extract, Mo: mass of the empty flask without extract, P: mass of *C. ambrosioides* powder.

Similarly, one hundred grams of the dried leaves of *C. ambrosioides* were distilled in the Clevenger glassworks for 3 hours. The essential oil obtained was dried on anhydrous sodium sulphate and stocked at 4°C. The experiment was repeated three times under the same conditions and the rate of oil was calculated as follows:

$$P = \frac{V}{M} \times 100$$

P: rate of the essential oil, M: Mass in grams of dried leaves, V: Volume in millilitre of the dried essential oil. The hydrosol was collected after 2 hours of hydrodistillation using a separating funnel and stocked at 4°C

Antimicrobial assay: Antimicrobial screening was performed with reference strains *Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922,* and clinical strains *Salmonella OMB, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Pseudomonas aeruginosa* and *candida albicans.* The clinical strains were obtained from "Laboratoire de Microbiologie et de Contrôle Qualité des Denrées Alimentaires (LAMICODA) of the University of Lomé. Plant extracts for the assay were prepared as follows: Upon dilution to desired concentration the emulsion was prepared by mixing 10 mL MH Broth with 1% Tween 20 and 10 mL of essential oil whereas the ethanol extract was prepared by dissolving the plant extract in sterile water using a concentration of 1 g/mL and the hydrosol extract was used without dilution. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts were determined using the Mueller Hinton (MH) broth microdilution assay in 96 well-plates according to National Committee for Clinical Laboratory (N.C.C.L.S, 2000). In brief, upon 24 hours colonies of microorganisms were suspended in 0.9% sterile water to a turbidity of 0.5 Mac Farland standards (10^8 cfu/mL). These suspensions were diluted with MH broth to inoculate 96 well plates containing 2-fold serial dilutions of the extracts. The final volume and final microbial density in wells were 200 µL and 10^5 cfu/mL, respectively. Drug concentrations ranged from 7.812 to 500 mg/mL, 3.125 to 50µL/mL and 3.125 to 50µL/mL of extract, essential oil and hydrosol respectively. The plates were incubated at 37°C for 24 h. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth. MBC was recorded as a lowest extract concentration killing 99.9% of bacterial inocula. MBC values were determined by counting microbial colonies from 100µl of bacterial suspension removed from subculture demonstrating no visible growth and inoculated Muller Hinton Agar, which had been incubated at 37°C for 48 h.

Determination of antioxidant activity

DPPH free radical scavenging : The ability of the samples to scavenge DPPH (2,2-diphenyl-picrylhydrazyl) free radicals was evaluated as described previously (Velàzquez *et al.*, 2003). This protocol was based on the decrease of absorbance at 517 nm due to the stable free radical DPPH in the presence of a radical donor $H^{\bullet}$. The assay was performed in 96-well plates and each well of the plate received 300µL of solution constituted of 100µL of methanol, 100µL of DPPH radical (20µg/mL) diluted in methanol and 100µL of different concentrations (5 to 0.078 mg/mL) for hydroethanolic extract. For essential oil and its hydrosol, a single concentration of 100µL/mL was constituted. A methanolic solution of ascorbic acid was added to 100µL of the DPPH solution as a positive control. A negative control was also constituted using the methanolic solution of DPPH as sample. The plates were incubated for 15 min at 30°C in dark. The test was performed in triplicate and absorbance's were recorded at 517nm using a multiwell plate reader (Epoch, bioTek, Winooski, US). The anti-radical activity was evaluated as a percentage of free radical scavenging and as µg ascorbic acid equivalent per mL (µg AAE/mL) of sample respectively for hydroethanolic extracts, essential oil and hydrosol.

ABTS free radical scavenging : The 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) assay was performed according to Re *et al*. (1999). The $ABTS^{\bullet+}$ radical was generated by dissolving 37.7mg of ABTS and 6.48mg of potassium persulfate in 9.802mL of water. The mixture was homogenized and kept in darkness for 16h. The solution was then diluted in ethanol to obtain an absorbance of approximately 0.70±0.02 at 734nm. At that

wavelength, a linear absorbance as a function of the $ABTS^{\bullet+}$ radical concentration was obtained up to an absorbance of 2. Each drug was diluted as follow: the hydro ethanolic extract was dissolved in water to obtain a concentration of 1mg/mL (w/v) and the essential oil diluted by 1/2 in methanol. The hydrosol was not diluted. The assay was performed by incubation of 200 µL of chemically generated $ABTS^{\bullet+}$ with 50 µL of drug samples in wells within 15 min at room temperature in darkness. The decrease in absorbance of controls and samples were recorded at 734 nm. The amount of $ABTS^{\bullet+}$ radical scavenged was calculated using ascorbic acid standard curve. The results were expressed as µg AAE/mg.

Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP): The presence of reductants in samples induces the reduction of iron Fe^{3+} a ferricyanide complex to the ferrous form Fe^{2+} as a result of a mono electronic electron transfer (Oyaizu, 1986). Therefore, Fe^{2+} can be evaluated by measuring and monitoring the increase in density of the blue-green color at 700 nm (Amarowicz *et al.*, 2004). Thus, the iron (Fe^{3+}) reduction capacity of *Chenopodium ambrosioides* samples were determined as previously described (Aïra, 2012) and miniaturized in 96 well plates (Ablassé, 2017). One millilitre of different concentrations of extract (0.5–2.5 mg/mL), hydrosol (400-1000µL/mL) and essential oil diluted in methanol (10-50µL/mL) were mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%) was added. Finally, the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicates an increase of reduction capability. Ascorbic acid is used as positive control. Results were evaluated as µg AAE/ml or mg.

Anti-inflammatory activity

Inhibition of lipoxygenase activity: Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1,4-diene structures (Alain *et al.*, 2012). The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed by absorbance measuring at 234 nm. Gallic acid and quercetin were used as a reference drug. Briefly, 146µl of the enzyme solution (400U/mL) prepared with borate buffer (0.2M; pH 9.0) were mixed with 3.7µl of samples, 98.83µg/mL, 12.2µL/mL and 12.3µL/mL respectively of hydroethanolic extracts, essential oil (1% DMSO in buffer) and hydrosol. The reaction was then induced by adding 150µL of linoleic acid (1.25mM) afterward and the optical densities were recorded at 234 nm at different times. Borate buffer and enzyme were used as negative control, substrate and enzyme as positive control. The test was repeated at least three times. The experimental conditions were adapted from those previously described (Alain *et al.*, 2012, Alitonou *et al.*, 2010).

The percentage inhibition of lipoxygenase (%I) was calculated according to the following equation:

$$\%I = \frac{ODcontrol - ODsample}{ODcontrol} \times 100$$

OD control = enzyme activity without sample
OD sample = enzyme activity with sample.

Carageenan-induced paw oedema in rats: Oedema was induced by injecting into the aponeurosis of the left hind leg of the rats using 0.1 mL of 1% (W/V) fresh carageenan suspension prepared with a normal saline solution according to the method described by Toudji *et al.*, (2018) with several modifications. Briefly, after the rats had been fasting for 12 hours, the test groups (4 and 5, n= 3) were treated orally for three days with

400 and 800 mg/Kg of the hydroethanolic extracts respectively. Thirty minutes after the treatment, the oedema was induced by injecting carageenan fresh suspension. For the control and references groups the extract was replaced by respectively (1) no treatment, (2) 10 mg/Kg normal saline solution and group (3) received ketoprofen 20mg/Kg. The measurement of the oedema was carried out before and after induction of oedema using dislocation of the water column in a plethysmometer at 0, 1, 2, 3, 4, 5 hours and on the third day after carrageenan injection. Blood sampling on EDTA tubes was performed in all groups by retro orbital puncture after petroleum ether anaesthesia for white blood cell count. The anti-inflammatory activity was assessed by the percentage of oedema inhibition and calculated using the following formula:

$$1 - \frac{V}{Vo} X100$$

Where V_O was a median volume of control group and V was a median volume of the test groups

Toxicological Assessment of *Chenopodium ambrosioides* leaves

***In Vitro* cytotoxicity using peripheral blood mononuclear cells (PBMCs):**

Extraction of PBMCs: Blood samples were purchased from the National Blood Transfusion Center (CNTS) of Togo. Each sample (20mL) was mixed with 15mL of phosphate buffered saline (PBS). The mixture was slowly poured into 15mL of pancol. Tubes were centrifuged at 2000rpm using a Bench Top 14k centrifuge (HuMax, Wiesbaden, Germany) for 20min. The PBMCs were collected in tubes containing 50mL of RPMI+++ (gentamicin+, L-Glutamine+, Streptomycin+). The tubes were centrifuged twice at 1300rpm for 8min. A suspension of cells was prepared in one millilitre of RPMI+++ with 10% fetal calf serum (FCS) and the viability of cells was determined using the trypan blue exclusion technique with Neubauer cell counter using an inverted microscope.

Co-culture of PBMCs with hydroethanolic extract, essential oil and hydrosol from *Chenopodium ambrosioides* leaves : Five concentrations of the essential oil ranging from (0.4, 0.2, 0.1, 0.05 and 0.025µL/mL) in DMSO 10%, seven dilutions of hydrosol (20, 10, 5, 2.5, 1.25, 0.75 and 0.312%) and seven concentrations of hydroethanolic extract (100, 50, 25, 12.5, 6.25, 3.12 and 1.56mg/mL) were co-cultured with 2×10^5 PBMCs/well in duplicate and unstimulated wells, 10% and 100% DMSO were used as controls. The plates were incubated for 24 hours at 37°C, 5% CO_2 and wet atmosphere conditions. After incubation, the supernatants were removed and 100µL of FACS buffer was added to the cells in the wells. These cells were recovered in haemolysis tubes; then 200µL of FACS buffer was added for washing. After washing cells were stained with propidium iodide (PI) in order to evaluate the *in vitro* toxicity of the plant materials as described by Ataba *et al*., (2020). The percentage of dead cells was evaluated by flow cytometry using Cytoflex flow cytometer (Beckman Coulter, Brea, Califinia, USA).

***In Vivo* Toxicological Assessment**

Experimental animals and housing : The experiment was conducted as previously described using 24 healthy Wistar rats weighing 90 to 130g which were purchased from Faculty of Sciences, University of Lomé (Toudji *et al.*, 2017). They were kept in cages and acclimatized in the animal house of "Laboratoire de Microbiologie et Contrôle Qualité des denrées alimentaires" (LAMICODA) University of Lomé, at room temperature and 12-hours photoperiod/ dark cycle. Animals were provided with food and water *ad libitum* and they were acclimatized for a period of 8 days before starting the experiment.

Acute oral toxicity investigation : Acute toxicity at a single dose was evaluated following the Organization for Economic Co-operation and Development (OECD) guideline, method 423 (OECD, 2001). After acclimatization, rats were fasted overnight, marked and weighed. Animals were divided randomly into 7 groups (n=3). Each group received orally: group 1 (control, saline water); groups which received essential oil: group 2 (50 µL /Kg), group 3 (100 µL/Kg), group 4 (200µL/Kg); group treated with hydrosol, group 5 (2mL/100g); group treated with fresh leaves extract, group 6 (2mL/100g) and group treated with hydroethanolic extract, group 7 (5g/Kg). Extracts for experiments were prepared as follows, tween 80 (2% solution prepared with distilled water) was used for dissolving essential oil. Fresh leaves extract (50±10g mixed with 100mL distilled water) and ethanolic extract (50mg/mL with distilled water). The experiment was conducted on the 7 groups each received a single dose of the drugs. The animals were observed individually during the first five hours, then daily throughout the 14 days of the experiment. Signs and symptoms of toxicity were recorded. Observations focused on the determination of death and time of occurrence, including changes in skin, fur, mucous membranes and eyes, respiratory and circulatory systems, central nervous and autonomic systems, somatomotor activity and behaviour. Attention was paid to the potential occurrence of, seizures, salivation, diarrhoea, lethargy, drowsiness, and coma.

Repeated dose 28-day oral toxicity study: The repeated dose 28-day oral toxicity study was performed with only hydroethanolic extract and following OECD test guideline-407 (OECD, 2008). Twelve female rats (92±20 g bw) and 12 male rats (107±19 g bw) were used. The animals were randomly assigned to three groups (n = 8: 4 females and 4 males). Each rat in Group I (control group) received only saline water. Groups II, and III received the hydroethanolic extract dosed at 200 and 500 mg/kg bw, respectively for 28 days. The animals were gavered at approximately the same time each day. The animals were monitored for signs of toxicity and mortality twice a day throughout the experimental period of 28 days. The weight of each animal was recorded daily throughout the course of the experiment. After treatments, on 29th day the body weight of each rat in the experiment was measured and recorded. They were anaesthetized with petroleum ether and blood samples were collected in EDTA tubes and anticoagulant free tubes via retro-orbital puncture and assessed for haematological and biochemical parameters. Animals were sacrificed according to OECD recommendation, and the heart, lungs, liver, spleen, kidney, and testis or ovaries were analysed for macroscopic visual characteristics and excised immediately and weighed. The blood cell count was performed on the Sysmex automated system SN A4201 (Sysmex, Kobe, Japan). Biochemical parameters were determined with Automatics Biochemical Analyzers, models Roch/Hitachi cobas C 311 (Hitachi, Tokyo, Japan) and Selectra PoM (Elitech, Netherland).

Phytochemical screening of *Chenopodium ambrosioides* leaves

Histochemistry of C. ambrosoides leaves: The main leaf rib was isolated and thin sections were made. The sections were deposited on an object-holder slide and treated for 2 minutes with the characteristic reagent of each metabolite to be detected, 1% aluminium trichloride to detect tannins, 1N sodium hydroxide for flavonoid and Dragendorff's reagent for alkaloids. The sections were then washed with distilled water and observed with a Motic BA 200 microscope. In order to perform the structural arrangement of leaf tissues and to localize the secretory structures of the essential oil, fine cuts were made on the main rib of leaf. The cuts were soaked in chlorine solution for 15 to 20 minutes. The cuts

were washed abundantly with water and soaked in acetic acid for 5 minutes. Then they were washed once with water and stained with the carmino-green reagent for 5 min. The stained cuts were washed with abundant water and observed with the microscope.

Determination of total phenolic content: Total phenolic content from hydro ethanolic extract were quantified using Folin-Ciocalteu's method (Singleton *et al.*, 1999) . Thus, 125 µL of Folin-Ciocalteu's reagent (0,2N) was added to 25 µL of 10 mg/mL of hydroethanolic plant extract dissolved in water. After 5 min incubation at room temperature, 100 µL of sodium carbonate in water (75g/L) were added and after 60 min of incubation, the absorbencies were read at 760 nm using a multiwell plate reader (Epoch, bioTek, Winooski, US). All the assays were carried out at least in triplicate. Gallic acid was used as standard and results were expressed as µg/gallic acid equivalent per gram of extract.

Determination of flavonoids content : The flavonoid content of the hydroethanolic extract was assessed using the aluminium trichloride method previously described (Arvouet *et al.*, 1994). Briefly, 100µL of the extract (1mg/mL) diluted in methanol was mixed with 100µL of an $AlCl_3$ 2% methanolic solution in the wells of a 96-well plate. Quercetin was used as the reference flavonoid for calibration curve. A blank was constituted of 100µL of extract and 100µL of methanol. The experience was repeated three times and the absorbance were recorded at 415 nm and the results expressed in µg Quercetin Equivalent for 1g of extract.

GC-MS analysis : The chemical analysis of studied samples was performed using an Agilent Technologies model 7890B gas chromatograph (GC), equipped with a capillary column type HP-5MS 5% Phenyl Methyl Silox4635344H of 30m length, 0.25mm diameter and 0.25µm film thickness, with mass spectrometry (MS), 5977B MSD and an injector set at 250°C. The injection mode was manually in a split mode (leakage ratio: 1/50, flow rate: 66ml/min). Column temperature program 50 to 250°C at 5°C.min^{-1}. The apparatus is managed by a "MassHuter" system that manages the operation of the apparatus and allows the acquisition of chromatographic data. The mass spectrometer was operated at 70ev field. The carrier gas is helium at a flow rate of 1.2ml/min. The quantitative analysis was carried out with the application Quantitative Analysis version B07.01/Build 7.1.524.0. The instrument is connected to a computer system managing a NIST mass spectra library.

Statistical analysis: Microsoft Excel (2013) spreadsheet was used to draw the standard curves. Differences between means were tested using Anova one-way analysis of variances test and the Student t-test of the Graph Pad software. Statistical differences were considered significant at $p<0.05$.

RESULTS

Ethnopharmacological study: One hundred traditional healers (THs, 12 males and 88 females), mean age 58±16 years were interviewed in Kara town for several uses of *C. ambrosoides*. Four names were recorded for the plant in the local languages, namely *Doma koye* and *Dom gneou* in Kabye language, *Gbanssoukou* in Bassar and Kotokoli languages and *Magbedode in* Ewe language. Concerning the use of the plant parts, 15 THs use the whole plant, while 85 prefer the use of leaves only. The source of information from 89 THs were obtained within family whiles the rest were sourced from neighbours. The main treatments that were indicated included malaria (36%), intestinal worms (20%), wounds (15%) and inflammation (6%). The mode of preparation and administration route included the juice obtained by crushing the fresh materials for syrup (50%), or to be applied directly on the cataplasm (7%), the decoction for bathing (27%) and the sauce

obtained by crushing the plant materials and preparing with caesame (16%) used for its nutrients. The notable adverse effect was vomiting which was observed by 30 THs.

Antimicrobial activities : To evaluate the antimicrobial activities of leaves of *C. ambrosioides*, the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined. Thus, essential oil inhibited the growth of all microbial strains with MICs ranging from 3.125 to 50 µL/mL. The lowest MIC was obtained on *S. aureus* and the highest MIC was obtained on *P. aeruginosa*. However, the activity of the hydroalcoholic extract on six microbial strains was only obtained at high concentrations (500 mg/mL). The hydrosol inhibited only the growth of *C. albicans*. (Table 1).

Table 1: Antimicrobial activities of essential oil, hydrosol and ethanolic extract of the leaves of *Chenopodium ambrosioides*

Sample/ Microorganisms	**Essential oil (µL/mL)**		**Hydrosol (%)**		**Hydroethanolic extract (mg/mL)**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
S. aureus	3.125±0.00	50±0.00	-	-	500±0.00	>500
S. aureus ATTC 29213	6.25±0.00	500±0.00	-	-	500±0.00	>500
S. aureus ATTC 29923	6.25±0.00	>500±0.00	-	-	500±0.00	>500
E. coli ATTC 25922	25±0.00	200±0.00	-	-	-	-
K. pneumoniae strain a	12.5±0.00	12.5±0.00	-	-	-	-
K. pneumoniae strain b	12.5±0.00	12.5±0.00	-	-	-	-
P. aeruginosa	50±0.00	200±0.00	-	-	-	-
S. OMB	6.25±0.00	6.25±0.00	-	-	500±0.00	>500
C. freundii	12.5±0.00	>500±0.00	-	-	500±0.00	>500
C. albicans	12.5±0.00	200±0.00	50±0.00	50±0.00	500±0.00	>500

- : No activity

Antioxidant activity: The IC_{50} of the hydroethanolic extract obtained with the DPPH method was 50 times lower than that of ascorbic acid (94.57±3.13 µg/mg and 1.88±0.32 µg/mg respectively). The essential oil of the leaves of *C. ambrosioides* had the highest antioxidant activity 8358±937.30 µg AAE/mL with FRAP method, (Table 2).

Table 2. Antioxidant activities of essential oil, hydrosol and ethanolic extracts of the leaves of *Chenopodium ambrosioides L*.

Samples	**Antioxidant activities**

HDE (µgAAE/mg)	FRAP	ABTS	DPPH
	32.48 ±1.59	45.33±2.47	-
HE (µg AAE/mL)	8358±937.30	2108±60.15	1597 ±194.20
HD (µg AAE/mL)	30.51±1.12	1274±4.15	271.60±31.64

AAE: Ascorbic acid equivalent, IC_{50}: Concentration that inhibit 50% of DPPH, HDE: Hydroethanolic extract; HD: Hydrosol 0.8µL/mL, HE: Essential oil 0.4µL/mL, QE: Quercetin equivalent, GAE: Gallic acid equivalent Results are expressed as mean ± SD, n= 8 and compared to p<0.05

Anti-inflammatory activity of *Chenopodium ambrosioides* leaves

Lipoxygenase inhibition: The essential oil of *C. ambrosioides* had an interesting anti-inflammatory activity through the capacity to inhibit lipoxygenase activity from 95.14±1.83% (Figure 2A); 98.11±0.90% up to 96.82±0.40% at the 10th min of the kinetics compared to the reference molecules. The activity of hydrosol was not different from that of quercetin (Figure 2B); while hydroalcoholic extracts did not exceed 31% (Figure 2C).

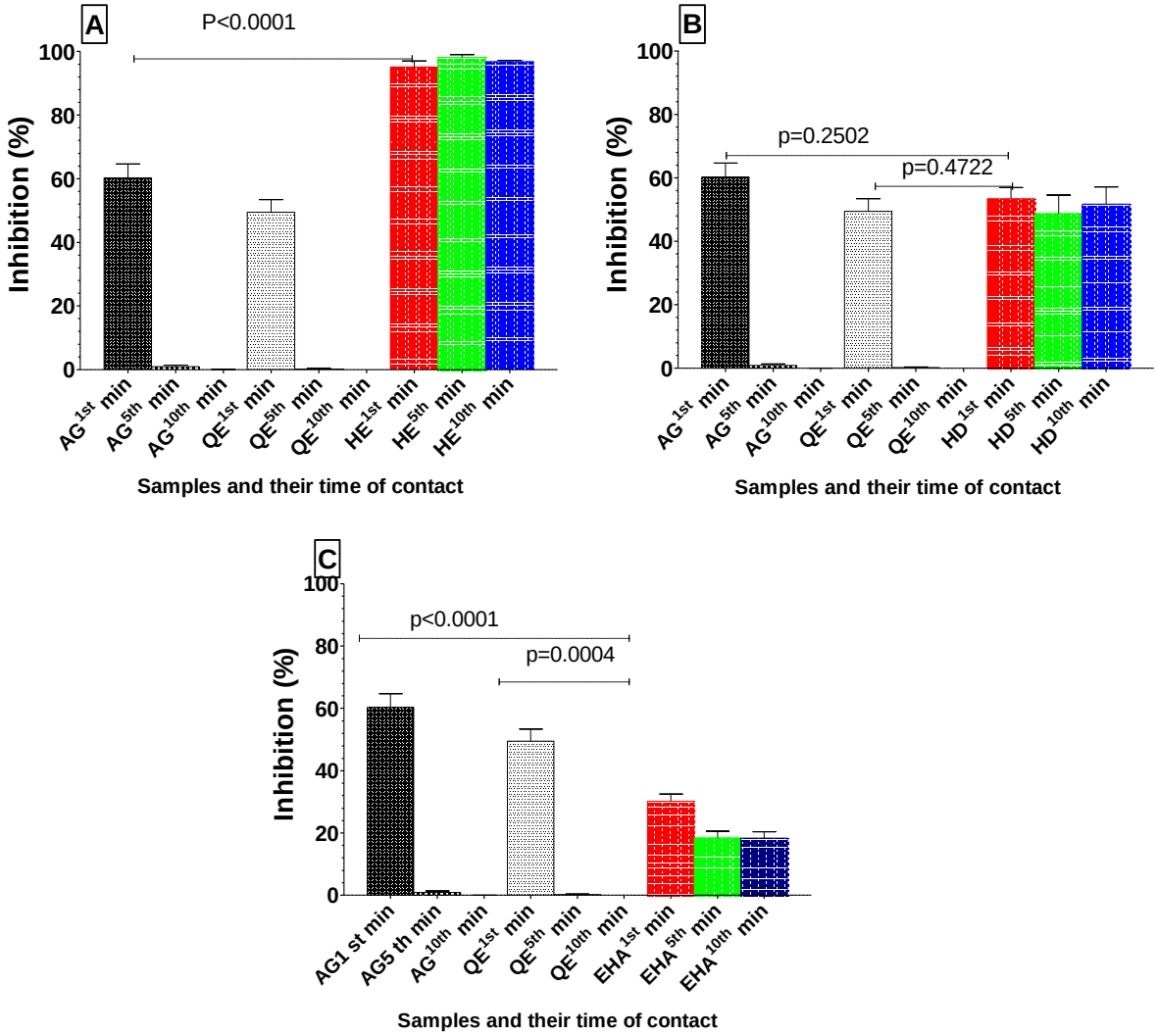


Fig. 2: Lipoxygenase inhibition by *C. ambrosioides*.

A: essential oil (12.22µL/mL); B: Hydrosol (12.33µL/m); C: Hydroethanolic extract (98.83µg/mL). AG (gallic acid 10µg/mL), $AG^{1st\ min}$: first minute of contact, $AG^{5th\ min}$: five minutes of contact, $AG^{10thmin}$: ten minutes of contact; QE (Quercetin 10µg/mL), $QE^{1st\ min}$: first minute of contact, $QE^{5th\ min}$: five minutes of contact, $QE^{10thmin}$: ten minutes of contact were used as control. HE: essential oil, $HE^{1st\ min}$: first minute of contact, $HE^{5th\ min}$: five minutes of contact, $HE^{10thmin}$: ten minutes of contact. HD: Hydrosol, $HD^{1st\ min}$: first minute of contact, $HD^{5th\ min}$: five minutes of contact, $HD^{10thmin}$: ten minutes of contact. EHA: Hydroethanolic extract. $EHA^{1st\ min}$: first minute of contact, $EHA^{5th\ min}$: five minutes of contact, $EHA^{10thmin}$: ten minutes of contact. Box whiskers (tukey) with outliers show the percentage of inhibition of lipoxygenase. P values were determined by Mann-Whitney U-test.

Inhibition of carageenan induced paw oedema and white blood cells count: Table 3 shows the inhibitory effect of *C. ambrosioides* hydroalcoholic extracts and reference drug ketoprofen. From the first hour to the third day of the test, carrageenan induced paw oedema in rats, which was not inhibited by oral treatment with hydroethanolic extract of *C. ambrosioides*. Only the reference drug ketoprofen inhibited the oedema from 71.21% (4H) to 90.90% (5H).

Table 3: Effect of *Chenopodium ambrosioides* hydroethanolic extracts on carageenan-induced paw oedema in rats

Treatment	**mg/Kg**	**Oedema volume (%)**					
		1H	2H	3H	4H	5H	Third day
Saline solution	10	0.21±0.08	0.24±0.07	0.33±0.08	0.33±0.08	0.33±0.08	0.11±0.11
Ketoprofen	20	0.06±0.02 (73.02)	0.05±0.03 (81.25)	0.05±0.04 (85.00)	0.10±0.08 (71.21)	0.03±0.02 (90.90)	0.11±0.12 (0.00)
C. ambrosioides	400	0.29±0.03 (NI)	0.46±0.12 (NI)	0.46±0.04 (NI)	0.43±0.03 (NI)	0.47±0.07 (NI)	0.21±0.04 (NI)
C. ambrosioides	800	0.24±0.03 (NI)	0.38±0.03 (NI)	0.51±0.12 (NI)	0.51±0.06 (NI)	0.53±0.07 (NI)	0.31±0.10 (NI)

Values are expressed as mean±S.E.M. (n = 3) NI, No inhibition

Similarly, no inhibition effect was noted based on white blood cell counts. It was noted that there was a drastic decrease of inflammation cell counts on the first day and on the third day from the rats treated with 400 mg/Kg of hydroethanolic extract and ketoprofen respectively. However, these reductions were not statistically different when compared to the control at $p<0.05$ as shown, Figure 3.

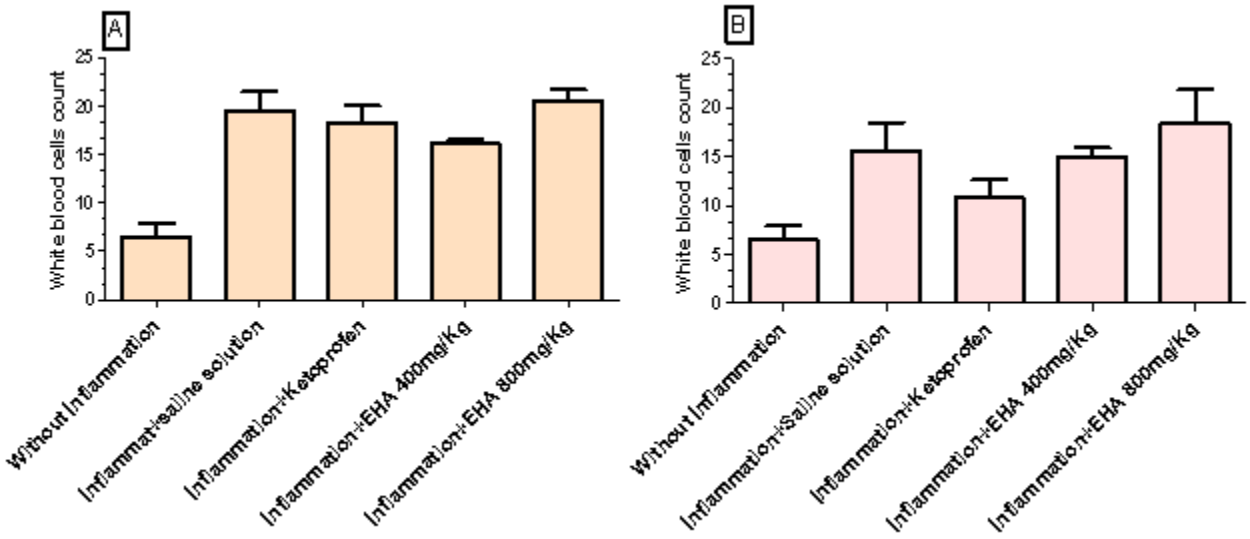
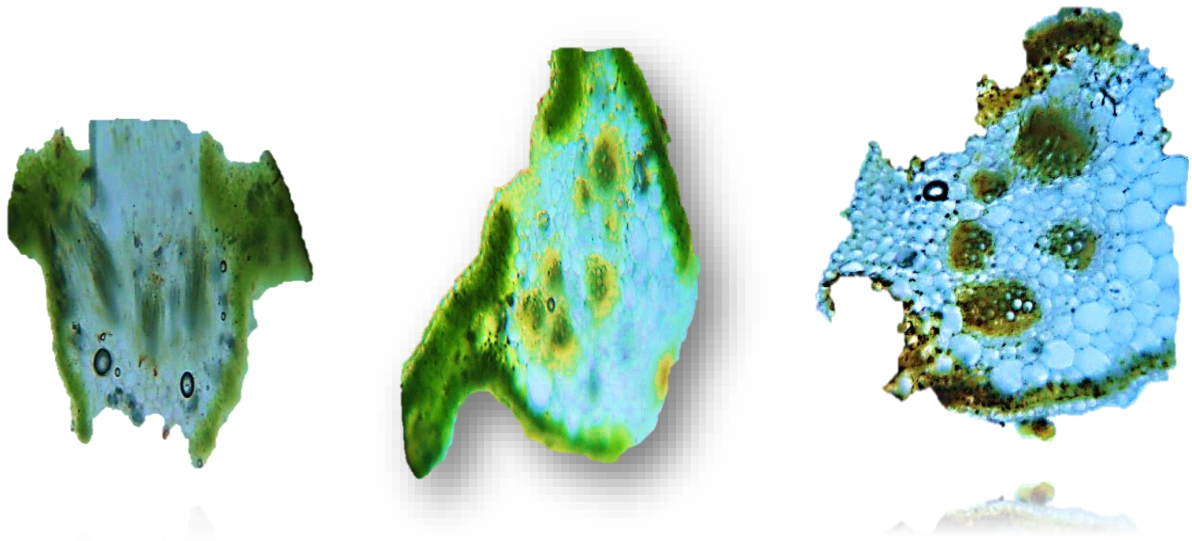


Fig. 3 Effect of *Chenopodium ambrosioides* on rats white blood cells count
***A**: cells count of day 1; **B**: cells count of day 3. **EHA**: hydroethanolic extracts. Values were expressed as mean ± SD (n=3); No statistically difference at p<0.05*

Phytochemical screening

Chemical groups and structuration of the leaves of *Chenopodium ambrosioides* leaves : *Chenopodium ambrosioides* leaves containing tannins and flavonoids had green-black (Fig. 4A) and yellow (Fig. 4B) colours respectively and were localized particularly at the edges and in some areas inside of the leaves. The screening for alkaloids with Dragendorff reagent was negative (Fig. 4 C). Furthermore, the use of carmine green localized glandular cells secreting the essential oil were towards the anal part of the leaves. Others tissues were also localized as shown (Fig. 5.)



(A) Green-black coloured tannins **(B) Flavonoids coloured yellow** **(C) Negative search of alkaloids**

(D) **(E)** **(F)**

Fig. 4: Chemical compound groups found in the leaves of *Chenopodium ambrosioides L.*; (A): Green-black coloured tannins, (B): Flavonoids coloured yellow, (C): Negative search of alkaloids.

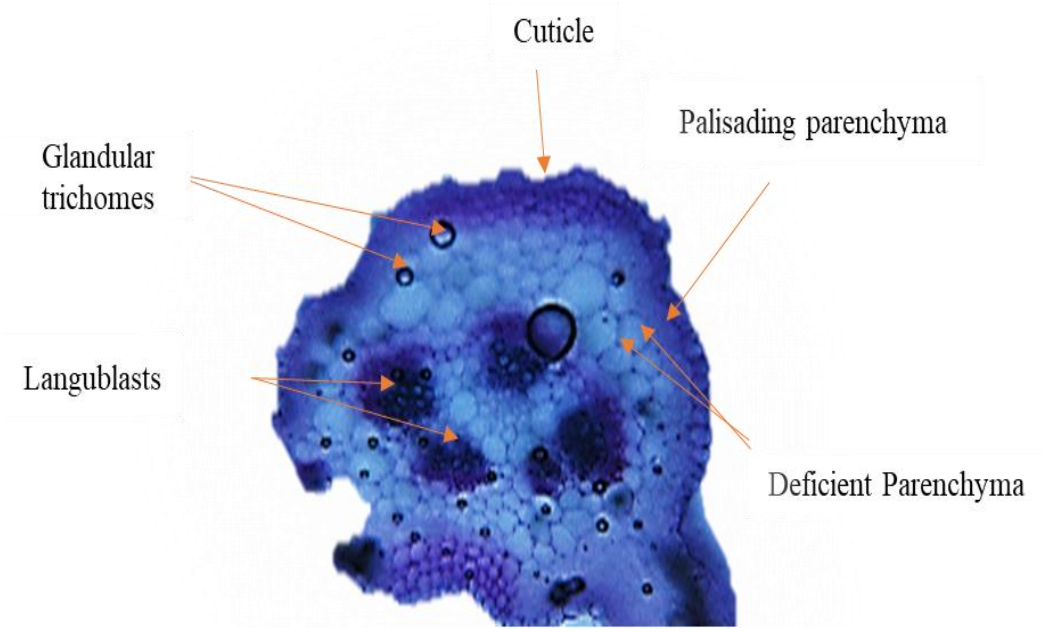


Fig. 5: Structuration of the leaves of *Chenopodium ambrosioides L.*

Total phenolic content (TPC) and Total flavonoids content (TFC)

Values of TPC and TFC were respectively 324.8±17.3 µg/mg gallic acid equivalent and 63.2±8.7µg/mg quercetin equivalent.

Gas chromatography of hydroethanolic extract and essential oil of the leaves of *Chenopodium ambrosioides L.* : A total of eight (8) components of essential oil and 10 of hydroethanolic extracts were identified using GC-MS (Fig. 6A) and (Fig. 6B) respectively. Hydrocarbon monoterpenes such as 2-carene (37.79%), ortho-cymene (33.27%) and α-terpinene (20.94%) represented the major components (93.08%) of the essential oil (Table 4). Similarly, hydro ethanolic extract contained terpenic alcohols such as Phytol (50.45%), 5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0] heptan-2-ol (22.7%) and 4-Isopropyl-5-methylhex-2-yne-1,4-diol (14.41%), (Table 5)

A

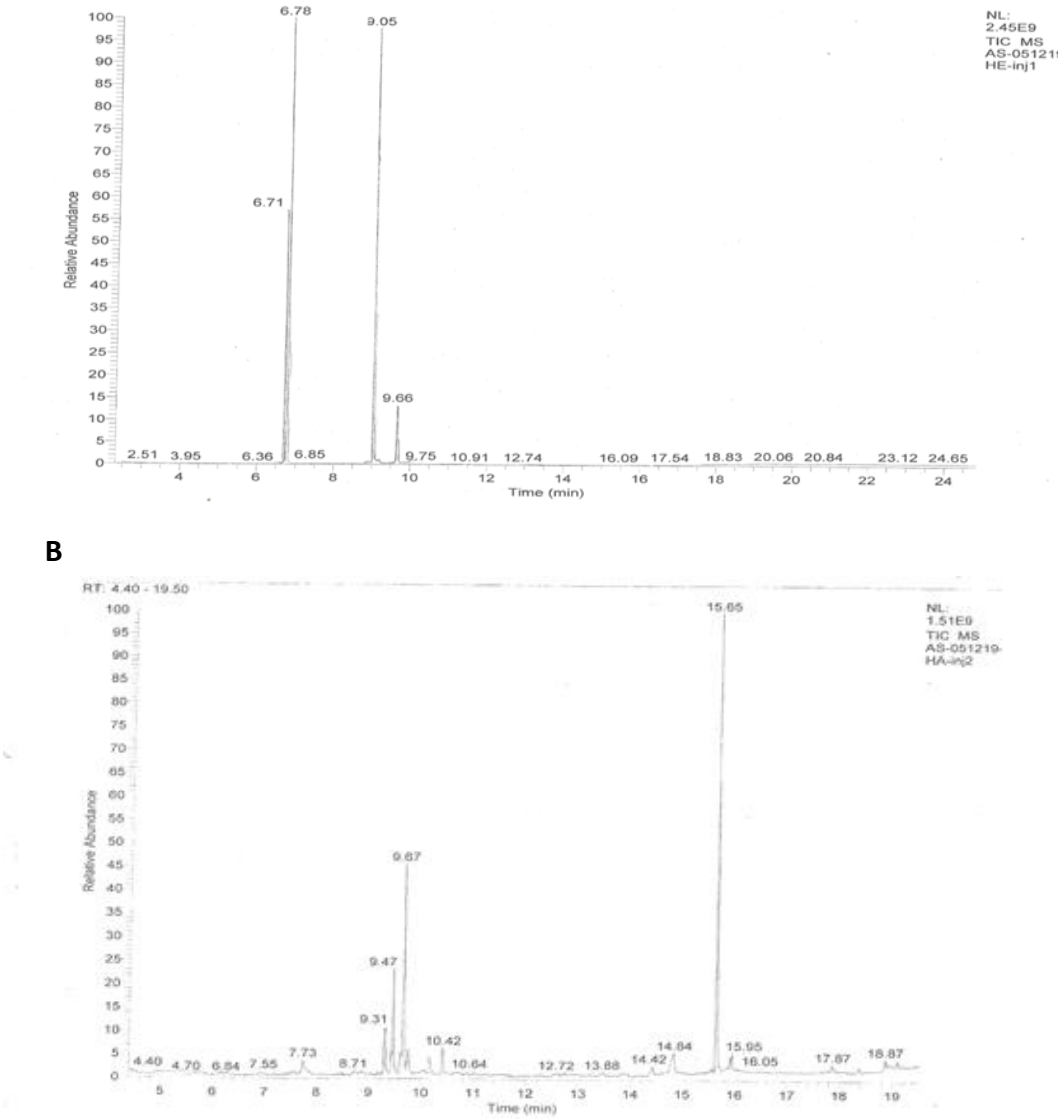
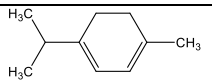
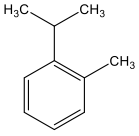
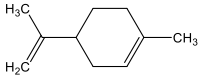


Fig. 6 Chromatograms of essential oil and hydro ethanolic extract of *Chenopodium ambrosioides* leaves. *The peaks represent the mass spectra of essential oil components (A) and the mass spectra of hydroethnolic components (B).*

Table 4: Chemical composition of *Chenopodium ambrosioides* essential oil

Retention time (minutes)	Compounds	Structure	Percentage (%)
6.71	α-terpinene		20.94
6.78	O-cymene		33.27
6.85	Limonene		0.09

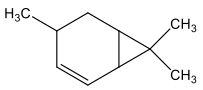
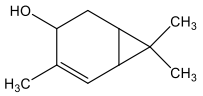
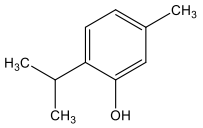
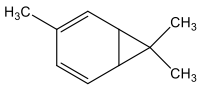
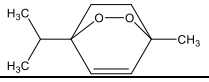
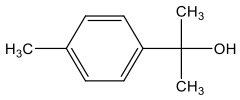
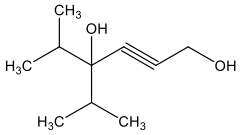
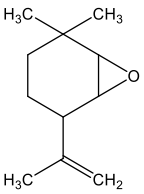
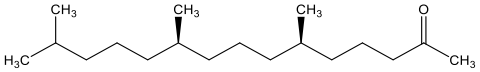
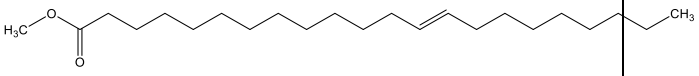
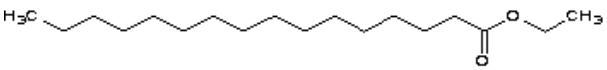
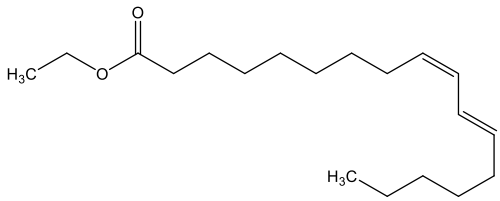
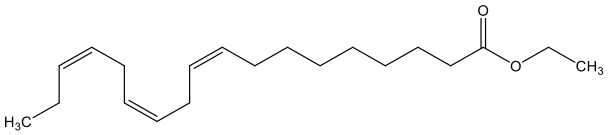
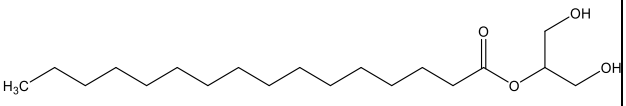
7.16	4-Carene		0.11
8.35	Trans-2-caren-4-ol		0.06
8.45	Hydroxy-p-cymene		0.08
9.05	2-Carene		37.79
9.19	Ascaridole		0.5

Table 5: Chemical composition of hydroethanolic extract

Retention time (minutes)	Compounds	Structure	Percentage (%)
8.45	p-Cymene-8-ol		Tr
9.31	4-Isopropyl-5-methylhex-2-yne-1,4-diol		14.41
9.67	5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0] heptan-2-ol		22.7
13.88	Hexahydrofarnesyl acetone (phytone)		Tr
14.41	13-Docosenoïque ester methylic acid		Tr
14.84	hexadecanoique ehyl ester acid		Tr
15.65	Phytol		50.45

15.91	Ethyl ester linoleic acid		Tr
15.96	9, 12,15-Octadecatrienoate éthyle		Tr
17.87	2-hydroxy-1-(hydroxy methyl) ethyl ester Hexadecanoic acid		Tr

TR: *traces*

Toxicity of *Chenopodium ambrosioides* leaves

***In vitro* cytotoxicity of *Chenopodium ambrosioides* leaves on PBMCs:** The cytotoxicity of *Chenopodium ambrosioides* leaves was evaluated by flow cytometry using PI staining after PBMCs were cultured with the plant extracts. According to the NF EN ISO 10993-5 standards classification, essential oil, hydrosol and hydroethanolic extract of *C. ambrosioides* leaves induced toxicity in a dose-dependent manner on PBMCs. The essential oil at 2µL/mL induced 14.60±3.77 % of $CD4^{+}PI^{+}$cells, however, this oil was very toxic at 100µL/mL (P=0.0069) (Fig. 8).

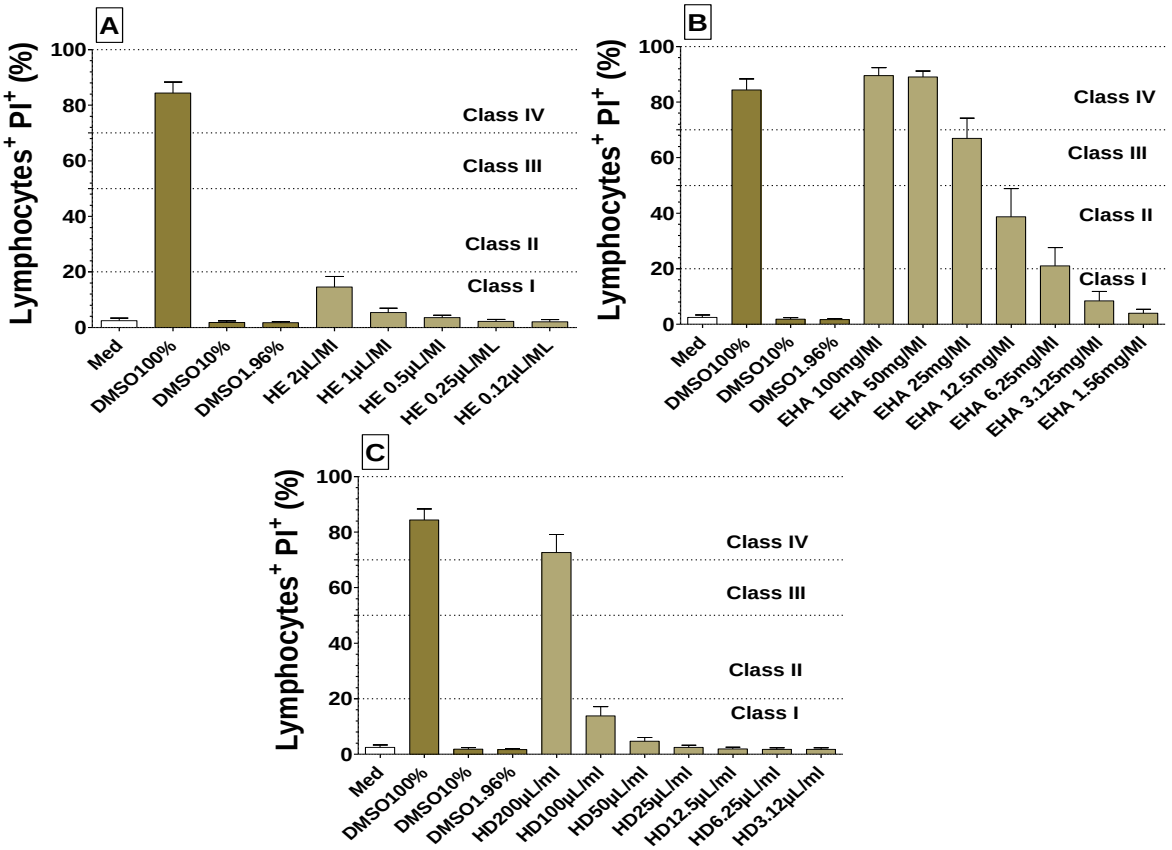


Fig. 8: Cytotoxicity of *Chenopodium ambrosioides* leaves. : Human PBMCs (2.10^5 cells/well) were left alone (Medium) or stimulated with Hydroethanolic extracts (EHA), Hydrosol (HD), essential oil (HE) and DMSO at various concentrations for 24 h. Cells were stained with propidium iodide dye (PI) and acquired by flow cytometry. Box whiskers (tukey) with outliers show the percentage of lymphocytes expressing PI (n = 12) of HE (A), EHA (B) and HD (C). P values were determined by Mann-Whitney U-test. The NF EN ISO 10993-5 standards classifications were indicated by class I (not cytotoxic), class II (moderate cytotoxicity), class III (Benign cytotoxicity) and class IV (severe cytotoxicity).

***In vivo* evaluation of essential oil, hydrosol and hydroethanolic extract of *Chenopodium ambrosioides* leaves toxicity**

Acute oral toxicity: Observations were based on changes in skin, hair, salivation and behaviour, including various manifestations of tremor, convulsions, diarrhoea, sleep, coma and death. With 200µL/Kg of essential oil agitations and motor difficulties were noted; followed by violent jumps and death of 50% of animal's which occurred after 3h30min and 100% of death in 72h after treatment. This test determined the dose of 5000mg/Kg as Maximum Tolerated Dose (MTD) for the hydroethanolic extracts. For the essential oil, the MTD was 100 µL/Kg. The lethal dose 50 (LD_{50}) was determined using Trevan curve (Tuo *et al*., 2005). The curve gives the percentage of mortality as a function of logarithms dose. Thus, the LD_{50} was obtained by projecting 50% mortality on the curve and from there the LD50 was determined on the X-axes. The calculated LD_{50} of the essential oil was 131.63µL/Kg. Thus, considering these results, only the hydroalcoholic extract was used for the subacute toxicity test.

Subchronic toxicity: In the evaluation of subacute toxicity of hydroethanolic extracts at 500 and 200mg/Kg among the 6 groups of 4 rats that were treated, all the female rats that

received the 500mg/kg survived until the last day of the test. However, for all other groups, even the controls, each recorded one dead rat. Thus, regardless of the sex, and the dose used, both rats increased in weight during the first week. Throughout the rest of the test period, the animals treated and control lost weight (Fig. 9). No significant difference in organs weight was noted between treated and control rats except the weight of livers and kidneys of female rats treated with 200mg/kg which were significantly lower than that of the controls (P=0.0141) and (P=0.0479) respectively (Table 8). There were no major variations of biochemical and haematological parameters between control rats and which had extracts (Table 6 and 7). However, there was only an increase in the urea level of females which received 200mg/Kg of extract P=0.0237 (Table 6).

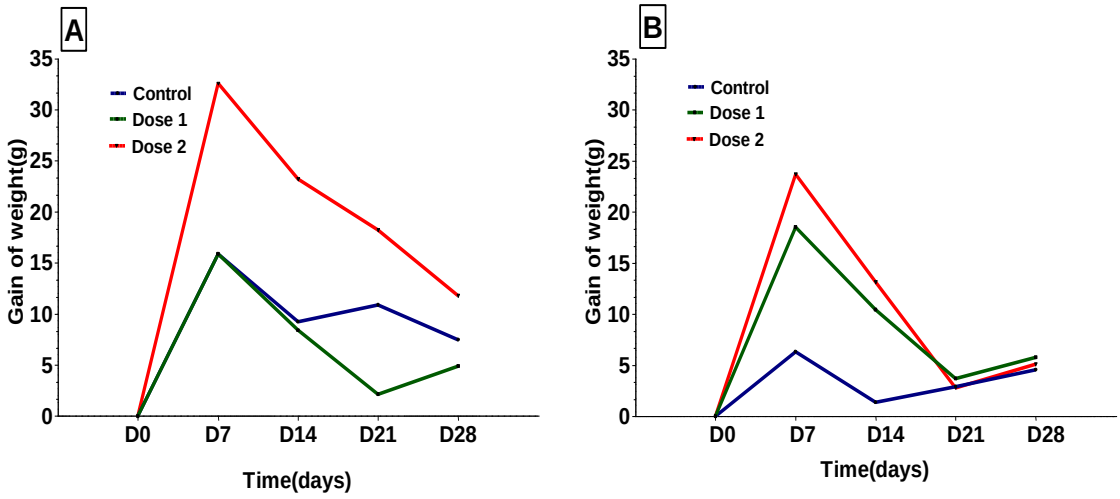


Figure 9: Body weight gain in rats treated with *Chenopodium ambrosioides L.* Hydroethanolic extract repeated oral doses (Dose 2=200mg/kg/bw, and Dose 1= 500mg/kg/bw) for 28 days. **A**: Males; **B**: Females

Table 6: Effect of hydroethanolic extract of *Chenopodium ambrosioides* leaves on biochemical parameters

Parameters	**Control**	**200 mg/Kg 200 mg/Kg**	**500 mg/Kg**
	Males		
ALT (U/L)	51.67±15.39	61.67±6.17	54±10.82
AST (U/L)	132.0±26.50	178.3±39.35	186.7±66.68
ALP (U/L)	180.0±61.20	253.0±51.29	234.0±34.12
Creatinine (U/L)	34.00±4.58	41.00±4.93	43.67±3.38
Cholesterol (g/L)	0.74±0.07	0.61±0.05	0.72±0.04
HDL (g/L)	0.36±0.05	0.34±0.08	0.39±0.06
Protein (g/L)	70.90±3.12	69.83±3.04	73.17±2.64
Urea (g/L)	0.41±0.04	0.40±0.06	0.46±0.06
Glucose (g/L)	0.73±0.17	0.63±0.18	0.81±0.11
Females			

ALT (U/L)	51±19.50	50.67±5.24	41.00±7.09
AST (U/L)	196.3±76.85	162.7±40.18	126.3±29.61
ALP (U/L)	146.7±24.84	151.3±20.34	110.3±28.26
Creatinine (U/L)	40±5.77	41.33±3.53	44±3.22
Cholesterol (g/L)	0.49±0.09	0.69±0.07	0.62±0.07
HDL (g/L)	0.29±0.05	0.43±0.06	0.40±0.06
Protein (g/L)	73.60±2.70	74.30±1.01	73.30±1.72
Urea (g/L)	0.43**[a]**±0.03	0.59**[b]**±0.03	0.50±0.07
Glucose (g/L)	0.45±0.21	1.05±0.38	0.78±0.16

*Values are expressed as Mean ± SD. n=3; a<b. P<0.05. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; HDL: high-density lipoprotein.**a**: urea value of control females; **b**: Urea value of females treated with hydroethanolic extracts 200mg/Kg*

Table 7: Effect of hydroethanolic extract of *Chenopodium ambrosioides* leaves on haematological parameters

Parameters	**Control**	**200 mg/Kg200**	**500 mg/Kg**
	Males		
WBC (10^3/µL)	8.7±2.53	6.77±1.27	11.43±1.29
RBC (10^6/µL)	8.67±0.47	7.69±0.31	7.75±0.29
HGB (g/dL)	14.47±0.47	13.50±0.29	13.07±0.95
HCT (%)	50.00±1.41	45.27±1.84	44.40±3.18
MCV (fl)	57.84±1.52	58.47±0.38	57.17±2.03
MCH	16.73±0.38	17.57±0.43	16.83±0.63
MCHC (g/dL)	28.23±0.52	29.87±0.74	29.43±0.12
PLT (10^3/µL)	724.7±116.4	929.0±119.7	696.7±122.7
Females			
WBC (10^3/µL)	10.07±1.,03	8.60±1.06	11.27±2.08
RBC (10^6/µL)	7.78±0.59	7.94±0.23	8.43±0.17
HGB (g/dL)	13.23±0.68	14.07±0.15	13.93±0.15
HCT (%)	43.93±2.51	47.03±0.68	46.93±0.41
MCV (fl)	56.73±1.88	59.23±1.02	55.77±0.96
MCH	17.10±0.71	17.77±0.48	16.53±0.27
MCHC (g/dL)	30.13±0.23	29.90±0.31	29.70±0.06
PLT (10^3/µL)	1141±209.2	728.3±57.01	834.7±149.8

*Values represent the mean ± SD (n = 3/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. Differences between the treated group and the control group were not significant P<0.05*

Table 8: Effect of hydro ethanolic extract of *Chenopodium ambrosioides* on organs weight

Organs (g)	Control	200 mg/Kg	500 mg/Kg
	Males		
Heart	0.7±0.13	0.67±0.09	0.57±0.18
Lungs	1.67±0.07	1.58±0.23	1.39±0.38
Liver	6.24±1.63	5.15±0.43	4.67±1.67
Spleen	0.59±0.11	0.57±0.15	0.68±0.02
Kidney	0.92±0.24	1.02±0.03	0.80±0.26
Testis	1.72±0.71	1.75±0.24	1.72±0.09
Females			
Heart	0.75±0.17	0.49±0.04	0.53±0.12
Lungs	1.51±0.29	1.24±0.20	1.34±0.22
Liver	5.08±0.47^{a}	3.75±0.30^{b}	3.77±0.78
Spleen	0.53±0.22	0.46±0.02	0.55±0.1
Kidney	0.91±0.10^{c}	0.69±0.09^{d}	0.76±0.16
Ovaries	1.56±0.24	2.12±0.64	1.69±0.30

Values are expressed as Mean ± SD. n=3; b<a; d<c. p<0.05. a, c: Liver and kidney weights of control females; b and d: Liver and kidney weights of females treated with hydroethanolic extracts, 200mg/Kg

DISCUSSION

The use of plants as an alternative in disease treatment is important in developing countries (Hostesttman *et al.*, 2002). The aim of this study was to evaluate the bioactivities of *Chenopodium ambrosioides* leaves and to identify the components of its essential oil and its hydroethanolic extracts. The survey, which was carried out in Kara town, revealed that *Chenopodium ambrosioides* is well known by native people and it is used to treat malaria, inflammation and wounds. This is in agreement with previous study (Song *et al.*, 2011) Frausin *et al,* 2015). However, some pathologies treated with *C. ambrosioides* such as tuberculosis, in Ghana (Nguta, 2015) and rheumatism in China (Jiangsu, 1986) were not reported during the survey. The survey revealed that respondents mostly used the leaves of the plant, it was demonstrated that 58% of Maya people also used leaves of *C. amrosioides* as anthelmintics (Kliks, 1985). *C. ambrosioides* was also used by 15% of respondents to heal wounds. This prompted a further investigation into the antimicrobial activity of its hydroethanolic extracts, essential oil and its hydrosol against some bacterial strains including gram-positive, gram-negative and yeast. All of these bacteria strains have developed antibiotic resistance according to the results of the susceptibility tests. Nevertheless, the essential oil inhibited the growth of all pathogens with MICs ranging from 3.125µL/mL on Gram-positive bacteria to 50µL/mL on Gram-negative bacteria. These results are similar to those of Alain *et al.* (2012) and Owolabi *et al.* (2009) who reported that the essential oil inhibited all the strains tested. However, the MICs ranged from 1560µg/mL on Gram-positive bacteria to 6860µg/mL on Gram-negative bacteria and 1250µg/mL, respectively. The MICs differences could be linked to the difference in bacterial strains or in the chemical composition of samples or possible antagonistic reactions between molecules. In fact, α-terpinene and 1,8-Cineole extracted from an aromatic plant inhibited the growth of *E. coli* and *S. aureus* with low MICs and p-Cymene from the same plant showed no activity on the same microorganisms at the concentrations tested (Carson *et al.*, 1995). The essential oil in the study of Owolabi *et al.* (2009) was dominated

by α-terpinene, p-cymene, ascaridole and 1,8-cineole. The resistance of Gram negative bacteria could be explained by the structure of the cell wall; compared to the cell envelope of Gram positive, because of the additional membrane, separating the periplasmic space from the cytoplasmic membrane, which limits the diffusion of hydrophobic compounds (Tian *et al.*, 2008). It should be noted that the hydroethanolic extracts showed a low activity. Its anti-microbial activity was found at a high concentration (500mg/mL). These results are similar to those of Brito *et al.* (2007). Any inhibitory activity was obtained with discs impregnated with 8mg of a hydroalcoholic extract against strains of *S. aureus* and *E. coli*. Zulane *et al.* (2012) tested *C. ambrosioides* extracts against 13 strains of pathogens. The hydroethanolic extracts did not inhibit the growth of yeasts, nor Gram positive, nor even Gram-negative bacteria; only the hexane extract at 200µg/ml inhibited yeast growth.

Furthermore, the antioxidant activity of hydroethanolic extracts, essential oil and hydrosol of *C. ambrosioides* leaves was evaluated using three methods: FRAP, DPPH and ABTS. Human body produces free radicals for self-protection against external agents. Whereas uncontrolled production could initiate an oxidative reaction that may result in numerous deleterious effects (Antolovich *et al.*, 2002). It is known that natural antioxidants can scavenge and react with free radicals, and hence terminate the free radical reaction (Bardaweel *et al.*, 2014). Recently, herbal medicines containing free radical scavengers are drawing attention of the pharmaceutical research for their importance in preventing and treating several diseases and disorders (Dharmendra *et al.*, 2009). Among herbal medicines, a number of essential oils (Erkan *et al.*, 2008,Graça, 2010) and extracts (Ouadja *et al*., 2018, Karou *et al*., 2005) isolated from several medicinal and aromatic plants were shown to possess considerable antioxidant potential and, consequently, protect against some cardiovascular and degenerative diseases. Our findings demonstrated that hydroalcoholic extract showed a low antioxidant activity compared to ascorbic acid. Using DPPH method, this activity was 50 times lower than that of ascorbic acid. However, Muhammad *et al.*, (2016) obtained good antioxidant activity with aqueous plant bark extract using ABTS (10.22±0.9mM) and 34.1% DPPH remaining with the petroleum ether bark extract. Other studies have obtained good anti radical activity with methanolic extract of leaves (51.80% hydroxyl radical inhibition) (Martha *et al.*, 2019). According to this study results, regardless of the method applied, the essential oil showed good activity compared to that of the hydrosol. These results are in accordance with those of Beatriz *et al.*, (2012) who inhibited the DPPH radical up to 84.89% with the essential oil of *C. ambrosioides* leaves dominated by ȁ-terpinene. However, Alain *et al.*, (2012) obtained a low radical inhibition with three samples of the studied plant essential oils dominated by α-terpinene. The antioxidant activity of plant extracts must be interpreted taking into account the activities of all molecules even if they are in the minority, as it is possible to have potentiation and/or synergy reactions of the molecules. On the other hand, there are no data available in the literature on the composition and activities of *C. ambrosioides* hydrosol. The hydrosol of the current study obtained a great antioxidant activity with ABTS method. Its antioxidant activity would be linked to a small quantity of essential oil molecules or new molecules resulting from the hydrolysis of essential oil molecules during distillation. The survey revealed that *C. ambrosioides* is used for its anti-inflammatory capacity. Thus, hydroethanolic extracts, essential oil and hydrosol of *C. ambrosioides* were screened for their antiinflammatory activity firstly by measuring their ability to inhibit the lipoxygenase (LOX) activity and afterward by

measuring the capacity of the hydroalcoholic extract to inhibit the carageenan induced paw in rats. LOX is known to catalyse the oxidation of unsaturated fatty acids notably linoleic acid to 13-hydroperoxy linoleic acid which is a pro-inflammatory molecule (Alain *et al*., 2012). Thus, the results showed that from the first to the tenth minute of contact with the enzyme, the essential oil demonstrated a strong antiinflammatory activity inhibiting LOX. This was more pronounced than that of the standard molecules, quercetin and gallic acid. However, four samples of essential oil of *C. ambrosioides* from Benin with similar chemotypes, α-terpinene, p-cymene and ascaridole, at 100 ppm, showed weak LOX inhibition activity (Alain *et al*., 2012). Generally, essential oils have a complex molecular composition and molecules do not act individually. In these conditions, mechanisms of potentiation and/or antagonism of molecules can occur. Consequently, the elucidation of the relationship between composition and anti-inflammatory activity of essential oils was difficult. However several monoterpenic molecules of essential oils have shown promising anti-inflammatory activities either by reducing the activity of pro-inflammatory molecules or by completely inhibiting their production (Rita *et al*., 2013). The high content of monoterpenic molecules of the studied oil would therefore explain the effectiveness of its anti-inflammatory activity. Similarly, the hydrosol and the hydroethanolic extracts exhibited LOX inhibitory activities with a relatively low percentage compared to that of essential oil. However, the anti-lipoxygenase activity of the hydrosol was statistically not different to that of quercetin moreover; its action was still present with the test time (53.45±3.57%; 48.78±5.82% and 51.72±5.41% respectively from the first to the tenth minute). On the other hand, carageenan induced paw in rats was not inhibited by the extract of *C. ambrosioides*. Similarly, neither the reference drug ketoprofen nor *C. ambrosioides* extract had exhibited inhibition effect regarding inflammation cells count. These results were similar to those of Calado *et al*. (2015) which induced osteoarthritis by injecting sodium monoiodoacetate in knee of rats and treated them with *C. ambrosioides* extract for 10 days. From this test, any decreased of knee diameter was noted until the third day of the inflammation induction. The diameter reduction was noted on the seventh day of the test. On the day 10, they obtained a reduced number of inflammation cells only on the group treated with 5mg/Kg. When they observed synovial cartilage microscopically, they reported there was no oedema inhibition. This result suggests that the inhibition of the inflammation would be link to the reduction of the inflammation cells or the inhibition of their production by the plant extract as we showed with lipoxygenase method above. Rios *et al*., (2011) also founded that ethanolic extract and hexanic fraction of *C. ambrosioides* significantly inhibited an inflammation induced experimentally. Treatments have decreased level of the proinflammatory cytokines in the serum, indicating a systemic anti-inflammatory effect of *C. ambrosioides*. However, a pure compound, 12-hexadecanoic acid molecule extracted from *C. ambrosioides* leaves showed good anti-inflammatory activity (Lohdip *et al.*, 2013). Essential oils and plant extracts sometimes exerted toxicological effects (Liaquat *et al*., 2018; Emma *et al*., 2015). To evaluate the toxicity of *C. ambrosioides* leaves through its hydroethanolic extracts, its essential oil and its hydrosol, several experiments were carried out. First, *in vitro* cytotoxicity on peripheral blood mononuclear cells (PBMCs); afterward *in vivo* toxicity using the animal model by measuring the toxicity through the body weight of animals and organs mass, biochemical parameters and through blood cells count. All these tests have shown that the hydroethanolic extracts, the hydrosol and the essential oil of the leaves of *C. ambrosioides*

exerted cytotoxic effects depending on concentration. The hydroethanolic extracts of the plant at high concentrations studied by Valério *et al.* (2015) exhibited cytotoxicity on RAW 264.7 macrophages. The cytotoxicity of two chemotypes of *C. ambrosioides* essential oil has been attributed to carvacrol, caryophyllene (Monzote *et al.*, 2009), and to neral (Koba *et al.*, 2009). In addition, ascaridole was found to be toxic to HaCaT cells (Ali *et al*., 2016). The acute toxicity of essential oil using oral administration caused the death of three rats treated with 200µL/kg/bw. However, no signs of toxicity of the hydrosol and hydroalcoholic extract were detected in the rats at 2mL/100g/bw and 5000mg/Kg/bw respectively. Thus, the hydroethanolic extracts of the plant was subject to sub-acute toxicity study up for 28 days. Punctually, a decrease in the weights of rats liver and kidney were noted which consequently induced, an increase in the urea value of the females treated with 200mg/kg of extract. These observations could be linked to a localised disorder between the livers and the kidneys of the animals. Similarly, hydroethanolic extracts at 50mg/Kg induced an increase in kidney weight and urea values after 15 days of treatment in mice (Pereira *et al*, 2010). Studies of the toxicity of the hydrosol are not available. Our results suggest that its cytotoxicity would be due to the same molecules responsible for the toxicity of its essential oil, since both are sensitive to the dilutions made during cytotoxicity. The toxicity of *C. ambrosioides* leaves could be attributed to their chemical composition, the type of cells tested and to the administration route. To know the molecules, which exhibited bioactivities above, hydroethanolic extracts and essential oil were screened for their chemical composition. Histochemical tests of the leaves revealed the presence of flavonoids coloured yellow and tannins coloured green-black. The search for alkaloids was negative. But, in addition to phenolic compounds, alkaloids were present in the hydroethanolic extracts of *C. ambrosioides* leaves from Brazil (Jesus *et al.*, 2018) and in an aqueous extract of fresh leaves from Morocco (Hallala *et al.*, 2010). The chemical composition of plant extracts could be related to the genetic factors of the plant species and climatic conditions. The presence of phenolic compounds in plant extracts may confer beneficial activities to humans' body. Therefore, it was prompted to evaluate the content of phenolic compounds in ethanolic extracts. Thus, our results show that the hydroethanolic extracts of the leaves contain high levels of total phenolics 324.8±17.3 µg/mg gallic acid equivalent and flavonoid content was 63.2±8.7µg/mg quercetin equivalent. Qualitatively, these results were similar to those of previous studies. Alarcon *et al.*, (2008) found in herbal teas of the plant, total phenols content of 36±2mg gallic acid equivalents/L ; Luz *et al*., (2017) found in the ethanolic extract 126.3±4.91mgEAG/100g and 147.26±19.68 mg EQ/100g respectively phenolics and flavonoids content. In fruits aqueous extract, 1076 ± 0.3 μg/mL gallic acid equivalent and the highest amount of flavonoid content was found in aqueous bark extract, that is, 1997.09 ± 1.5 μg/mL catechin equivalent (Muhammad *et al.*, 2016). The phenolic content of extracts may be due firstly to the difference between the plant organs used and secondly to the difference in the polarities of the extraction solvents. In this regard, it is known that as the extraction yield is directly proportional to the polarity index of the solvents, it increases progressively with the increase of the polarity of the solvents (Muhammad *et al*., 2016). In addition, several phenolic compounds are not water-soluble; their solubility depend on the number of hydroxyl groups, molecular weight and chain length, however, the addition of water to organic solvents increases the solubility of polyphenols (Sripad *et al.*, 1982). Therefore, the time of sample collection, the storage period of the extracts before analysis

and the moisture content of the extracts are factors to be taking into account, which could influence the content values found. Similarly, to the production of the extracts, the production of the essential oil by hydro distillation of the leaves of *C. ambrosioides* leaves with Clevenger revealed among 1.03±0.15% (V/m) of essential oil similar to that of Lomé (0.8%) (Koba *et al*., 2009) and to those found in 4 regions of Morocco (0.9, 1, 1.1 and 1.2%) (Elhourri *et al.,* 2016) but 3.7 and 6.4 higher than those of India (Lohani *et al*., 2012) . The level of essential oil of plants could be attributed to several factors, including rainfall, all factors favouring the development of the plant (Sudetbio, 2012) and the extraction methods used. The analysis of hydroethanolic extracts and essential oil by GC/MS facilitated the identification of samples components. The major compounds of the studied plant essential oil were 2-carene (37.79%), O-cymene (33.27%) and ȧ-terpinene (20.94%). This essential oil was obtained from Kara, northern part of Togo benefiting a dry tropical climate and a mountainous relief. Essential oil from *C. ambrosioides* grown in Lomé, southern Togo enjoying humid tropical climate with salty soil, did not contain 2-carene component but rather contained the neral and geraniol components that were not found in the essential oil of Kara; the major components of Lomé essential oil were ascaridole, p-cymene, neral, geraniol (Koba *et al*., 2009). Many other chemotypes have been characterised on soils with climatic and pedological conditions different from those of Kara (Elhourri *et al*., 2016 ; Álvarez *et al*., 2012). The chemical composition of essential oil could be influenced by several factors, the most cited includes the climatic and pedological conditions, genetic factors, the plant organ used and also the drying process (Elhourri *et al.,* 2016 ; Anwar *et al.,* 2009). Similar to essential oil, the composition of plant extracts would also be influenced by the same factors. The GC-MS analysis show that the hydroethanolic extracts was dominated by phytol (50.45%), 5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0] heptan-2-ol (22.70%) and 4-Isopropyl-5-methylhex-2-yne-1, 4-diol (14.41%). Similar results were published by Martha *et al.*, (2019) in Brazil, who also found that their apolar fraction obtained from hydroethanolic extracts of the leaves of *C. ambrosioides* was dominated by phytol. However, an extract of the same plant harvested in India was dominated by tetra decanoic acid which was not identified in the current extract although phytol was present in both samples (Subramaniam *et al.,* 2017). It should be noted that 12 out of 21 compounds were not identified in the studied extract.

The compounds of the hydrosol could not be identified. This will be the subject for future study.

CONCLUSION AND APPLICATION OF RESULTS

The results of the current study showed that *C. ambrosioides* was an aromatic plant. Its essential oil was dominated by hydrocarbon monoterpenes and the hydro alcoholic extract composed mainly of terpene alcohols. The expression of these molecules showed that the plant possessed the pharmacological activities exploited by traditional medicine. All these activities have been dominated by the essential oil. However, the toxicity of the oil limits the use of *C. ambrosioides* in health care. Orally, the plant could be a source of antioxidant and anti-inflammatory drugs at very low doses. The data from this study deepened knowledge for the discovery of new plant-derived medicinal molecules and helped to more understand certain practices of traditional healers.

List of abbreviations

CO_2: Carbon Dioxide, COX-2: Cyclooxygenase-2, DMSO: Dimethyl Sulfoxid, FCS: Fetal calf, serum, IC50: The half maximal inhibitory concentration, ISO: International Organization for Standardization, PBMC: Peripheral Blood Mononuclear Cells, PBS: Phosphate-buffered saline, PI: propidium iodide, RMPI: Roswell Park Memorial Institute Medium, TH : traditional herbalist, GC-MS: Gas Chromatography coupled with Mass Spectral Analysis, DPPH: 2,2-diphenyl-picryl-hydrazyl, DPPH: 2,2-diphenyl-picryl-hydrazyl, ABTS: 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), ATCC: American Type Culture Collection, AAE/mL: Equivalent Ascorbic acid per mL AAE/mL, FRAP: Ferric ions (Fe^{3+}) reducing antioxidant power assay, FACS: Fluorescens activated cell sorting, OECD: Organization for Economic Co-operation and Development.

Ethics approval and consent to participate: The study has the authorisation from the ethical board "Comité de Bioéthique pour la Recherche en Santé (CBRS)" of the Ministry of Health in Togo. The healthy blood donors gave their informed consent. The study using animal model was approved by the Committee for Animal Experimentation Ethics of ESTBA-UL (N° 0003/2020-07/ESTBA-UL) A minimum number of animals were used to obtain reliable results.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was in part funded through grants awarded to GK and LEL from the German Research Foundation (DFG Grant LA2746/1-1) within the "German- African Cooperation Projects in Infectiology.

Acknowledgements: We thanks all the traditional herbalist for their contribution and collaboration.

REFERENCES

Ablassé R. 2017. Phytochimie et potentiel génoprotecteur de la pulpe des fruits de Detarium microcarpum Guill. et Perr. (Caesalpiniaceae). Thèse de doctorat, Université Ouaga I Pr JOSEPHE KI-ZERBO, 144p.

aïra R. 2012. Activité anti-oxydante, et caractérisation phénolique du fruit de palmier amazonien Oenocarpus bataua (patawa). Thèse de doctorat,Université des Antilles et de la Guyane,215p.

Alain G. A., Philippe, PT. Fidèle, PN.Jean, A.Félicien, Y.Boniface, M.Chantal, V.Pierre, CK.S.Dominique. 2012. Chemical composition and biological activities of essential oils of Chenopodium ambrosioides L. collected in two areas of Benin. International Journal of Biosciences (IJB) ISSN: 2220-6655 (Print) 2222-5234 (Online) Vol. 2, No. 8, p. 58-66, 2012 http://www.innspub.net.

Alarcon E., AM. Campos , AM. Edwards , E.Lissi , CA. Lopez. 2008. Antioxidant capacity of herbal infusions and tea extracts: A comparison of ORAC-fluorescein and ORAC-pyrogallol red methodologies. Food Chemistry 107: 1114–1119.

Aline C. Q., L.F.D.Thays, B.B.M.Carolina, H.A.C.S.Luiz, A.J.X.João, B.A.Givanildo, B.P.Flávia, and S.A.M.Magna. 2014. Antileishmanial Activity of Medicinal Plants Used in Endemic Areas in Northeastern Brazil.Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine, 9p.

Alitonou G., F.Avlessi, DCK.Sohounhloue, JM.Bessiere, C.Menut. 2010. Chemical and Biological lnvestigation

on Volatile Constituents of Pentadesma butyracea Sabin (Clusiaceae) From Benin. Journal of Essential Oil Research 22, 138-140.

Álvarez H. , RC.Isabel , D.Georgina , B.Robert , M.Rachel. 2012. HPLC Determination of the Major Active Flavonoids and GC–MS Analysis of Volatile Components of Dysphania graveolens (Amaranthaceae). Phytochemical analysis 9p; DOI 10.1002/pca.2405.

Amarowicz R. , R.B.Pegg , M.P.Rahimi , B.Bart , J.A.Weil. 2004. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry, 84: 551-562.

Antolovich M., P.Prenzler, E.Patsalides , S.McDonald , K.Robards. 2002. Methods for testing antioxidant activity. Analyst, 127:183–198.

Anwar F., AI.Hussain, TH.Sherazi , MI.Bhanger. 2009. Changes in composition, antioxidant and antimicrobial activities of essential oil of fennel (Foeniculum vulgare Mill.) fruits at different stages of maturity. J. Herbs Spices Med. Plants. 15(2): 187-202

Arvouet A., B.Pourrat, P.Legret. 1994. Standardisation d'un extrait de propolis et identification des principaux constituants. Journal de pharmacie de Belgique, 49, 462–468. .

Ataba E. , G.Katawa , M.Ritter , AH.Ameyapoh , K.Anani , OM.Amessoudji , PE.Tchadié , T.Tchacondo , K.Batawila , Y.Ameyapoh , A.Hoerauf , LE.Layland , SD.Karou. 2020. Ethnobotanical survey, anthelmintic effects and cytotoxicity of plants used for treatment of helminthiasis in the Central and Kara regions of Togo. BMC Complementary Medicine and Therapies, 20:212; doi.org/10.1186/s12906-020-03008-0.

Bardaweel S. K. , K.A.Tawaha , M.M.Hudaib. 2014. Antioxidant, antimicrobial and antiproliferative activities of Anthemis palestina essential oil BMC Complement. and Altern. Med., (3), 8 http://www.biomedcentral.com/1472-6882/14/297.

Bayala B. 2014. Etude des propriétés anti-oxydantes, anti-inflammatoires, anti-prolifératives et antimigratoires des huiles essentielles de quelques plantes médicinales du Burkina Faso sur des lignées cellulaires du cancer de la prostate et de glioblastomes. Sciences agricoles. Thèse de doctorat, Université Blaise Pascal - Clermont-Ferrand II, 224p. Français. NNT : 2014CLF22502.

Carlos E., GA.Afonso, AFBF.Jose, RN.Johnny, NMG.Rosane, MMA.Flávia, CGM.Márcia , RFN.Flávia. 2017. Chenopodium ambrosioides L. Improves Phagocytic Activity and Decreases Bacterial Growth and the Systemic Inflammatory Response in Sepsis Induced by Cecal Ligation and Puncture. Frontier in microbiology 8: 148.

Carolina M. J., N.J.Gulab, D.D.Onkar, M.F.Marcelo. 2008. Composition and Antifungal Activity of the Essential Oil of the Brazilian Chenopodium ambrosioides L. Journal of Chemical Ecology, 34:1213–1218.

Carson C. , TV.Riley. 1995. Antimicrobial activity of the major components of the essential oiI of Melaleuca alternifolia. Journal of Applied Bacteriology, 78: 264-269.

Cécile P. 2005. Assainissement microbiologique de l'air et des systèmes de ventilation au moyen d'huileS essentielles. Thèse de doctorat, école polytechnique férérale de Lausanne, 177p.

Chevallier A. 1996. The Encyclopedia of Medicinal Plants Dorling Kindersley. London, ISBN9-780751-303148.

Chiasson H. , N.J.Bostanian , C.Vincent. 2004. Acaricidal properties of a Chenopodium-based botanical. J Econ Entomol. 97(4): 1373-1377.

Chuan Q. B. , L.L.Zhi , Z.L.Qi. 2011. Nematicidal Constituents from the Essential Oil of Chenopodium Ambrosioides Aerial Parts. CODEN ECJHAO E-Journal of Chemistry, ISSN: 0973-4945; 8(S1), 143-148.

Cloyd R. A. , H.Chiasson. 2007. Activity of an Essential Oil Derived from Chenopodium ambrosioides on Greenhouse Insect Pests Journal of Economic Entomology 100(2): 459-466. .

Dharmendra D., K.Prashant, SK.Jain. 2009. In-vitro Anti-oxidant activity of the ethyl

Elhourri M., I.Mostafa , A.Ali , L.Adrae , B.Saadia , E.Ghizlane. 2016. Chemical characterization of essential oils from Chenopodium ambrosioides (L.) (Chenopodiaceae) from four regions of Morocco]. Journal of Materials and Environmental Science 7 (11): 4087-4095.

Erkan N., G.Ayranci, E.Ayranci. 2008. Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella Sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem., 110:76–82.

Fernando J. P. , C.C.Graciomar , V.S.P.Paulo , C.A.F.Walmir , M.S.Sanara , B.F.Josias , S.P.Wanderson , C.G.M.M´arcia , A.S.Lucilene , M.M.A.Fl´avia , M.M.R.Jos´e , N.M.G.Rosane , N.S.R.Maria , R.F.N.Fl´avia. 2008. Efficacy of the intralesional treatment with Chenopodium ambrosioides in the murine infection by Leishmania amazonensis. Journal of Ethnopharmacology, (115): 313-319.

González M. J., J.M.Marioli. 2010. Antibacterial activity of water extracts and essential oils of various aromatic plants against Paenibacillus larvae, the causative agent of American Foulbrood. Journal of Invertebrate Pathology 104: 209–213.

Graça M. M. 2010. Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. Molecules, 15:9252–9287.

Hallala A., S.Benalia, M.Markouk, K.Bekkouchea, M.Larhsinia, A.Chaitb, A.Romanec, A.Abbada, M. K. E. Abdounid. 2010. Evaluation of the Analgesic and Antipyretic Activities of Chenopodium ambrosioides L. Asian Journal of Experimental Biological Sciences, 1 (1) : 189-192.

Hameed S. 2014. Antibacterial and antifungal activities of the crude extracts from the stem of Chenopodium ambrosioides linn, an indiginous medicinal plant. African Journal of Pharmacy and Pharmacology,7(48) DOI 10.5897/AJPP2013.4010.

Hostesttman K. , A.Marston. 2002. Twenty years of research into medicinal plants: results and perspectives. Phytochemistry: 275-285.

Ibironke G. F. , K.I.ajiboye. 2007. Studies on the anti-inflammatory and analgesic properties of Chenopodium ambrosioides leaf extract in rats. International journal of pharmacology 3 (1): 111-115.

Iwalewa E., LJ.McGaw, V.Naidoo, JN.Eloff. 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology Vol. 6 (25), pp. 2868-2885, 28 December,

Available online at http://www.academicjournals.org/AJB .

Jesus R. , BF.Robson , FB.Thiele , FSA.Camilla , VB.Bianca , JM.Natália , CC.Ritiel , CVS.Roberto , VD.Tanise , VB.Bianca , MAC.Marli , FB.Liliane. 2018. In vitro antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from Chenopodium ambrosioides L. Brazilian Jounal of Microbiology, 296–302.

Jiangsu. 1986. New Medical College, Encyclopedia of Chinese Medicinal Substances. Shanghai People's Publisher, Shanghai, China, 329-333.

Kliks M. 1985. Studies on the traditional herbal anthelmintic Chenopodium ambrosioides L.: ethonopharmacological evalution and clinical fields trials. Social Science and Medicine 21,879–886.

Koba K., C.Guyon, R.Christine, C.Jean-Pierre, S.Komla, L.Nicod. 2009. Chemical Composition and Cytotoxic Activity of Chenopodium ambrosioides L. Essential Oil from Togo. Bangladesh Journal of Scientific and Industrial Research, 44(4), 435-440.

Kuete V. 2014. 22-Physical, Hematological, and Histopathological Signs of Toxicity Induced by African Medicinal Plants. Toxicological Survey of African medicinal plants, pp. 635-657; https://doi.org/10.1016/B978-0-12-800018-2.00022-4.

Lohani H. , NK.Chauhan , KKSZ.Haider , HC.Andola. 2012. Comparative aroma profile of wild and cultivated Chenopodium ambrosioides L. from Uttarakhand. Journal of Essential Oil Bearing Plants ,15:657-661.

Lohdip A., JC.Aguiyi. 2013 Some pharmacological activities of hexadec-12-enoic acid isoleted from Chenopodium ambrosioides Linn. Global Journal of Pure and Applied Chemistry Research Vol.1, No. 2, pp. 12-21

Lohdip A. M., Ov.Uduah, O.O.Ogunga , T.S.Akingbemisihi. 2017. Assessment of some physicochemical properties and antimicrobial activity of an insoluble extract from the stem of Chenopodium ambrosioides LINN. Ewemen Journal of Natural Product Research 2(1) 5-9; ISSN: 2504-8643.

Luz V., G.Mondragón , E. S. Govea , AY.Andrade , J.Ramí , JTS.Castro. 2017. Potential application of epazote (Chenopodium ambrosioides L.) as natural antioxidant in raw ground pork, LWT - Food Science and Technology, doi: 10.1016/j.lwt.2007.05.076.

Magbéfon T. , K.E.Kwashie , A.A.KODJO , A.Amégnona , Z.Comlan , G.Messanvi. 2009. Infected wound healing and antimicrobial effects of Chenopodium ambrosioides and Mitracarpus scaber. International Journal of Biology and Chemistry Sciences, 3(3): 623-627, ISSN 1991-8631.

Martha R. , A.Carlos , S.Veronica , MV.Juan , GL.Mercedes. 2019. Antioxidant, intestinal immune status and anti-inflammatory potential of Chenopodium ambrosioides L. in fish: In vitro and in vivo studies. Fish and Shellfish Immunology 86: 420–428.

Medzhitov R. 2010. Inflammation New Adventures of an Old Flame. Cell. 771: 1-6; DOI 10.1016/j.cell.2010.03.006.

Monzote L. , MR.Nance , M.García , R.Scull , WN.Setzer. 2011. Comparative chemical, cytotoxicity and antileishmanial properties of essential oils from Chenopodium ambrosioides. Natural Product Communications; 6:281-286.

Monzote L. , W.Stamberg , K.Staniek , L.Gille. 2009. Toxic effects of carvacrol, caryophyllene oxide, and ascaridole from essential oil of Chenopodium ambrosioides on mitochondria. Toxicology and Applied Pharmacology, 240: 337–347.

Muhammad A. , H.Tanveer , F.Saleha , A.Mehrban. 2016. Analysis of Antimicrobial and Antioxidant Activities of Chenopodium ambrosioides: An Ethnomedicinal Plant. Journal of Chemistry,11 pages http://dx.doi.org/10.1155/2016/4827157.

N.C.C.L.S. 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 5th ed. Approved standard M7-A5. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Nguta J. M. 2015. Medicinal plants used to treat TB in Ghana. International Journal of Mycobacteriol, http://dx.doi.org/10.1016/j.ijmyco.2015.02.003.

Noumi E., A.Yomi. 2001. Medicinal plants used for intestinal diseases in Mbalmayo Region, Central Province, Cameroon, Fitoterapia 72: 246-254.

Oyaizu M. 1986. Studies on products of browning reactions: antioxi-dative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics 44:307-15.

Pereira W. , BP.Ribeiro , AIP.Sousa , ICPB.Serra , NS.Mattar , TS.Fortes , AS.Reis , LA.Silva , ESB.Barroqueiro , RNM.Guerra , FRF.Nascimento. 2010. Evaluation of the subchronic toxicity of oral treatment with Chenopodium ambrosioides in mice. Journal of Ethnopharmacology, 127: 602–605.

Peter K. , J.A.H.Gustavo , M.C.Eduardo , G.L.Melesio , L.Zaira. 2018. In Vitro Bioactivity of Methanolic Extracts from Amphipterygium adstringens (Schltdl.) Schiede ex Standl., Chenopodium ambrosioides L., Cirsium mexicanum DC., Eryngium carlinae F. Delaroche, and Pithecellobium dulce (Roxb.) Benth. Used in Traditional Medicine in Mexico. Hindawi, Evidence-Based Complementary and Alternative Medicine, 11 pages https://doi.org/10.1155/2018/3610364.

Re R., N.PELLEGRINI , A.PROTEGGENTE , A.PANNALA , M.YANG , C.RICE-EVANS. 1999. Antioxidant activity applying an improved ABTS radical cation decoloration assay. Free Radical Biology & Medicine, 26: 1231–1237.

Rita C., NA.Luciana , PS.Damião. 2013. A Review on Anti-Inflammatory Activity of Monoterpenes. Molecules 18, 1227-1254; doi:10.3390/molecules18011227.

Ruth T. D. , V.F.Ingrid , T.G.Liliane , C.F.Gilberto , E.N.Alexandre , M.S.B.Christiane , M.W.Theodoro , M..S.Marcia, B.C.Alexandre, M.Angela. 2015. Characterization and evaluation of the cytotoxic potential of theessential oil of Chenopodium ambrosioides .Revista Brasileira de Farmacognosia, http://dx.doi.org/10.1016/j.bjp.2015.08.012.

Salifou S., F.D.Dadjo , A.Sabbas , D.Rodolph , F.B.Cyprien. 2013. Antiparasitic effects of the water extract from Chenopodium ambrosioides L. (Chenopodiaceae) against some gastrointestinal nematodes in West African Long Legged goats. Journal of Parasitology and Vector Biology Vol. 5(2), pp. 13-16.

Singleton V., R.Orthofer , RM.Lamuela-Raventos. 1999. Analysis of total phenols and oxidization substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-177.

Song M. J., S.M.Lee , D.K.Kim. 2011. Antidiabetic effect of Chenopodium ambrosioides. Phytopharmacology 1(2): 12-15.

Sripad G., V.Prakash , M. N. Rao. 1982. Extractability of polyphenols of sunflower seed in various solvents. Journal of Bioscience, Vol. 4. pp. 145-152.

Subramaniam J. , K.Murugan , A.Jebanesan , P.Pontheckan , D.Dinesh , M.Nicoletti , H.Wei , A.Higuchi , S.Kumar , A.Canale , G.Benelli. 2017. Do Chenopodium ambrosioides-Synthesized Silver Nanoparticles Impact Oryzias melastigma Predation Against Aedes albopictus Larvae? Journal of Cluster Science, 28:413–436, DOI 10.1007/s10876-016-1113-9.

SudetBio. 2012. Plantes aromatiques et médicinales : premiers pas dans la production bio en Languedoc Roussillon. Agriculture biologique, 12p. www.sud-et-bio.com/sites/default/files/Brochure_plantes_aromatiques_et_medicinales.pdf .

Tian F., B.Li , B.Ji , J.Yang , G.Zhang , Y.Chen , Y.Luo. 2008. Antioxidant andantimicrobial activities of consecutive extracts from Galla chinensis : thepolarity affects the bioactivities. Food Chemestry. 113, 173–179.

Toudji G., E.Thiombiano, S.D.Karou, K.Anani, Y.Adjrah, H.E.Gbekley, M.Kiendrebeogo, Y. Ameyapoh, J.Simpore. 2018. antibacterial and anti-inflammatory activity of crude extracts of of three Togolese medicinal plants against ESBL Klebsiella pneumoniae strans. Afr J Tradit Complement Altern Med., 15 (1): 42-58, https://doi.org/10.21010/ajtcam.v15i1.5.

Toudji G. A., D.kossivi , S.D.Karou , Y.Adjrah , K.Anani , Y.Ameyapoh , J.Simpore. 2017. Acute and sub-acute toxicity of Pithecellobium dulce (Roxb.) Benth. stem bark hydroalcoolic extract on Wistar rats. Journal of pharmcy and pharmacognosy Research, 5(5), 310-319.

Tuo C., FA.Coulibaly , BN.Djyh , AJ.Djaman , FG.Guede. 2005. Etude de toxicité aigüe de l'extrait toial aqueux de Phyllanthus amarus (Schum et Thonn) chez les souries. Journal of sciences pharmacology and biology, Vol. 6, n°1, pp. 48-52.

Valério É. , WLR.Barbosa , RM.Finger , MF.Muzitano , MH.Araújo , F.Vasconcelos , F.M.Teixeira. 2015. Physicochemical characterisation, microbiological quality control and toxicity evaluation of the hidroethanolic extract from Chenopodium ambrosioides Linn.International Journal of Pharmaceutical Sciences and Research Vol. 6(10): 4190-4197. DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(10).4190-97.

Velàzquez E., HA.Tournier, PMdd.Buschiazzo, GSaavedra , GR.Schinella. 2003. Antioxidant activity of Paraguayan plant extracts. Fitoterapia, 74: 91-97.

Wanderson S. , PS.Girlaine , VV.Mariana , RFL.Nathalia , AP.Fabiana , CF.Daniele , VMS.Shirley , M.Thiago , RN.Johnny , PSA.Ana , NF.Eduardo , SMV.Leosvaldo , RSN.Lauro , FB.Frederico , AP.Elyzabeth , CCS.Katia , N.Flavia , GPC.Marsen. 2018. Anti-arthritic properties of crude

extract from Chenopodium ambrosioides L. leaves.journal of Pharmacy and Pharmacology, 7:1078–1091.

Zulane L. S., F.O.Fernando, O.C.Aline , A.M.S.Luiz , H.R.Maria , S.S.Juliana , L.A.João. 2012. Biological activities of extracts from Chenopodium ambrosioides Lineu and Kielmeyera neglecta Saddi. Annals of Clinical Microbiology and Antimicrobials, 11:20.