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Anti-inflammatory and anti-nociceptive activities of essential oil of *Waltheria indica*

[Actividades antiinflamatorias y nociceptivas del aceite esencial de *Waltheria indica*]

Opeyemi N. Avoseh[1], Isiaka A. Ogunwande[2], Oladipupo A. Lawal[1], Joshua Atabo[1], Roberta Ascrizzi[3] & Flamini Guido[3]

[1]***Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway Ojo, Ojo, Lagos, Nigeria***
[2]***University Drive, A leku Area, Osogbo, Nigeria***
[3]***Dipartimento di Farmacia, Università di Pisa, Pisa, Italy***
Contactos | Contacts: **Opeyemi N. AVOSEH** - ***E-mail address:*** **opeyemi.avoseh@lasu.edu.ng**

Abstract: This paper reports for the first time volatile compounds, anti-nociceptive and anti-inflammatory activities of essential oils from the leaves of *Waltheria indica* L. (Stericullaceae) growing in Nigeria. The essential oil was hydro-distilled and characterized by gas chromatography-flame ionization detection (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS) analyses. The anti-inflammatory activity was evaluated on carrageenan induced rat paw edema while the anti-nociceptive test was based on hot plate model. The hydro-distillation afforded 0.41% (dry weight basis) of light green oil. Forty compounds representing 99.8% were identified in the oil. The main constituents of the oil were limonene (34.7%), sabinene (21.2%) and citronellal (9.7%). The anti-nociceptive property of the essential oils statically inhibited edema development (p<0.001) at a dose of 200 and 400 mg/kg independent of time of exposure. However, the 100 mg/kg *Waltheria indica* essential oils (WIEO) displayed a relatively low inhibition (p<0.01-p>0.5) which declines as exposure time increases. The anti-inflammatory activities shows a steady rate and non-dose dependent activity (p<0.001) up to the 3rd h of inflammation study. Conversely, a sharp reduction at the rate of p<0.5, 0.1 and 0.01 for the 100, 200 and 400 mg/kg WIEO doses respectively. Overall, the results presented sustain and establish the anti-nociceptive and anti-inflammatory properties and justifies the need for further evaluation and development of the essential oils from this plant.

Keywords: *Waltheria indica*; Essential oil; Monoterpenes; Anti-nociceptive activity, Anti-inflammatory activity.

Resumen: Este artículo informa por primera vez de compuestos volátiles, actividades anti-nociceptivas y antiinflamatorias de aceites esenciales de las hojas de *Waltheria indica* L. (Stericullaceae) que crecen en Nigeria. El aceite esencial fue hidro-destilado y se caracterizó por cromatografía de gases-detección de ionización de llama (GC-FID) y cromatografía de gases junto con análisis de espectrometría de masas (GC-MS). La actividad antiinflamatoria se evaluó en el edema de pata de rata inducido por carragenano, mientras que la prueba antinociceptiva se basó en el modelo de placa caliente. La destilación hidráulica proporcionó 0,41% (en peso seco) de aceite verde claro. Cuarenta compuestos que representan el 99.8% fueron identificados en el aceite. Los principales componentes del aceite fueron el limoneno (34,7%), el sabineno (21,2%) y el citronelal (9,7%). La propiedad anti-nociceptiva de los aceites esenciales inhibió estáticamente el desarrollo del edema (p<0.001) a una dosis de 200 y 400 mg/kg independientemente del tiempo de exposición. Sin embargo, los aceites esenciales de *Waltheria indica* de 100 mg/kg (WIEO) mostraron una inhibición relativamente baja (p<0.01-p>0.5) que disminuye a medida que aumenta el tiempo de exposición. Las actividades antiinflamatorias muestran una tasa constante y una actividad no dependiente de la dosis (p<0.001) hasta la tercera hora del estudio de inflamación. Por el contrario, una fuerte reducción a una tasa de p<0.5, 0.1 y 0.01 para las dosis de 100, 200 y 400 mg/kg de WIEO respectivamente. En general, los resultados presentados sostienen y establecen las propiedades anti-nociceptivas y antiinflamatorias y justifican la necesidad de una mayor evaluación y desarrollo de los aceites esenciales de esta planta.

Palabras clave: *Waltheria indica*; Aceite esencial; Monoterpenos; Actividad anti-nociceptiva; Actividad antiinflamatoria.

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INTRODUCTION

Waltheria indica L. belongs to the family Malvaceae. *Waltheria indica* is a small shrub growing up to 2 to 6 feet tall with velvety hairs covering all parts of the plant. The oblong to oval leaves are up to 6 inches long and 2 inches wide with toothed edges and conspicuous veins. The fragrant yellow flowers grow in small, dense clusters in the leaf axils (Wagner *et al*., 1990). *Waltheria indica* is commonly used in traditional medicine in Africa, South America and Hawaii, mainly against pain, inflammation, conditions of inflammation, diarrhea, dysentery, conjunctivitis, wounds, abscess, epilepsy, convulsions, anemia, erectile dysfunctions, bladder ailments and asthma (Zongo *et al*., 2013). A cosmetic formulation including extract of *W. indica* is known for its dermal activity (Pauly *et al*., 2014). Oral administration of *W. indica* significantly delayed the onset and progression of cataract in Naphthalene induced cataract which may be due its antioxidant activity (Atif *et al*., 2014). The cytotoxicity study clearly showed the dose dependent cytotoxic effect of extract in HeLa cell line (Krishna & Sudarsanam, 2015). The presence of potent NF-κB inhibitors compounds in the decoction of the aerial parts of *W. indica* supports its traditional use in inflammatory-related diseases and cancer chemoprevention (Monteillier *et al*., 2017). Crude extract of *W. indica* significantly inhibited carrageenan induced rat paw edema (Yougbare-Ziebrou *et al*., 2016; Chandekar *et al*., 2017), displayed analgesic (Yougbare-Ziebrou *et al*., 2016) and antioxidant effects (Mongalo *et al*., 2013; Yougbare-Ziebrou *et al*., 2016; Veeramani & Alagumanivasagam, 2017). The ethanolic extract of *W. indica* had inhibitory activity against *Candida utilis* (Hernández *et al*., 2009) and significantly inhibited the growth of several pathogens (Hernández *et al*., 2009; Olajuyigbe *et al.*, 2011; Mongalo *et al*., 2013; Olakunle *et al*., 2017). Extracts of *W. indica* significantly inhibited the enzymes PDE4A1α (smooth muscle contraction), 5- LOX and PLA2 (inflammatory activities) and rat trachea relaxation thereby which validate its use in traditional management of asthma and other conditions of inflammation (Zongo *et al*., 2014).

Chrysosplenol E, waltherione A and waltherione C were the major compounds responsible for potent NF-κB inhibitory action of *W. indica* (Monteillier *et al*., 2017). The major compounds identified by GC/MS analysis of crude extracts of *W. indica* were 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, tetradecane, nonadecane, tetracosane, phytol and squalene (Banakar & Jayaraj, 2018). The chemical investigation of the dichloromethane root extract of *W. indica* led to the isolation and characterization of 8-deoxoantidesmone, waltheriones E–L and antidesmone (Cretton *et al*., 2014). The compounds waltheriones G, waltheriones H and waltheriones K showed potent and selective growth inhibition toward *Trypanosoma cruzi* (Cretton *et al*., 2014). Previously, adouetin X, waltheriones A, waltheriones C, betulinic acid, 3β-acetoxy-27-trans-caffeoyloxyolean-12-en-28-oic acid methyl ester and 3β-acetoxy-27-cis-caffeoyloxyolean-12-en-28-oic acid methyl ester were identified from the plant (Cretton *et al*., 2015), while waltherione C exhibited the highest and selective antitrypanosomal activity towards *T. cruzi* (IC_{50}=1.93 μM) with low cytotoxicity (IC_{50}=101.23 μM)). Other phytochemical compounds of *W. indica* includes oxyanin A, vitexicarpin, chrysosplenol E, flindulatin, 5-hydroxy-3,7,4'-trimethoxyflavone and waltheriones M-Q and 5(R)-vanessine (Cretton *et al*., 2016). Some of these compounds are known to possess anti-candidan activity (Cretton *et al*., 2016). The flavonoids namely (−)-epicatechin, quercetin, and tiliroside identified from W. indica significantly and dose-dependently inhibited the production of the inflammatory mediator nitric oxide (NO), and the cytokines (tumor necrosis factor (TNF)-α and interleukin (IL)-12), in lipopolysaccharide (LPS) and interferon (IFN)-γ activated murine peritoneal macrophages, without displaying cytotoxicity (Rao *et al*., 2005).

Ameliorating the synthesis of Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 COX-2 (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b). Nevertheless, their long-term use has many side effects including gastrointestinal erosion, peptic ulcers, nephrotoxicity, leucopenia and allergic manifestations. To overcome these effects, natural substances, such as medicinal plant provide a suitable alternative and can also be a source of new anti-inflammatory drugs. *W. indica*, is used in Nigeria ethnomedically for the treatment of asthma, cough, diarrhea and inflammation. The present study, the first of its kind, was undertaken to analyse the chemical constituents, anti-nociceptive and anti-inflammatory actions of essential oil of *W. inidca* with a view to lend supports to the use of *W. indica* for the treatment of inflammatory diseases in

traditional medicine.

MATERIALS AND METHODS

Collection of W. indica leaf sample

The leaves of *W. indica* were collected from the Botanical Garden, Lagos State University, Nigeria, in June 2017. Botanical identification was accomplished by Mr. Onabanjo at the Herbarium, University of Lagos where a voucher number (LUH 7819) was deposited. Prior to hydrodisitillation process the leaf samples were air-dried under laboratory shade for two weeks (27ºC) to reduce the moisture contents. In addition, sediments and other unwanted materials were separated from the samples. Afterwards, samples were pulverized to coarse powder.

Hydrodistillation of the essential oils

In this process 375 g of air-dried and pulverized leaves of *W. indica* was used. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification as described previously (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b). The distillation time was 3 h and conducted at normal pressure. The volatile oils were collected separately into clean weighed sample bottles. The oils were kept under refrigeration (4ºC) until the moment of analyses.

Chemical analysis of the oil

Gas chromatography (GC-FID) analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60ºC for 10 min, rising at 5ºC/min to 220ºC. The injector and detector temperatures were maintained at 250ºC; carrier gas nitrogen (2 mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 μL. The relative proportions of the oil constituents were percentages obtained by FID peak area normalization.

Gas chromatography-mass spectrometry (GC-EIMS) analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm; film thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature 220ºC and 240ºC, respectively; oven temperature programmed from 60ºC-240ºC at 3ºC/min.; carrier gas helium at a flow rate of 1 mL/min; injection volume 0.2 μL (10% *n*-hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was m/z 30-300 at a scan rate of 1 scan/sec.

The identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to a series of *n*-alkanes (C_6-C_{36}). Further identifications were also made possible by the use of a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data as described previously (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Drug and Chemicals

Carrageenan drug (Batch Number: SLBR0530V) of analytical grade was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Acetylsalicyclic salicylate injection (RX, Nigeria Ltd; Batch Number: MT2056) and Diclofenac Injection (FITZKING LINK LIMITED, Nigeria Ltd; Batch Number: 180606) were purchased from Lagos State University Pharmacy.

Study Animals

Wistar rats (150-200 g) of both sexes were accommodated in the Biochemistry Department animal facility of Lagos state University, Ojo-Lagos. The animals were kept in a metal steel cage, where they had unrestricted supply to water and standard pellet food. They were acclimatized for two weeks before commencement of experiment. The animals were assigned at random to a group of 5 consisting of 6 animals per group:

Group 1
Control group (Saline solution)
Group 2
Diclofenac treated group 100 mg/kg (Standard Group)
Group 3
100 mg/kg of essential oil of *W. indica* (WIEO)
Group 4
200 mg/kg of essential oil
Group 5
400 mg/kg of (WIEO)

The rationale for selecting the studied doses was that animals of similar weight were grouped together to obtain average weight. The weight recorded was similar across the groups of animals. The dose was therefore determined from the weight of animals in the assigned group. WIEO was dissolved in a saline vehicle and administered orally using canula to the animal in the order of 100, 200 and 400 mg/kg.

All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval No: 012/2017/LASU/BCH).

Toxicity study

WIEO was tested for acute toxicity study. Wistar rats were administered 500, 1000, 1500 and 2000 mg/kg of *W. indica* per oral route. One group received normal saline that served as a negative control. The animals were observed for 12 h continuously for changes in their behavior. Mortality for the next 14 days was also noted.

Carrageenan-induced paw edema in rats (Anti-inflammatory Analysis)

Carrageenan induced rat paw edema experiment was carried out according to a modification form of an established procedure as described previously (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b). Thirty Wistar rats (both sexes, 150-200 g each) divided into 6 animals in each groups used for study. The animals were induced by subcutaneous injection of 0.1 mL of 1% freshly prepared carrageenan in saline in the right hind paw. In addition, 1mL of all other solutions was administered for all doses. Paw volume of the injected rats was measured every hour for four hours using a plethysmometer (Ugo Basile, Italy).

Hot Plate test for anti-nociceptive study

The experiment was carried out according to the method described previously (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b). Thirty (30) mature Wistar rats of both sexes were randomly divided into 5 groups of equal rats. The animals were fasted for 12 h with provision of clean water *ad libitum*. Each mouse was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55°C within the restraining glass cylinder. Group 1 mice received 10 mL/kg of saline solution and served as control. Group 2 mice received sodium salicylate (10 mg/kg (ASA) (standard control) and groups 3, 4 and 5 received 100, 200 and 400 mg/kg of WIEO respectively (p.o.). Animal response to the heat varies and such changes includes kicking of hind foot and jumping about, licking of foot, raising the foot, holding the foot tightly to its body or shaking of the foot. The reaction time was recorded 30, 60, 90 and 120 min after the administration of the treatments. The maximum reaction time was fixed at 30 s to prevent any injury to the tissues of the paws. If the reading exceeds 30 s, it would be considered as maximum analgesia.

Statistical analysis

Repeated Measures Two way ANOVA Analysis using Bonferotti multiple comparisons post hoc test was performed using GraphPad Prism (version 7.02), San Diego CA, USA, www.graphPad.com) to compare activity between the control groups and rat treated with the test compounds and values were considered significant at $p<0.05$ and above. Results were expressed as mean ± SEM (Ogunwande *et al*., 2019a; Ogunwande *et al*., 2019b).

RESULTS AND DISCUSSION

Chemical constituents of the essential oil

A total of forty compounds representing to 99.8% of oil contents were identified from WIEO. The essential oil was obtained in a yield of 0.08% (v/w), calculated on a dry weight basis. The volatile compounds were displayed in Table N° 1, along with their percentages and retention indices calculated on HP-5 column. The GC chromatogram was shown in Figure N° 1. The main classes of compounds identified in the oil were monoterpene hydrocarbons (68.5%) and oxygenated monoterpenes (25.5%). The sesquiterpene were less common, represented by oxygenated sesquiterpenes (3.6%) and sesquiterpene hydrocarbons (2.2%). The major compounds of the oil were limonene (34.7%), sabinene (21.2%) and citronellal (9.7%). The other prominent monoterpene compounds were linalool (4.1%), (*E*)-β-ocimene (3.8%), myrcene (3.5%), α-pinene (2.6%) and linalool isobutyrate (2.2%). β-Caryophyllene (1.2%) and isospathulenol (1.1%) are the only sesquiterpene compounds occurring above 1%.

The authors are not aware of any information on volatile compounds of *W. indica*. This study therefore represents the first of its kind. Although an

extract of *W. indica* was recently analysed by GC/MS, however, the main compounds present in the extract such as 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, tetradecane, nonadecane, tetracosane, phytol and squalene (Banakar & Jayaraj, 2018) were not found in the composition of the essential oil.

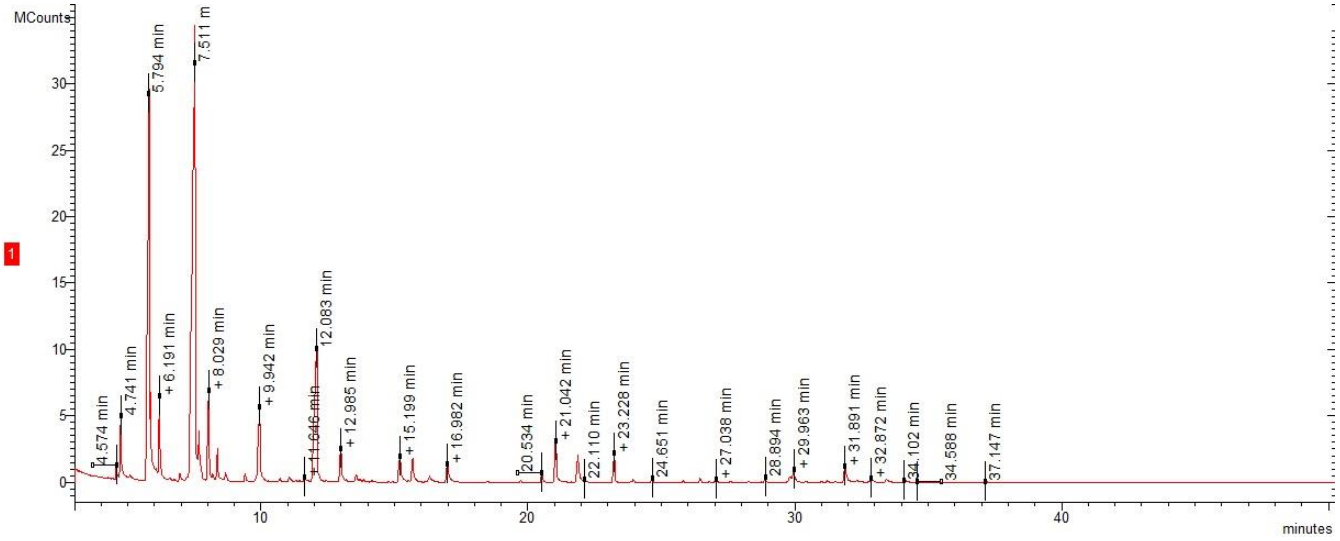

Figure N° 1
GC chromatogram of essential oil of *W. indica* on HP-5 capillary columns (30 m x 0.25 mm, 0.25 µm film thickness)

Acute toxicity

Test doses of 500, 1000, 1500 and 2000 mg/kg body weight of WIEO showed no adverse effects on the behavioural and physical responses in the tested rats following an observation for 14 days. There was no mortality, flesh or skin peeling, swollen limb or neck, and no weight loss was observed. Therefore, a higher dose of 400 mg/kg given to rats in this study was considered to be safe.

Anti-nociceptive action of the essential oil

Figure 2 shows the analgesic activity of WIEO using hot plate test. Hot plate test was chosen for evaluation of the anti-nociceptive activity of WIEO due to its ability to assess centrally-mediated nociceptive effects (Otuki *et al*., 2005a; Otuki *et al*., 2005b). The plot displayed minimum pain analgesia against the time of inhibition which was measured by animal response to heat characterised with hind paw licking and flicking or jumping measured at the 30th, 60th, 90th and 120th min following drug administration (Figure N° 2); high pain analgesia is displayed by rats with high pain tolerance. In this study, it was found that the essential oils showed high activity for all the doses when compared with the control (saline solution). The 100 mg/kg WIEO displayed a time dependent activity at the 30th min (p<0.01) and (p<0.001) at the 60th minutes but inhibition were reduced considerably to a non-significant (ns) value at the 90th and 120th min. The higher doses (200 and 400 mg/kg) displayed high inhibitory activity of thermal stimuli with statistical significant of p<0.001 during the entire period of exposure to heat sensitivity.

Waltheria species are traditionally recognised plant materials for the treatment of swellings, toothache, wounds, inflammation and rheumatism among the West Africans. In this study, we investigated the anti-nociceptive and anti-inflammatory properties of the essential oils of the leaves of *W. indica*. The central analgesic and thermal pain sensitivity activity of the extract was evaluated using the hot plate test. Nociception refers to the central nervous system (CNS) and peripheral nervous system (PNS) processing of mechanical, thermal and chemical stimuli, which triggers nociceptors and its pathways.

Table No. 1
Compounds identified in the essential oil of *W. indica*

Sr. No	Compounds [a]	LRI[b]	LRI[c]	Percent composition
1	α-Thujene	931	926	0.4
2	α-Pinene	941	932	2.6
3	Sabinene	976	968	21.2
4	Myrcene	993	988	3.5
5	α-Terpinene	1018	1014	0.3
6	Limonene	1032	1030	34.7
7	Sylvestrene	1033	1032	0.6
8	(*E*)-β-Ocimene	1052	1044	3.8
9	γ-Terpinene	1062	1060	1.2
10	*cis*-Sabinene hydrate	1070	1068	0.4
11	Terpinolene	1088	1088	0.2
12	Linalool	1101	1100	4.1
13	*cis*-p-Menth-2-en-1-ol	1124	1122	0.2
14	*neo*-Isopulegol	1144	1144	0.3
15	Citronellal	1155	1157	9.7
16	4-Terpineol	1178	1178	1.7
17	α-Terpineol	1191	1190	0.4
8	Citronellol	1230	1232	1.5
19	Neral	1240	1240	1.3
20	Geraniol	1257	1258	0.5
21	Geranial	1271	1273	1.2
22	Citronellyl aetate	1350	1350	0.5
23	Linalool isobutyrate	1373	1371	2.2
24	Geranyl acetate	1385	1383	1.5
25	β-Elemene	1392	1387	0.2
26	β-Caryophyllene	1420	1419	1.2
27	*trans*-α-Bergamotene	1438	1435	0.1
28	α-Humulene	1456	1453	0.1
29	Bicyclogermacrene	1495	1495	0.2
30	β-Bisabolene	1509	1507	0.2
31	Germacrene B	1556	1556	0.2
32	Spathulenol	1576	1576	0.4
33	Caryophyllene oxide	1581	1581	0.8
34	Isospathulenol	1639	1637	1.1
35	*epi*-α-Cadinol	1640	1640	0.2
36	α-Cadinol	1654	1652	0.3
37	α-Bisabolol	1683	1681	0.3
38	*epi*-α-Bisabolol	1686	1686	0.3
39	β-Sinensal	1691	1690	0.1
40	β-Bisabolenal	1765	1765	0.1
Total				**99.8**
Monoterpene hydrocarbons (Sr. No. 1-9, 11)				**68.5**
Oxygenated monoterpenes (Sr. No. 10, 12-24)				**25.5**
Sesquiterpene hydrocarbons (Sr. No. 25-31)				**2.2**
Oxygenatedsesquiterpenes (Sr. No. 32-40)				**3.6**

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5 column; [c] Literature retention indices (NIST, 2011); Sr. No. serial Number

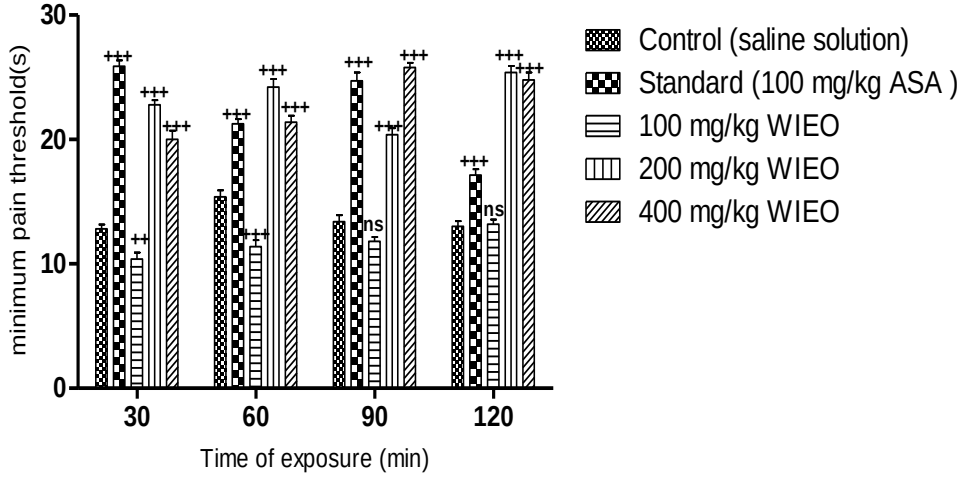

Figure N° 2
Effect of WIEO on heat induced pain. Control, Standard, and WIEO represent 1 mL saline solution, 100 mg/kg of Acetyl Salicylate and 100, 200 and 400 mg/kg of WIEO respectively. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ and ns= non-significant statistically compared to control

The cascade of events that occur during pain transmission in both, the PNS and CNS arises from the direct or indirect action of chemical mediators (Collins & Hourani, 1993). These mediators include arachidonic acid metabolites (prostaglandins and leukotrienes), peptides (kinins, tachykinins, calcitonin gene related peptide, galanin, cholecystokinin, vasoactive intestinal peptide), serotonin, acetylcholine, cytokines, nerve growth factor, glutamate, nitric oxide, adenosine triphosphate (ATP), adenosine diphosphate (ADP), among others. These mediators can be produced or released following tissue injury or by exogenous irritants (formalin, acetic acid, capsaicin, heat etc.).

Our result showed that WIEO also had a significant ability to prolong the response latencies indicating significant increase of the nociceptive threshold in treated animals as compared to the untreated groups typical of opioid drugs. The aqueous extract of stems of *W. indica* at Ouagadougou (Burkina Faso) administered at doses of 100, 200, 300 mg/kg reduced significantly ($p < 0.05$) the number of abdominal writhing in a dose-dependent manner (Yougbare-Ziebrou *et al*., 2016). Similar activity was reported by Owemidu *et al*. (2018), in that a dose of 200 mg/kg showed high thermal resistance which was attributed to the presence of phytochemicals such as terpenoids and alkaloid. Studies had confirmed that the analgesic effect of plant extracts were linked to their ability to inhibit the expression of certain pain mediators such as cytokines (TNF α, IL-12), prostaglandins, serotonin etc (Rao *et al*., 2005). A related species, *W. ovata* also displayed a dose dependent analgesic effect with inhibition ranging from 50 to 91%; an activity which could be inferred on suppression of mediators or enzymes during an inflammatory event (Herrera-Calderon *et al*., 2016). The analgesic effect of *W. indica* extract could be related to the inhibition of the mediators involved in nociceptive activities.

Anti-inflammatory activity

The evaluation of the anti-inflammatory activity in vivo was conducted using the model of carrageenan-induced paw edema. This is a well-defined model of acute inflammation and has been applied in the study of anti-edematous effect of extracts due to the production of different inflammatory mediator in the Wistar rat. This development is time dependent characterised by biphasic release of mediators. The initial phase involves the release of mediators such as histamine, serotonin and bradykin last within the first

1 h, while the latter phase is characterized by infiltration of leukocytes and prostaglandins biosynthesis (Di Rosa *et al.*, 1971; Posadas *et al.*, 2004; Antonio & Brito, 1998).

The inhibitory activities of WIEO were highly significant. As shown in Figure N° 3, mediators released in both phases were significantly inhibited. In the 1st to the 3rd h, edema was significantly reduced (p<0.001) by all the analysed doses (100, 200 and 400 mg/kg).The extract activities at these doses are also equivalent to that of the standard drug used (Ibuprofen). However, at the 4th h, there were significant reduction in the inhibitory activities of all doses; the 100, 200 and 400 mg/kg exhibited p<0.5, non-significant (ns) and p<0.01 respectively. This show that prolonged exposure to the essential oils of *W. indica* beyond the 3rd hour has no significant activity on the inflammation. Our results confirmed previous findings that WIEO exhibits a noticeable anti-inflammatory effect in accordance with traditional uses of the plant.

In the present study, oral treatment with WIEO markedly inhibited carrageenan-induced paw oedema in rats in a dose and time dependent property. This treatment steadily attenuated the paw oedema induced by carrageenan, as well as by numerous inflammatory mediators participating in the carrageenan-induced inflammation such as bradykinin, histamine, substance P and platelet-activating factor (Stochla & Maslinski, 1982; Hwang *et al*., 1986; de Campos *et al*., 1994; Gilligan *et al*., 1994). This evidence suggests that the anti-inflammatory actions of the essential oil of *W. indica* are related to the inhibition of one or more inflammation mediator pathways involved in the effects of these mediators.

Analgesic and anti-inflammatory activities found in this model have also been reported for several essential oils components (de Cássia da Silveira e Sá *et al*., 2013; Barreto *et al*., 2014) . In this connection, it should be noted that the 2 major components of the oil namely limonene and sabinene which are reported to have anti-inflammatory properties under cell culture systems. Limonene has been identified as an anti-inflammatory and anti-tumor candidate. In a previous study, at low concentration of limonene (7.34 mM), it decreased the production of ROS in eotaxin-stimulated HL-60 clone 15 cells. In the same study, NF-kappa B formation, chemotaxis and eosinophil migration were significantly reduced by limonene at 14.68 mmol/L (Hirota *et al.*, 2010). Limonene has also been reported to be an effective inhibitor of lipopolysaccharide (LPS)-induced NO and prostaglandin E2 production in RAW 264.7 cells. In addition, other cytokines (TNF-α, IL-1β, and IL-6) were also suppressed (Yoon *et al.*, 2010).

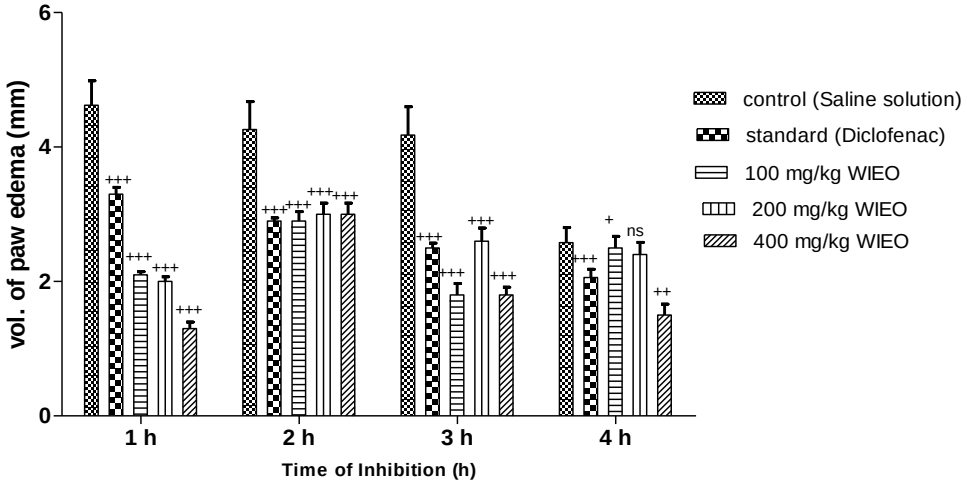

Figure No. 3
Effect of WIEO on Carragenan-induced inflammation. Control, Standard, and WIEO represent 1 mL saline solution, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg/kg of WIEO respectively. ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 and ns= non-significant statistically compared to control

Sabinene isolated from *Oenanthe crocata and Zornia diphylla* Pers, showed anti-inflammatory activity by inhibiting nitric oxide production in lipopolysaccharide (LPS) triggered (Valente *et al*., 2013; Kumar *et al*., 2014). Therefore, the mechanism of pain and anti-inflammatory of WIEO could be attributed to the ability of the major constituents to inhibit several pain mediators synthesized during the study. However, it should be considered that minor and major components, as well as synergistic interactions amongst the substances, could contribute to the studied pharmacological properties.

CONCLUSION

The present results suggest that the essential oils of *W. indica* might be the key players in the centrally-mediated inhibiting anti-nociceptive effect and the anti-inflammatory activity. The presence of Limonene (a monoterpenoid commonly found in lemon) and other constituents acting in synergy were suggested as agents causing the effect. The study therefore confirmed the ethno pharmacological basis of the use of *W. indica* in traditional medicine for treating pain, swellings, rheumatoid arthritis etc. Further study on the mechanism of pro-inflammatory inhibition will provide further basis for this application.

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