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Evaluation of antinociceptive activity of hydroalcoholic extract from leaves of *Oxytenanthera abyssinica* (A. Rich.) Munro (Poaceae) on strain Wistar rats

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Abstract

Oxytenanthera abyssinica (A. Rich.) Munro (Poaceae), an endemic plant to Africa, is used in traditional medicine in several African countries as Togo to treat many painful diseases. This study aimed to investigate the antinociceptive activity of hydroalcoholic leaves extract and reveal the phytochemical profile of leaves powder and extract. The phytochemical screening was based on colorimetric reactions and differential precipitations. For the antinociceptive assessment, writhing, glutamate and tail immersion tests were used. The extract doses tested were: 100, 200 and 400 mg/kg p.o. Chemical groups such as: tannins, saponins and steroids are revealed in the powder and extract. Alkaloids and flavonoids were only revealed in the extract. The extract produced a significant antinociceptive effect in writhing and glutamate tests but not in tail immersion test. The results obtained suggest that *O. abyssinica* leaves extract possesses antinociceptive activity and its therapeutic targets would be predominantly localize in periphery. The glutamatergic system would be particularly involved.

Keywords: Antinociceptive, Glutamatergic, Traditional medicine, *Oxytenanthera abyssinica*, phytochemical, Togo

1. Introduction

Pains is the major cause of psychological and physical distress. More than 90 percent of medical consultations are due to pain (Marchand, 2008) [17]. To relieve it, pain drugs are currently used through medical prescription or by self-medication (Naïm *et al.*, 2010) [20]. Despite modern analgesics administration, more than 50 % of post surgical patients continue to complain for intense pain (Vial *et al.*, 2000) [23] and some cancer patients often continue to suffer from persistent pain (Brahimi *et al.*, 2016) [4]. According to WHO (2015), about 83% of the world's population does not have access to pain relief and the majority are in low- and middle-income countries (<http://www.who.int/mediacentre/factsheets/fs402/fr/>). It adds to this figure day by day the people with chronic pathologies. In fact, it is estimated that 80 % of patients with AIDS and 67 % of patients with cardiovascular diseases or chronic obstructive bronchopneumonia will experience moderate to severe pain at the end phase of their life (<http://www.who.int/mediacentre/factsheets/fs402/fr/>). Pain is thus a public health problem and one of this problem factors is unavailability of essential and appropriate analgesics. Indeed, it has been revealed a shortage of adapted pain relieve drugs for cancer patients in university hospital centers in Togo despite patients suffering (Kpassagou *et al.*, 2016) [15]. Apart from ineffectiveness of conventional analgesics now available against chronic pains, many sides effects such as respiratory depression, ulcer, nausea, vomiting, pruritus are also related to their administration. Considering the above and, more importantly, the cost of pain medical care, more than 80 % of the population in Africa use traditional medicine for their health care (<http://www.who.int/mediacentre/news/releases38/fr/>). In Togo, *Oxytenanthera abyssinica* (A. Rich.) Munro (a bamboo spices) is used in traditional medicine as across sub-Saharan countries to treat several diseases (Louppe *et al.*, 2008; Yessoufou *et al.*, 2013; Ezeja *et al.*, 2014; Kokutse *et al.*, 2014; Wahab, 2015; Kantati *et al.*, 2016) [16, 25, 5, 14, 24, 13] and especially those with painful components (Borokini *et al.*, 2013; Ibeh *et al.*, 2013; Honfo *et al.*, 2015) [3, 11, 7]. But, to our best knowledge few pharmacological studies on this plant have been performed. In Togo, there is no report on pharmacological studies on *O. abyssinica* and among those carried out wide world, none concern its antinociceptive activity. So the results of phytochemical screening of the leaves main powder and hydroalcoholic extract of *O. abyssinica*, and assessment of antinociceptive activity of extract are reported in the present paper.

2. Materials and Methods

2.1 Vegetal material

Fresh leaves of *Oxytenanthera abyssinica* commonly known as Simouh "Kotokoli" or Pamplo "Ewe" were collected from their natural habitat in December 2016 in Kpéwa, Kara region (Togo). The plant has been identified by Pr Atsu K. GUELLY, the Head of the Department of Botany and Plant Ecology, Faculty of Sciences, University of Lomé (Togo), department in which herbarium voucher specimens were deposited and enregistered under the following number : TOGO15189. Fresh leaves were picked, washed and dried at laboratory air-conditioned temperature (20°C). The dried leaves were then reduced into powder using electrical grinder, packed and then stored in a dry place prior to use.

2.2 Animal material

Wistar rats (100–120 g) of either sex were used. The animals were obtained from the Laboratory of Physiology of Faculty of Sciences (FDS)-University of Lomé. The animals were kept in standard laboratory condition (room temperature: 25°C) and fasting for 18 h before experiment. Water access was ad libitum.

2.3 Chemicals and Drugs

Acetic acid, glutamate, NaCl, alcohol 95° (ethanol) were obtained from Laboratory of Animal Physiology. The others were obtained from the following Laboratories: acetylsalicylate of DL-Lysine (Aspégic) from SANOFIS, sodium diclofénac from FAES FARMA, Tramadol hydrochloride from MEDIS.

2.4 Extraction

The leaves powder (800 g) were macerated with 10 l of aqueous ethanol 70 % (v/v) and subjected to intermittent shaking every 30 minutes interval for 72 h. The collected filtrate after maceration was evaporated under vacuum at 45°C (Rotavapor Buchi R 210). The extraction yield was 3.2 % and the extract of *Oxytenanthera abyssinica* (EOA) was stored in a refrigerator at 4 °C until time of use.

2.5 Phytochemical Screening test.

Hydroalcoholic extract and the main powder of *O. abyssinica* leaves were assessed qualitatively to detect the presence of phytochemical constituents. The tests were based on colorimetric and precipitation reactions (Houghton *et al.*, 2012)^[8].

➤ Alkaloids detection

Six hundred mg of the powder or extract of *O. abyssinica* were dissolved in 9 ml of 10 % HCl.

Solution was then clarified by filtration. The filtrate was shared into three test tubes and in each tube was added one of the following reagents:

- Valser-Mayer, presence of alkaloids confirmed by the formation of yellowish-white precipitate
- Bouchardat, presence of alkaloids confirmed by formation of brown precipitate
- Dragendorff, presence of alkaloids confirmed by formation of orange precipitate.

➤ Polyphenolic compounds detection

A sample of 500 mg of extract or powder was dissolved in 10 ml of boiling water. The cooled solution after 15 minutes was filtered. The filtrate was divided into three portions which are used for the following investigations:

Tannins detection (Ferric chloride test)

The first portion of the filtrate was treated with few drops of 1 % ferric chloride solution. The change in color to bluish black indicates the presence of tannins.

Anthocyanins detection

One ml of the second portion is treated with 5 % HCl and to the mixture is added 50 % ammonia. The appearance of pink red turns blue violet indicates presence of anthocyanins.

Leucoanthocyanins detection

Five ml of the third portion is treated with 5 ml of concentrated HCl. The mixture is then heated for 15 minutes in a water bath set on 90°C. Appearance of a cherry or purplish red color indicates leucoanthocyanins.

Flavonoids detection

Fifty mg of the extract were dissolved in 2.5 ml of alcohol (95°). The solution was filtered. Two ml of alcohol chloride is added to 2 ml of the filtrate. The mixture is then treated with magnesium. The presence of flavonoids is confirmed by pink color turning to red.

Anthraquinones detection (Bornträger test)

About 200 mg of extract or powder was boiled with 2.5 ml of 10 % H₂SO₄ and filtered hot. The filtrate was shaken with an equal volume of toluene. After settling in another test tube, an equal volume of 10 % ammonia solution was added to the toluene phase. The mixture was shaken and the presence of a pink color indicated the presence of anthraquinones.

Saponins detection (Frothing Test)

One gram of the powder or extract was boiled with 100 ml of distilled water for thirty minutes and then filtered. The filtrate was shaken vigorously in test tubes for thirty seconds and was allowed to stand for fifteen minutes. Result was observed.

Sterols and terpenoids detection (Liebermann test)

One ml of acetic anhydride and one ml of chloroform were mixed with 100 mg of the powder or extract and then filtered. The filtrate was shared into two test tubes. The first served as control and into the second was added 1 ml of conc. H₂SO₄ carefully to the wall of the tube. Appearance of brownish or purple red ring at the junction of the two layers indicates the presence of metabolites. A gray or violet supernatant is characteristic of terpenoids, however, a blue to green-gray color indicates the presence of sterols.

2.6 Antinociceptive analysis

2.5.1- Acetic acid-induced writhing test

The wistar rats (25) were randomly organized in five groups of five animals and treated as following: group I, considered as control received distilled water (ED) per os when the groups (II, III, IV) were treated by 100, 200 and 400 mg/kg per os respectively. The group V which served as reference received 200 mg/kg per os of acetylsalicylate of DL-Lysine (ASP). Fifteen minutes after aspirin or 30 minutes after EOA administration, the writhes was induced by injection of acetic acid (2.5 %). Five minutes after acetic acid administration, the rats were observed and the writhing number was counted for 30 minutes as described previously (Singh *et al.*, 2001)^[22]. Inhibition percentage is calculated by the following formula:

$$\% I = \frac{[NCTe - NCTr]}{NCTe} \times 100$$
 (NCTe: negative control et NCTr : treatment groups).

2.5.2- Glutamate-induced paw licking test

Aiming to elucidate antinociceptive response of EOA, the participation of glutamate receptors was evaluated using the method described by Beirith *et al* (2002)^[1]. The animals were treated with EOA (100, 200, and 400 mg/kg, p.o.) while control group received NaCl (10 ml/kg p.o.) and the reference group was treated by diclofenac (10 mg/kg p.o.). Thirty minutes after treatments, 20 μ l (10 μ mol/paw) of glutamate was injected into the ventral surface of the right hind paw of rats. The rats were immediately after injection individually placed in an observation chamber for 15 minutes following glutamate injection. Their behavior response (injected paw licking) was observed and the period spent licking the injected paw was recorded as an indication of nociception.

2.5.3. Tail immersion test

The tail immersion test was performed in order to evaluate the central antinociceptive activity of EOA. Randomized rats in five groups of five were treated with extract (100, 200 and 400 mg/kg p.o.). The control group received NaCl 9 % (1 ml/kg, i.p.) and tramadol (25 mg/kg, i.p.) is used as reference (Foroud *et al.*, 2015)^[6]. After treatment, the animal is held in a vertical position and 3 cm from the end portion of the tail is immersed in hot water (50°C). The tail residence time in hot water (withdrawal latency) is considered as the reaction time

(Tr) and the control is denoted Tc. This reaction time is recorded at 0, 30, 60, 90, 120 minutes after treatment. A cut-off time of 30 s was maintained to avoid tail tissue damage.

The percentage of the maximal effect (PME) is calculated using the following formula:

$$PME = (Tr - Tc) / Tc \times 100.$$

2.5.4 Statistical analysis

The results were analyzed by the GraphPad Prism version 6 statistical software. One-way analysis of variance (ANOVA) followed by Tukey's test were used for the treatment of acetic acid-induced writhing and glutamate-induced paw licking test results. Two-way ANOVA followed by Dunnett's test were used for the treatment of tail immersion test results. Differences between groups were considered significant at $p < 0.05$.

3. Results

3.1- Phytochemical screening

Phytochemical analysis shows that *Oxytenanthera abyssinica* leaves are a rich source of polyphenols (tannins, flavonoids), alkaloids, steroids and saponins. These chemical components are more revealed in hydroalcoholic extract than in the direct leaves powder (Table).

Table 1: Phytochemical composition of powder and leaves extract of *Oxytenanthera abyssinica*

Chemical groups	Tests	Powder	Extract
Tannins	FeCl ₃ 1 %	+	+++
Flavonoids	Alcool 95° + Alcohol chloridric (Cyanidine reaction)	-	+++
Anthocyanins	HCl 5 % + Ammoniac 50 %	-	-
Leucoanthocyanins	Hot chloridric alcohol (90°C) (Shinoda reagent)	-	-
Anthraquinones	H ₂ SO ₄ 10 % + Toluene + Ammoniac 10% (Boroträger)	-	-
Saponins	H \geq 1 cm => IM \geq 100	+	+
Alkaloids	Bouchardat reagent	-	+++
	Dragendorff reagent	-	+++
	Valser-Mayer reagent	-	+++
Steroids	Acetic anhydrid + CHCl ₃	+++	+++
Terpenoids	(Liebermann reaction)	-	-

Absent (-), trace (+), abundant (++) , more abundant (+++)

3.2 Antinociceptive effects of extract

3.2.1 Effect of extract on acetic acid-induced writhing test

The writhing extent in rats induced by intraperitoneal administration of acetic acid 2.5 % was reduced by the EOA dose dependently (100, 200 and 400 mg/kg p.o.). The writhing number was significantly suppressed by 100 mg/kg

($p < 0.001$) and by 200 and 400 mg/kg ($p < 0.0001$). The three oral doses: 100, 200 and 400 have exhibited 39,19 %, 49,19 % and 63,78 % of writhes suppression respectively compared in percentage to the control,. The pain reduction effect by the dose of 400 mg/kg compared to 100 mg/kg was also significant ($p < 0.05$). The reference drug aspirin also

caused a significant antinociceptive effect ($p < 0.0001$) which is more important (78,10 %) than the highest dose effect of EOA (all compared to the control (Figure 1).

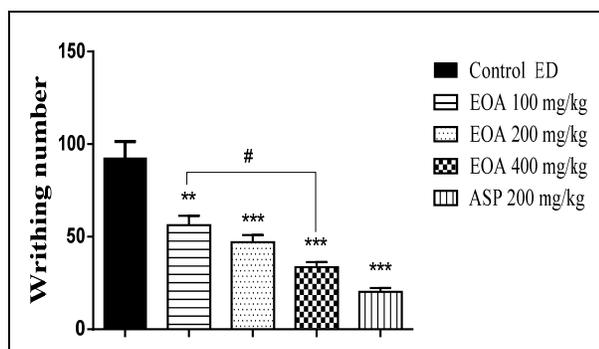


Fig 1: Antinociceptive effect of EOA (100, 200, 400 mg/kg, p.o.) and acetylsalicylic acid (ASP: aspirin 200 mg/kg, p.o.) in the nociception induced by acetic acid in rats. EOA: hydroalcoholic extract of *O. abyssinica*. The data were analyzed by ANOVA one-way followed by Tukey's test. The results are expressed as mean \pm SEM (n = 5). ** $p < 0.001$ and *** $p < 0.0001$ (treated compared to control); # $p < 0.05$ (EOA 400 vs. EOA 100).

3.2.2- Effect of extract on glutamate-induced paw licking

Injection of the glutamate induces a nociceptive response illustrated by the licking of the treated paw. The dose (100 mg/kg) of EOA does not significantly reduced the nociception induced by glutamate injection. In contrast, the doses (200 and 400 mg/kg p.o.) as well as the reference (diclofenac: 10 mg/kg p.o.) compared to the control exerted a significant antinociceptive activity ($p < 0.001$). The inhibition percentages obtained with 200 mg/kg and 400 mg/kg doses is 80.11 % and 89.54 %, respectively. The reference drug has shown more important pain reduction (96.36 %) (Figure 2).

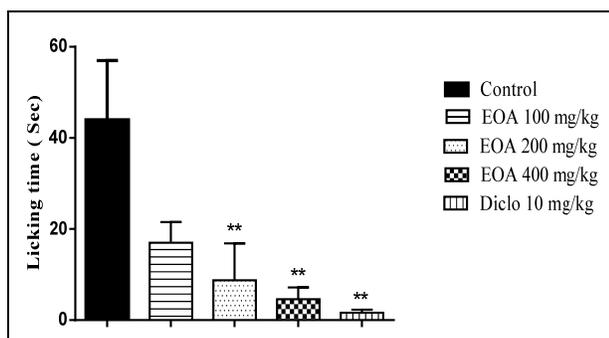


Fig 2: Antinociceptive effect of EOA (100, 200, 400 mg/kg, p.o.) and diclofenac (Diclo 10 mg/kg, i.p.) in the nociception induced by glutamate injection. EOA: hydroethanolic extract of *O. abyssinica*. The data were analyzed by ANOVA one-way followed by Tukey's test. The values expressed are mean \pm SEM (n = 5). ** $p < 0.001$ (treated compared to control).

3.2.3- Effect of extract on Tail immersion test

Rat's tail immersion in water (50° c) causes a pain. Animals react by tail removal from the thermal source (hot water). In this study, the evaluation was performed at 0, 30, 60, 90 and 120 minutes after treatment. The doses of: 100, 200, 400 mg/kg p.o. did not show significant antinociceptive activity ($p < 0.05$) compared to the control. However, 30 minutes after treatment, an appreciable increase in PME of 71.87 % and 65.62 % was noted with 100 and 400 mg/kg doses respectively, when the dose of 200 mg/kg showed 28.12 %. The reference drug, tramadol significantly ($p < 0.0001$)

reduced the nociceptive effect induced by the thermal source. The PME obtained with tramadol are: 358.33 %, 207.97 %, 188.88 % and 140.56 % respectively at 30, 60, 90 and 120 minutes after treatment.

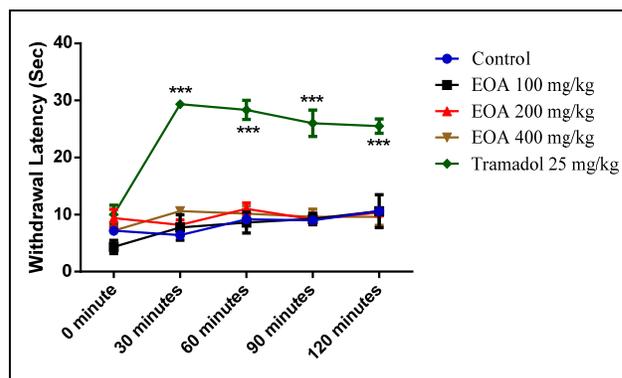


Fig 3: Antinociceptive effect of EOA (100, 200 and 400 mg/kg p.o.) and tramadol (25 mg/kg i.p.) on the withdrawal latency of the tail from water (50° c) in the rat. Each value represents the mean \pm SEM (n = 5). The data were analyzed by ANOVA two-way followed by the Dunnett's test. The values expressed are mean \pm SEM (n = 5). *** $p < 0.0001$ (treated compared to control).

4. Discussion

The aim of our work was to evaluate the antinociceptive activity of *Oxytenanthera abyssinica* (A. Rich.) Munro on pain induced by chemical and physical agents, and reveal chemical groups that are present in the leaves powder and extract. The present study reveal the presence of some chemical groups in the powder and in the hydroalcoholic extract of the plant leaves, and beyond its antinociceptive activity confirmed, some data give an idea about its mechanism of action. This study could constitute a scientific contribution for the plant use in traditional medicine for the management of pathologies with painful manifestation.

The phytochemical analysis has shown that the main leaves powder of *Oxytenanthera abyssinica* (A. Rich.) Munro contain like the hydroalcoholic extract tannins, steroids, and saponins. Indeed, the method of Houghton et al. (2012) [8] used for characterization revealed that tannins and saponins are less abundant in the powder than the extract. The hydroalcoholic extract, in addition to containing steroids (abundant) and saponins (trace) as in the powder, reveals abundant presence of tannins, flavonoids and alkaloids whereas flavonoids and alkaloids are absent in the powder. Ibeh et al. (2013) [11] in Nigeria found that methanolic extract of *Oxytenanthera abyssinica* contains steroids, tannins, alkaloids, flavonoids in abundant state while saponins are found in trace. Yessoufou et al. (2013) [25] in Benin also have demonstrated that ethanolic extract of *Oxytenanthera abyssinica* contains some bioactive compounds. According to their works, alkaloids, tannins, steroids and flavonoids were abundant whereas saponins, anthocyanins, leucoanthocyanins are present in state of trace. These results of the phytochemical screening corroborate with previous works in literature at the exception of anthocyanins and leucoanthocyanins. This difference can be related to the geographical area of harvest.

The acetic acid-induced abdominal constriction (writhes) is the preliminary test for the evaluation of antinociceptive activity of a plant extract. The writhing (response to pain behavior) expressed by the rats after acetic acid injection, reflect activation of local peritoneal sensitive receptors through inflammation mediators. The mediators involved in

this pain are prostaglandins, substance P, histamine, serotonin, bradykinin and cytokines (TNF- α , IL-1 β and IL-8) (Moniruzzaman *et al.*, 2015) [19]. These above compounds are implicated in nociceptive receptors activation and peripheral sensibilisation. In this study, the doses (100, 200 and 400 mg/kg p.o.) of EOA significantly reduced pain syndromes dose-dependently. Diatta *et al.* (2014) in Senegal has demonstrated that hydroalcoholic extract of *Zanthoxylum zanthoxyloides* showed similar results with 100 and 300 mg/kg per os. With 50.54 % and 72.9 % of writhes inhibition the doses of 100 and 300 mg/kg per os respectively have exhibited more important pain reduction than EOA effect at 100 mg/kg (39.19 %) and 400 mg/kg (63.78 %) per os. Aqueous extract of *Hyptis pectinata* administrated orally has shown a dose-dependent antinociceptive protection (Bispo *et al.*, 2001) [2]. But the doses of 100, 200 and 400 mg/kg of *H. pectinata* has shown 43, 51 % and 54 % of protection respectively. The highest dose (400 mg/kg) has shown less protection than EOA (400 mg/kg). Our results demonstrate that EOA would more effectively protect against writhes induced by acetic acid (Figure 1).

Glutamate has been known as the main excitatory neurotransmitter of the central nervous system. But recently glutamatergic receptors have been highlighted on peripheral sensitive neurons (Ritter *et al.*, 2014) [21]. So the manipulation of the peripheral glutamatergic system provides an approach for the management of peripheral pain (Carlton, 2001). To examine whether glutamate receptors could be involved in EOA-induced antinociception, the glutamate test has been performed. An intraplantar injection of glutamate solution causes painful behavior characterized by licking and biting the injected paw. Glutamate receptors also have an important role in sensitization of dorsal horn of spinal cord, since primary afferent fibres stimulation results in liberation of glutamate (Millan, 2002) [18]. In this experience the significant antinociceptive response ($p < 0.001$) was obtained with doses of 200 and 400 mg/kg, the protection induced was 80.11 % and 89.54 %, respectively. Quintans *et al.* (2014) in Brazil found that hexanic fraction of the ethanolic extract of *Combretum duarceanum* Cambess reduces glutamate-induced pain dose dependently. According to their studies, doses of 200 and 400 mg/kg reduced the nociceptive effect respectively by 54.1 % and 58.7 % ($p < 0.001$) compared to the control. These previous results show that EOA provides greater antinociceptive effect than the hexanic fraction of the ethanolic extract of *Combretum duarceanum* at the same dose of administration. Glutamate injected provoke activation of peripheral, spinal and supraspinal NMDA and non-NMDA receptors (Beirith *et al.*, 2002) [1] and stimulate peripheral release of nitric oxide (NO) or related substances (Moniruzzaman *et al.*, 2015) [19]. The EOA could have molecules that inhibit activation of central and/or peripheral NMDA and non-NMDA receptors. These molecules could also block the synthesis of NO. There was an oedema developed on the paw injected during the experiment. This phenomenon could confirm the work of Beirith *et al.* (2002) [1] on the involvement of nitric oxide (NO) which is a potent vasodilator. The anti-oedematous properties of EOA deserve to be investigated.

Based on the previous effects of *O. abyssinica* extract on nociception induced by acetic acid and glutamate injection, we aimed to evaluate its central effect on pain control. The results obtained have shown that none of the doses (100, 200 and 400 mg/kg) according to the method described by Foroud and Vesal (2015) [6] significantly increases the tail withdrawal

latency. However, the reference drug tramadol (25mg/kg, i.p.) significantly ($p < 0.0001$) increased the withdrawal latency beyond 100 % compared to the control at 30, 60, 90 and 120 minutes after treatment. This test is a popular tool used to control the spinal and supraspinal reflex (Jinsmaa *et al.*, 2004) [12]. Tramadol is an opioid agonist acting on the same receptors as morphine. Indeed in the spinal, the analgesia produced by tramadol involves the opioid receptors δ and $\mu 1/\mu 2$ ($\mu 2$ preferentially) whereas at the supraspinal level, basically the $\mu 1$ receptors are involved (Jinsmaa *et al.*, 2004) [12]. Our current evaluation has not shown that EOA is gifted of antinociceptive effect using tail immersion method.

5 Conclusion

EOA is confirmed to be rich in bioactive substances. This study has rationally proved *Oxytenanthera abyssinica* use in traditional medicine to treat pain or nervous and rheumatic diseases for its antinociceptive activities. The results show that the antinociceptive activity of EOA would be related to the presence of chemical groups revealed. This study has shown that therapeutic targets of these bioactive molecules on neurons would be localized in periphery and glutamatergic receptors would be mainly involved. Further studies are required to carry out anti-inflammatory and anti-oedematous activities of the plant leaves. Further more, antinociceptive activities of extract isolated steroids might be investigate.

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