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Antinociceptive evaluation of the aqueous bark extract of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. and Timler (Rutaceae) on albino wistar rats

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ABSTRACT

Objectives: This study aimed to investigate the phytochemistry and the analgesic activity of the aqueous bark extract of *Zanthoxylum zanthoxyloides* (AZZ) on albino Wistar rats.

Methodology and Results: Phytochemical screening was done using colorimetric reactions and precipitations. Writhing, glutamate, and hot plate tests were used for antinociceptive assessment. The animals were given the extract (400 and 800 mg/kg) and standard drugs, orally. The phytochemical screening has revealed chemical components like alkaloids and flavonoids. The extract displayed significant antinociceptive activity ($p < 0.05$). At 400 and 800 mg/kg, the extract reduced the writhing by 33.51% and 54.74% respectively. Licking reduction was observed after 15 and 20 minutes of glutamate injection in groups that received extract at 800 and 400 mg/kg, respectively. For the hot plate test, the extract effect was obtained from the 30th to the 90th minutes.

Conclusions and application of findings: This study finding corroborates the traditional use of *Zanthoxylum zanthoxyloides* species. This activity may be due to the presence of some chemical groups confirmed by the phytochemical screening such as flavonoids and alkaloids. The mechanism for the antinociceptive activity could be due to the inhibition of the synthesis of some inflammatory mediators such as prostaglandins and nitric oxide (NO). The plant *Z. zanthoxyloides*, particularly its bark, could be a potent source for the development of new analgesic drugs. Further researches about the safety and characterization of its effective ingredient are needed.

Keywords: Albino Wistar rats, *Zanthoxylum zanthoxyloides*, bark, folk medicine, antinociceptive.

INTRODUCTION

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja *et al.,* 2020). As a defensive mechanism, pain aids a living entity to be delivered of unpleasant stimuli in order to keep it away from injury. However, the production of hypersensitivity such that pain metamorphosizes from a short-term warning signal to a chronic devastating condition is a result of alterations in the pain pathway (Basbaum *et al.,* 2009). Although a tool for the immune system to protect the area damaged by mechanical, chemical, and thermal stimuli, pain causes a lot of discomfort and suffering to the patients (Boursinos *et al.,* 2009; Gunn-Moore, 2010). A variety of analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, as well as opioid analgesics are used to manage pain. These drugs have various adverse effects such as liver damage, cardiovascular problems, renal failure, hypertension, gastrointestinal tract ulcers, and respiratory problems (Wallace, 2001; Yasmen *et al.,* 2018). Thus, there is an urgent necessity for antinociceptive cure with less side effects. Medicinal plants represent an important health tool in the rural population lives, especially in the isolated region of developing countries. According to the World Health Organization (WHO), up to 80% of the population in many African and Asian countries depend on traditional and complementary drugs for their primary health care (Ridtitid *et al.,* 2008; Verma & Singh, 2008). Herbal therapies could provide an interesting option for emerging antinociceptive drugs. Zanthoxylum is the most abundant genus in Rutaceae family with approximatively 200 species distributed in the warm and tropical regions of Africa (Groppo *et al.,* 2012). One among these is *Zanthoxylum zanthoxyloides* (Lam) also called Senegal prickly-ash, a small shrub tree known all over the African continent. Several works have been reported on its pharmacological properties. *Z. zanthoxyloides* has been reported to display antibacterial activity (Tatsadjieu *et al.,* 2003; Anne *et al.,* 2013; Wouatsa *et al.,* 2013), antiplasmodial activity (Kassim *et al.,* 2005; Adebayo & Krettli, 2011;), antioxidant and anti-inflammatory activities (Chaaib *et al.,* 2003; Diatta *et al.,* 2014; Larsen *et al.,* 2015), and analgesic activity (Prempeh & Mensah-Attipoe, 2008). It is used traditionally to treat toothache, dental caries, and cancer (El-Said *et al.,* 1971; Adesina, 2005 ;), central nervous system disorders and malaria ((Denou *et al.,* 2016; Kantati *et al.,* 2016; Koudouvo *et al.,* 2016), inflammation and pain (Diatta *et al.,* 2014), malaria, chest pain, and heart palpitations (Sanogo, 2011; Diarra *et al.,* 2015). This present study was carried out to evaluate the possible antinociceptive effect and the phytochemical components of the aqueous bark extract of *Z. zanthoxyloides*.

MATERIALS AND METHODS

Plant material: *Zanthoxylum zanthoxyloides* bark was collected in the northern part of Togo (Pya) in February 2019. A botanist from the Botany and Vegetal Ecology Department authenticated the plant, and a voucher specimen has been deposited in the herbarium of the Department with reference number: Togo15491. The stem bark was air-dried and reduced into powder in the Department of Biochemistry, University of Jos, using a traditional mortar and its pestle.

Preparation of the aqueous extract: The maceration of the plant powder (500 g) was done for 24 h with 1.5 L of distilled water at laboratory temperature, and the filtrate collected after filtration. The same procedure was done twice with the substrate. The filtrate from the three days of maceration was

concentrated to dryness in an oven at 70 ºC and kept at - 4 ºC for further uses.

Phytochemical screening: Phytochemical screening was carried out in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, using the colorimetric and precipitation methods described by Harborne (1998), Evans (2002), and Sofowora (2008) with some modifications. The plant powder and the aqueous extract were assessed qualitatively to detect the presence of various secondary metabolites.

Animal material and ethical considerations: The Animal House Unit of University of Jos, Nigeria provided male Wistar rats weighing between 140-180g. The animals were housed under ambient temperature, with 12 h light and 12 h dark cycle, with free access to water and food. All animal procedures were executed in line with the guidance of proper care and use of laboratory animals after approval (No. UJ/FPS/F17-00379) from the Ethics Committee of the University of Jos, Nigeria. Before each experiment, the animals were housed in groups of five per cage based on their body weights, acclimated for 7 days and then overnight fasted with liberal access to water before experimentation.

Chemicals and drugs: Acetic acid, L-glutamate, sodium chloride, Ethanol 95% was obtained from Department of Biochemistry, University of Jos. The others were obtained from the following Laboratories: Tramadol hydrochloride from Richy Gold International Limited, Lagos-Nigeria; acetylsalicylate of DL-Lysine (ASP); and sodium diclofenac from Ghali Pharmacy, Jos-Nigeria.

Antinociceptive activities

Acetic acid-induced writhing test: Twenty male albino Wistar rats weighing 140-180g were randomly divided into four groups of five animals each and treated orally as follows: group I, as control received normal saline at (0.9% NaCl); group II, as standard drug received 200 mg/kg *per os* of acetylsalicylate of DL-Lysine (ASP); groups III and IV, received AZZ at 400 and 800 mg/kg body weight, respectively. Writhes were induced by injecting 0.1 mL of 2.5% acetic acid 15 or 30 minutes after drugs administration. Five minutes after writhes induction, the animals were observed and the number of writhes counted for 30 minutes as described previously by Singh *et al*. (2001).

Glutamate-induced paw licking test: This test aimed to evaluate the intervention of glutamate receptors during the antinociceptive response of *Z. zanthoxyloides* bark extract. The method used was described by Beirith *et al*. (2002) modified by Atchrimi et al. (2017). Twenty Wistar rats weighing 150-180g were grouped into four groups of five animals each. The four groups were given normal saline as control (10 ml/kg *per os*), diclofenac as reference drug (10 mg/kg *per os*) and AZZ (400 and 800 mg/kg, *per os*), respectively. Thirty minutes after treatment, 20 µL (10 µmoL/paw) of glutamate was injected into the ventral surface of the right hind paw of the rats and individually placed on observation for 20 minutes following glutamate injection. Their behavioural response (injected paw licking) was observed and the number of licking of the injected paw by the animal was recorded every five minutes as an indication of nociception.

Hot plate test: The central antinociceptive activity of the AZZ was evaluated using the hot plate method described by Eddy & Leimbach (1953). Male albino Wistar rats weighing 150-180 g were housed under standard laboratory conditions with free access to food and water. Four groups of five rats each were constituted and treated orally as follows: group I, as Control group received normal saline at 10 mL/kg body weight; while group II, as standard group received tramadol at 25 mg/kg body weight. AZZ was given to animals in group III at 400 mg/kg while animals in group IV received the extract at 800 mg/kg body weight. After treatment, the rats were placed on a hot plate analgesia meter (Orchid

scientific, India) maintained at 55 ± 1 ºC within the restraint. The latency period or reaction time in seconds was considered as the time taken by the rats to react to the thermal pain by licking their paws or by jumping (LeBars *et al.*, 2001). The reaction time was recorded at 0.5, 1, 1.5, 2 and 3 h after administration of the extract and standard drug.

Statistical analysis: Results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test were applied to determine the level of significance, and $p < 0.05$ was considered to be statistically significant.

RESULTS

Phytochemical screening: Nine secondary metabolites were sought in both extract and powder. Alkaloids, flavonoids, tannins, cardiac glycosides, and carbohydrates were present in varying concentrations in both the powder and the extract, while terpenoids and anthraquinones were absent in both powder and extract. Steroids were moderately present in the powder, but absent in the extract (Table 1).

Table 1. Phytochemical composition of powder and bark aqueous extract of *Zanthoxylum zanthoxyloides*

Secondary metabolites	Tests	Powder	Aqueous Extract
Alkaloids	Mayer reagent	+++	+++
Flavonoids	10% Lead acetate	+++	+++
Tannins	10% $FeCl_3$	+	++
Saponins	Frothing test	+	+++
Steroids	Lieberman-Burchard test	++	-
Terpenes	Lieberman-Burchard test	-	-
Cardiac glycosides	Keller-Kiliani's test	++	++
Carbohydrates	Molisch's test	+	+
Anthraquinones	Borntrager's test	-	-

+++: highly present, ++: moderately present, +: present, -: absent

Antinociceptive effects of the extract

Effect of the extract on acetic acid-induced writhes: The writhes were induced by injection of acetic acid (2.5%) 15 minutes after aspirin or 30 minutes after extract administration. Results showed significant reduction of writhes number in the standard ($p < 0.001$) and treated (AZZ 400 mg/kg and 800 mg/kg at $p < 0.01$ and $p < 0.001$, respectively) groups (Figure 1).

Effect of the extract on glutamate-induced paw licking test: The lickings were induced by injection of L. glutamine (10µmol). The number of licking was recorded every 5 minutes for 20 minutes. The results showed significant reduction in the number of licking in the standard group from the tenth to the twentieth minute ($p < 0.01$). Number of licking was significantly reduced in the group that received the extract (800 mg/kg) at 15 and 20 minutes ($p < 0.05$ and $p < 0.01$), but only at the twentieth minute in the group that received the extract at 400 mg/kg ($p < 0.05$) (Figure 2).

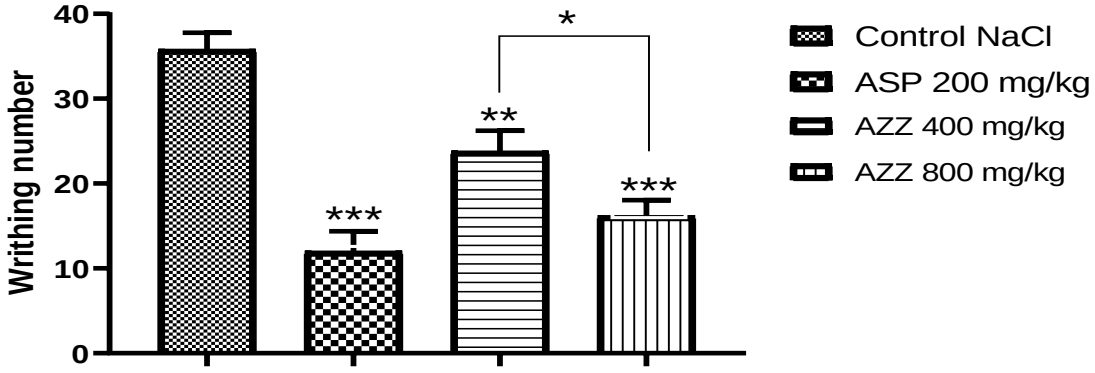

Figure 1. Antinociceptive effect of AZZ (400 and 800 mg/kg, p.o) and acetylsalicylic acid (ASP 200 mg/kg, p.o) on acetic acid induced nociception in rats. AZZ: aqueous extract of *Zanthoxylum zanthoxyloides* bark. The results were expressed as mean ± SEM (n=5). $^{**}p < 0.01$ and $^{***}p < 0.001$ (control group compared with treated groups), $^{*}p < 0.05$ (AZZ 800 mg/kg vs AZZ 400 mg/kg).

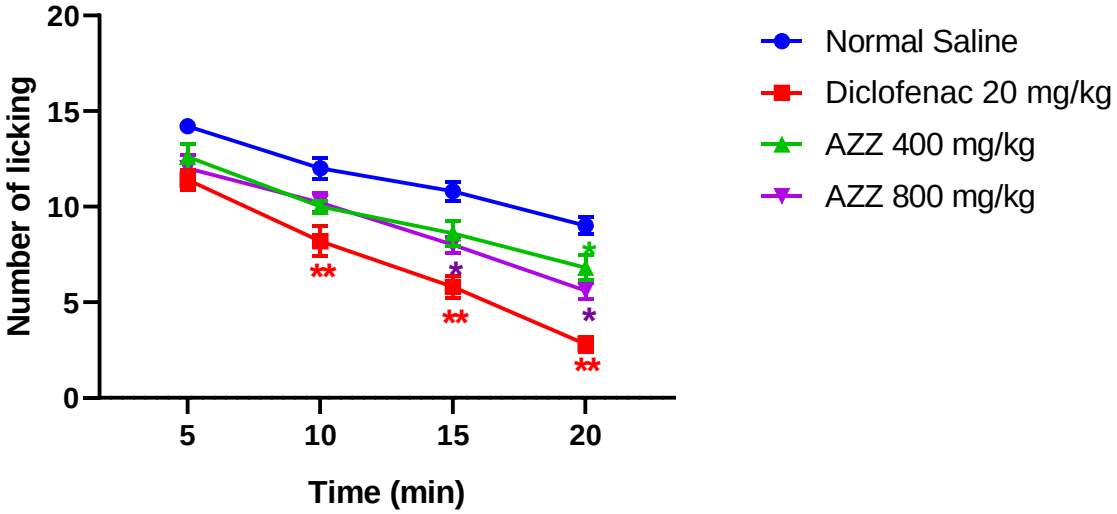

Figure 2. Antinociceptive effect of AZZ (400 and 800 mg/kg, p.o) and Diclofenac 20 mg/kg (p.o) on the nociception induced by L. glutamine. AZZ: aqueous extract of *Zanthoxylum zanthoxyloides* bark. The results were expressed as mean ± SEM (n=5). $^{**}p < 0.01$ and $^{*}p < 0.05$ (treated groups compared with control).

Effect of the extract on hot plate test: With the effect of AZZ on nociception induced by acetic acid and L-glutamate, we aimed to evaluate its central effect on pain. Thermal nociception is mainly undertaken to explore if drugs have any central analgesic aspect. Placing an animal on the hot plate analgesia meter causes pain, and the resulting action is jumping or paw licking. There was significant augmentation of reaction time in the standard group at $p < 0.001$ when compared to the control group. Data obtained showed significant augmentation ($p < 0.001$) of reaction time at 30, 60, and 90 minutes after administration of AZZ in groups III and IV (AZZ at 400 and 800 mg/kg, respectively) when compared with the control group; while in the standard group (tramadol 25 mg/kg), there was significant augmentation of reaction time from 30 to 180 minutes. At $p < 0.01$, data

showed significant difference from 30 to 180 minutes for group III (AZZ 400 mg/kg) and from 30 to 90 minutes for group IV (AZZ 800 mg/kg) when compared with the standard group. When the 400 mg/kg group was compared to 800 mg/kg group, there was significant augmentation ($p < 0.05$) of reaction time (Table 2). The antinociceptive effect was found to be dose-dependent.

Table 2. Effect of aqueous stem bark extract of *Zanthoxylum zanthoxyloides* on hot plate induced nociception

Time	Control (NaCl)	Standard (Tramadol 25 mg/kg)	AZZ 400mg/kg	AZZ 800 mg/kg
30 min	4.28±0.17	16.1±0.44 *	8.1±0.30 *	15.3±0.32 *
60 min	4.02±0.28	17.54±0.28 *	8.42±0.41 *	14.82±0.40 *
90 min	3.98±0.63	16.18±0.48 *	8.9±0.45 *	10.78±0.16 *
120 min	4.46±0.16	12.44±0.47 *	4.84±0.40	5.44±0.48
180 min	3.32±0.60	12.32±0.60 *	3.32±0.47	4.76±0.85

Values were expressed as mean ± standard error of mean (SEM, n=5), *$p < 0.05$ when compared control group to all treated groups.

DISCUSSION

The commonly useful models for drugs antinociceptive evaluation are acetic acid induced abdominal writhing; L-glutamine induced licking responses, and hot plate test. This work aimed to evaluate the analgesic effect of AZZ on pain induced by chemical and physical agents and reveal chemical components present in powder and aqueous extract. The presence of alkaloids, flavonoids, tannins, and saponins was noticed in both powder and extract. All these components were present at the same amount in both except tannins, which amount was high in the extract. Water in this study seems to extract more tannin from the stem bark of *Z. zanthoxyloides*. This study result is similar with the previous one of Anne *et al.* (2013) in Nigeria, who found in aqueous root extract of *Z. zanthoxyloides* flavonoids, tannins, saponins, phenolic compounds but in ethanol extract of the same part of the plant, they found alkaloids, tannins, flavonoids and phenolic compounds. Similar results were found during some previous works done on the root or stem of this plant (Adefisoye, Ako-Nai, & Bisi-Johnson, 2012; Adegbolagun & Olukemi, 2010; Adesina, 2005; Chaaib *et al.,* 2003). Ikumawoyi, Awodele, Rotimi, & Fashina (2016) in Nigeria using the root hydroethanolic extract of this plant found the presence of flavonoids, alkaloids, phenols, tannins, saponins and terpenoids. Kosh-Komba and coworkers in Central African Republic identified during their work using the hydroalcoholic extract reported the presence of alkaloids, and flavonoids in the bark but the root and leaves screening showed the presence of alkaloids, tannins, flavonoids and saponins (Kosh-Komba *et al.,* 2017). Zahoui *et al.,* (2010) during their work on the aqueous extract of *Zanthoxylum zanthoxyloides* root bark found the presence of sterols, polyterpenes, polyphenols, flavonoids and alkaloids. Using the root ethanolic extract of *Fagara zanthoxyloides*, Banso and Ngbede found the strong presence of tannins, good presence of alkaloids and glycosides and saponins were in trace. This little difference in composition can be explained by the type of extract used and the plant environment (Banso & Ngbede, 2006).

Acetic Acid induced abdominal writhing response, based on the peripheral system, is the preliminary test for analgesic activity

evaluation. After acetic acid injection, the writhing as response to pain behaviour reflects the activation of local peritoneal sensitive receptors through inflammation mediators such as prostaglandins, substance P, histamine, serotonin and cytokines (TNF-α, IL-1β and IL-8) (Atchrimi *et al*., 2017; Moniruzzaman *et al.*, 2015). In our study, 20 rats were randomly grouped into four and were treated respectively as follows: normal saline (control), aspirin at 200 mg/kg (standard), aqueous stem bark extract of *Zanthoxylum zanthoxyloides* at 400 and 800 mg/kg. The writhes induced by acetic acid injection (2.5%) was significantly reduced dose dependently in standard and extract treated groups at $p < 0.01$ and 0.001 respectively. This study result is similar with that of Diatta et al. (2014) in Senegal, who during their work showed that the leaves hydroethanolic extract of *Zanthoxylum zanthoxyloides* (EZZ) has dose dependent antinociceptive effect in acetic acid induced writhing on rats. In Togo, Atchrimi et al. (2017) showed the same effect using hydroethanolic leaves extract of *Oxytenanthera abyssinica* (EOA). The reduction of writhes was 50.54% (100 mg/kg) and 72.90% (300 mg/kg); 39.19% (100 mg/kg) and 63.78% (400 mg/kg) respectively for EZZ and EOA. Those extracts have exhibited more pain reduction than AZZ at 400 mg/kg (33.1%) and 800 mg/kg (54.74) per os. However, the standard drug used (aspirin at 200 mg/kg) exhibited pain reduction of 66.48%. This study results were not too far from those found by Bispo *et al*. (2001), who found that aqueous extract of *Hyptis pectinate*, at 100, 200 and 400 mg/kg, has shown writhing reduction of 43 %, 51 % and 54 % of protection respectively. According to Fialho *et al.* (2017), the production of prostaglandins could induce the sensitization of nociceptors to prostaglandins, which is related to abdominal constrictions. The writhing may be due to the production of prostaglandins (PG) in peritoneal exudates after acetic acid injection via the cyclooxygenase pathway (Oh *et al.*, 2015). The extract could have molecules that may block the production of inflammation mediators such as prostaglandins. The main excitatory neurotransmitter of the central nervous system is known to be glutamate. This last decade, glutamatergic receptors have been highlighted on peripheral sensitive neurons (Ritter *et al.*, 2014). Therefore, a perspective for the management of peripheral pain can be provided by the manipulation of the peripheral glutamatergic system (Carlton, 2001). Glutamate receptors also have an important role in sensitization of the dorsal horn of spinal cord, since primary afferent fibres stimulation results in liberation of glutamate (Atchrimi *et al*., 2017; Millan, 2002). The glutamate test was performed to investigate whether glutamate receptors are involved in L-glutamate induced antinociception. Painful behaviour characterized by licking and biting the injected paw is caused by an intraplantar injection of glutamate solution. In our study after injection, the significant antinociceptive response ($p < 0.05$) was obtained at 15 minutes and 20 minutes respectively with doses of 800 and 400 mg/kg. For the doses used (400 and 800 mg/kg), the nociceptive reduction was 24.44% and 37.77% respectively. The standard used (Diclofenac at 20 mg/kg) displayed nociceptive reduction of 68.88%. In Togo, Atchrimi et al. (2017) obtained similar results at dose of 200 and 400 mg/kg ($p < 0.001$) using hydroethanolic leaves extract of *Oxytenanthera abyssinica* with nociceptive reduction of 80.11% and 89.54% respectively for 200 and 400 mg/kg. Quintas *et al*. (2014) found that the hexanic fraction of the ethanolic leaves extract of *Combretum duarteanum* (HCD) displayed dose dependent effect on pain induced by glutamate injection. According to their studies, the reduction was 54.1 % and 58.7 % ($p< 0.001$) when the 200 and 400 mg/kg were compared to the control. These previous results show that AZZ provides lesser analgesic effect than the hexanic fraction

of the ethanolic extract of *Combretum duarteanum* and hydroethanolic leaves extract of *Oxytenanthera abyssinica*. AZZ could contain some metabolites that inhibit central and/or peripheral NMDA and non-NMDA receptors activation. In fact, glutamate injection provoked peripheral, spinal and supra-spinal NMDA and non-NMDA receptors activation (Beirith, Santos, & Calixto, 2002) and peripheral release of nitric oxide (NO) or related substances stimulation (Moniruzzaman *et al.,* 2015). Oedema was developed on paw injected during the experiment. Therefore, these metabolites contained in the extract could also block the synthesis of NO confirming the work performed by Beirith *et al.*, (2002) on the involvement of nitric oxide (NO) which is a powerful vasodilator. With regard to the results of the nociception induced by acetic acid and L-glutamate of the aqueous stem bark extract of *Z. zanthoxyloides*, we planned to evaluate its central effect on pain control. Hot plate test model is mainly undertaken to study if drugs have any central analgesic effect (Jinsmaa *et al.*, 2004; Xin, Bai, & Liu, 2017). In our study, 20 rats were randomly divided into four groups and were treated respectively as following: normal saline (control), tramadol at 25 mg/kg (standard), aqueous stem bark extract of *Zanthoxylum zanthoxyloides* at 400 and 800 mg/kg. The results obtained according to the method described by Eddy & Leimbach (1953) have shown that the reaction time was significantly increased in standard (tramadol) and extract treated groups when compared to the control ($p < 0.05$). Tramadol is an opioid agonist and acts on the same receptors as morphine. Analgesia production in the spinal cord by tramadol involves the opioid receptors δ and μ1/μ2 (μ2 preferentially) whereas at the supra-spinal level, the μ1 receptors are involved (Jinsmaa *et al.*, 2004). In the hot plate method, aqueous root bark extract of *Zanthoxylum zanthoxyloides* at 400 and 800 mg/kg showed statistical dose dependent increase in the reaction time at $p < 0.05$ (Prempeh & Mensah-Attipoe, 2008). These results are in line with our results. Damor *et al*. (2018) working on *Guazuma ulmifolia* in hot plate test found no statistical difference at 250 mg/kg but at 500 mg/kg, there was significant difference ($p < 0.05$). The extract significantly reduced acetic acid induced writhing, L-glutamine-induced hind paw licking as well as delayed the reaction time of animals to thermally induced pain. Its antinociceptive effect may be due to the presence of some components such as flavonoids, steroids, tannins. In fact, Just *et al.* (1998) have shown that flavonoids, tannins and steroids possess analgesic propriety.

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