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Antinociceptive Activity of Flavonoid-Rich Ethylacetate Fraction of *Ocimum gratissimum* L. Leaves in Mice

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ABSTRACT

Ocimum gratissimum L. in an economic plant of immense ethnomedicinal properties. This study evaluated the anti-nociceptive activity of the sequential extracts and flavonoid-rich ethylacetate fraction of *O. gratissimum* (EAFOg) in mice. The sequential extracts of the dried leaves in hexane, chloroform and methanol and the ethylacetate fraction were assessed in acetic acid-induced writhing and formalin test in mice. Further antinociceptive activity of the ethylacetate fraction (25, 50 and 100 mg/kg) was investigated in hot plate, acetic acid and formalin tests. The results revealed that antinociceptive activity was in the order of ethylacetate > methanol > chloroform > hexane. EAFOg demonstrated activity in the hot plate, acetic acid and formalin test, respectively. The antinociceptive activity of EAFOg (100 mg/kg) was not reversed by pre-treatment with the opioid antagonist (Naloxone, 1 mg/kg, i.p) when assessed in the hot plate test. Furthermore, the antinociceptive effect of EAFOg (100 mg/kg) was reversed by L-NAME (10 mg/kg) and yohimbine (2 mg/kg) but not atropine (2 mg/kg) when assessed in the acetic acid-induced writhing test. This suggests an interaction with the nitric oxide and adrenergic pathway. The results suggest that the flavonoid-rich ethylacetate fraction of *O. gratissimum* (EAFOg) may have beneficial effect against neurogenic and inflammatory pain.

***Keywords*:** Antinociceptive, *Ocimum gratissimum*, Ethylacetate, Flavonoid.

Introduction

Herbal medicines play an important role in disease prevention, in health promotion and healthcare management systems.[1] Medicinal plants are often believed to be safe and efficacious based on their long-standing use in various cultures. There has been an increasing acceptance and public interest in herbal medicines either as phytomedicines or nutraceuticals both in the developing and developed countries.[2;3] Medicinal plants have played a major role as a source of pharmacologically active substances. They contain phytochemicals which includes potent pharmacologically active substances such as flavonoids, alkaloids, terpenoids, tannins, saponins, steroids and a host of others.[4] Herbal medicines tend to look primitive and unscientific when compared to synthetic drugs, which are thought to be more reliable than those made from plants. However, a vast majority of people still relies on it in managing painful illnesses.[5] This is primarily because of the general belief that herbal drugs are without any side effects associated with synthetic analgesic drugs, besides being cheap and locally available.[6] Many synthetic drugs originated from plant sources including aspirin, turn-of-the century miracle drug, which is a chemical copy of the analgesic chemical in the bark of willow trees.[7] *Ocimum gratissimum* is an important medicinal plant in sub-Saharan Africa and Asia. The leaf of *O. gratissimum* is used in the folklore medicine for a variety of ailments in Nigeria and other West African countries.

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There is widespread usage and consumption of its leaves and stems as spice or tea due to its richness in essential oil. Moreover, the leaves are prepared in several ways as infusion, decoction, maceration in water or palm wine or in other alcohol beverages, sometimes the leaves can be rubbed on body parts or used as incense to bring relief to different painful ailments.[8] These painful ailments include gastrointestinal disorders such as diarrhea, ulcer, overactive motility; chest pain such as is associated with cough, pneumonia, and bronchitis;[9] inflammation and pain such as arthritis and hemorrhoids.[10,11] *O. gratissimum* leaf is known to be rich in essential oil (Ocimum oil) and phenolic compounds. Eugenol, thymol, and geraniol were the major volatile oil constituents found in *O. gratissimum.* Eugenol is the main component of *O. gratissimum* essential oils. Eugenol was reported to constitute about 40% of the constituents of *O. gratissimum* essential oils.[12] *O. gratissimum* leaves essential oils have been shown to possess antinociceptive activity.[13,14] While the essential oils have been shown to possess antinociceptive activity, but there has been no report of antinociceptive activity of the phenolic-rich fraction. Our earlier investigation showed the anti-nociceptive and anti-inflammatory effects of hydroethanolic extract in rodents.[15] Polarity based solvent extraction of medicinal plants is known to selectively extract components that are soluble in particular solvents. The extracted components vary from solvent extracts to whole extract and may be responsible for different biological effects.[16] Following a sequential extraction and fractionation techniques, we have isolated and reported the anti-inflammatory activity of the phenolic-enriched methanol extract[17] and flavonoid-rich ethylacetate fraction.[18] Here we present the antinociceptive activity of the sequential extracts and the flavonoid-rich ethylacetate fraction in experimental pain models.

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Materials and Methods

Plant collection and extraction

Botanical identification of plant material was done at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan by comparison with an existing herbarium specimen deposited at various periods. A voucher specimen of collected plant sample was also deposited in FRIN herbarium and given specimen number F.H.I 110191. Fresh leaves collected from an open space on the University of Ibadan campus in June 2015 was air dried at room temperature, powdered and sequentially extracted by maceration for 48 hours with solvents of increasing polarity (n-Hexane, Chloroform and Methanol) as earlier described.[18] The hexane (HEO*g*), chloroform (CEO*g*) and Methanol (MEO*g*) extracts were filtered and concentrated in a Rotary evaporator (Buchi Rotavapor R-124) under reduced pressure, dried in the oven at 40^{o}C and stored at room temperature. Using a 1L separatory funnel, 10 g of the MEO*g* extract was re-dissolved in water and partitioned with equal volume of ethyl acetate. The ethylacetate fraction was collected and concentrated in a rotary evaporator and dried in an oven. The yield of ethylacetate fraction of *O. gratissimum* (EAFO*g*) was 1%. This flavonoid-rich ethylacetate fraction was reported to be rich in rutin, ellagic acid, myricetin and morin.[18]

Experimental animals

Swiss mice (18 – 25 g) of either sex were obtained from the central animal house, University of Ibadan and kept in cages with wood shaven as beddings. The animals were housed at room temperature and 12 h/12 h light/dark cycle. All animals were allowed free access to water and fed with standard commercial rat chow pellets (Ladokun Feeds Nigeria Ltd). All experimental procedures on laboratory animals were conducted in accordance with established protocols under the guidelines of the Principle of Laboratory Animal Care (NIH publication No. 85 -23). The experiments were approved by the University of Ibadan Animal Ethics Committee (UI-ACUREC/app/2015/026).

Evaluation of antinociceptive activity of sequential extracts and fractions of O. gratissimum leaves

Acetic acid – induced writhing test

Acetic acid – induced writhing in mice as earlier described by[19] was used with slight modification. The mice were divided into five groups of five mice and pretreated with HEO*g*, CEO*g* and MEO*g* (400 mg/kg), indomethacin (10mg/kg p.o), or 1% tween 80 (10 mL/kg p.o). The choice of the experimental doses was guided by the results obtained in the acute toxicity test and based on our findings from our previous study.[15] Thirty minutes after treatment, 0.6% acetic acid (10 mL/kg) was intraperitoneally injected to the mice to induce the characteristic writhings. The onset time of writhing and the frequency of writhing occurring between 5 and 15 minutes after acetic acid injection was recorded. The results of the treatment groups were compared with those of vehicle treated controls. In another phase, the antinociceptive activity of the ethylacetate fraction (EAFO*g*) and residual aqueous fraction (AQFO*g*) were investigated by measuring the number of acetic acid-induced writhing and the latency of writhing.

Formalin-induced pain behaviour

The test was carried out in mice divided into five groups of five mice and pretreated with HEO*g*, CEO*g* and MEO*g* (400 mg/kg), indomethacin (10 mg/kg p.o), or 1% tween 80 (10 mL/kg p.o). Thirty minutes after treatment, 50 µl of formalin solution (2.5%) was injected into the sub-plantar surface of the mice left hind paw. A nociceptive score was determined for each 5 min interval by measuring the sum of duration (time spent licking plus elevating the paw) and the weighted pain score of [20] to assess pain behaviors in both phases. Severity of pain was rated using,[21] pain scoring measurements in the following manner: (0), normal weight bearing on the injected paw; (1), light resting of the paw on the floor; (2), elevation of the injected paw; and (3) for licking, biting and grooming of paw. The first 10 min was considered as the early phase and represents aphasic pain while the period between 15 and 60 min was recorded as the late phase (representing tonic pain). In the second phase of the experiment, the effect of the ethylacetate fraction (EAFO*g*) and residual aqueous fraction (AQFO*g*) was investigated by measuring both the pain score and paw licking time.

Evaluation of central antinociceptive activity of flavonoid-rich ethylacetate fraction (EAFOg)

Five groups of mice (n = 5) were administered with group 1 (1% tween 80, 0.1 mL/10g, as control), groups 2-4 (25, 50, 100 mg/kg EAFOg) and group 5 (Morphine, 5 mg/kg) orally. The mice were placed singly in the UgoBasile hot/cold plate (35100, Italy) maintained at 55 ± 1 ^{0} C and observed for the reaction time due to the thermal stimulation. Reaction time was recorded when the animals licked their fore-and hind paws or jumped; before treatment and at 60, 90, 120 and 150 min after administration of extract and morphine. The mice which reacted within 20 s before treatment were selected for the experiment. [22]

The mean percentage maximum possible effect (% MPE) was calculated as:

$$\% \text{ MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Cut-off time} - \text{Post drug latency}} \times 100$$

To test for the involvement of opioidergic receptor in the antinociceptive activity of the ethylacetate extract, another set of mice were pretreated with naloxone (1 mg/kg) for 15 min before the animals were treated with either EAFOg (100 mg/kg) or morphine, respectively. The animals were tested in the hot plate as earlier described.

Evaluation of effect of flavonoid-rich ethylacetate fraction (EAFOg) on neurogenic and inflammatory response in formalin-induced paw licking

The animals were pretreated with EAFOg (25, 50 and 100 mg/kg) and indomethacin (10 mg/kg) one before being injected with formalin. The control group was pre-treated with vehicle (1% tween 80). Formalin solution (0.05 mL of 2.5% formalin) was injected into the sub-plantar of the left hind paw. The formalin injection evoked a characteristic spontaneous paw licking, biting and grooming. The time spent licking the injected paw was recorded and considered as indicative of pain. The responses were observed for the first 10 min (early phase) and from 15 – 30 min (late phase) after formalin injection.

Evaluation of peripheral antinociceptive activity of flavonoid-rich ethylacetate fraction (EAFOg)

In the acetic acid-induced nociception, writhing was induced by intraperitoneal injection of 0.6% acetic acid (0.1 mL/10 g body weight). Five groups of mice of five mice each were used in the study comprising the vehicle (10 mL/kg; 1% tween 80), indomethacin (10 mg/kg), or EAFOg (25, 50, 100 mg/kg). After 1 hour, acetic acid was administered intraperitoneally, mice were placed singly in a transparent observation box. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. The number of writhes occurring between 5 and 20 minutes were recorded.

The involvement of cholinergic, adrenergic, nitric oxide pathway in the antinociceptive effect of EAFOg was evaluated by pretreatment with atropine (2 mg/kg), Yohimbine (2 mg/kg) and L-NAME (10 mg/kg) intraperitoneally. After 15 min, the animals were then treated with EAFOg (100 mg/kg). Acetic acid was injected intraperitoneally and the number of writhes counted.

Statistical analysis

Data were reported as mean ± standard error of the mean, and statistical significance was taken for $P< 0.05$. Statistical analysis was done using one-way analysis of variance (ANOVA), significant main effects were further analyzed by *post hoc* test using Newman-Keuls Multiple comparison test to compare the treatment groups. Graphs and statistical analysis were done using Graph pad prism (version 5).

Results and Discussion

The study evaluated the antinociceptive activities of polarity-based solvent extracts and fractions of *Ocimum gratissimum* leaves. *O gratissimum*

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leaves gave an increasing percentage extractive yield in solvent of increasing polarity. Sequential extractive values revealed the solubility and polarity particulars of the metabolites in the leaf extracts. The HEO*g* with the lowest extractive yield obtained as the non-polar solvent used was expected to collect fat soluble compounds mainly terpenes, fatty acids or fatty acid esters. A recent report demonstrated the cardioprotective potential of an isolated compound "gratissinol" from chloroform extract of *O gratissimum* leaves.[23] Polar solvents are frequently employed for the recovery of polyphenols from plant matrix. The most suitable of these solvents are (hot or cold) aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. The presence of multiple constituents in the methanol extract, even though some of them might be in low concentrations but they are important from the aspect of quality, safety and efficacy of the herbal medicines.[24] The hexane, chloroform and methanol extracts administered at 400 mg/kg significantly ($p < 0.05$) inhibited the frequency of writhing when compared to control mice. The onset of writhing was not significantly different in all treatment groups. However, MEO*g* (400 mg/kg) showed a higher percentage inhibition of number of writhing at 52% when compared to 50% of indomethacin (10 mg/kg). HEO*g* (400 mg/kg) and CEO*g* (400 mg/kg) showed comparable activities at 40% and 41%, respectively which is lower than MEO*g* and indomethacin (Table 1). EAFO*g* (400 mg/kg) significantly (P<0.001) reduced the number of acetic acid-induced writhes with maximal inhibition of (63%) as well as a significant (*p<0.001*) delay in the onset of writhing to about (6 min). AQFO*g* (400 mg/kg) showed a significant (*p<0.01*) reduction in the number of writhes with maximal inhibition (33.5%) but no significant delay in the onset of writhes as compared to control. Indomethacin showed a significant (P<0.001) inhibition of number of writhes (51%) but no delayed in the onset of writhing. This result revealed a pronounced antinociceptive effect with EAFO*g* much greater than observed with AQFO*g* (Figure 1A & B). The acetic acid-induced writhing test indicated that the MEO*g* (400 mg/kg) significantly reduced the number of writhing movements much more than observed with the other extracts by hexane and chloroform. Similarly, the ethylacetate fraction demonstrated a greater antinociceptive activity than the residual aqueous fraction. Acetic acid-induced pain-like effect by causing dilation of arterioles and venules, increasing vascular permeability and thereby releasing inflammatory mediators such as histamine, prostaglandins into the peritoneum. Acetic acid induces pain by increasing fluids of PGE_2 and $PGF_{2\alpha}$ at the peritoneal receptors.[25,26]

Injection of formalin in mice paw induced a biphasic response which was scored by the severity of pain and paw licking time (Table 2). Treatment with the extracts and indomethacin before formalin injection only caused significant (p <0.05) antinociceptive effect in the late phase but not in the early phase. MEO*g* showed a significantly (p <0.05) higher percentage of inhibition on the score of pain (42%) as well as the licking time (62%) in the late phase. CEO*g* and HEO*g* showed significant ($p < 0.05$) percentage inhibition on the score of pain (35 and 15%) as well as the licking time (38 and 56%), respectively. The EAFOg showed a significant (p <0.05) reduction of neurogenic pain in 1st phase and significant (p <0.001, Figure 2A) inhibition of inflammatory pain in the 2nd phase. EAFOg showed a more pronounced reduction in the biting and licking response when compared to AQFOg and indomethacin (Figure 2B).

Injection of formalin into right hind paw in mice is known to evoke a characteristic biphasic response captured by pain score and licking and biting time. Pain score is a qualitative assessment of pain behavior following injection of formalin, licking and biting time is the recorded time (sec) in which animals bite the injected paw.[27] Formalin-induced pain is caused primarily by peripheral tissue inflammation. It involves a phase of inflammation in which a variety of chemical mediators alter the functions of peripheral afferent fibers.[28] Formalin induces swelling and inflammation; it may also produce sensations other than pain, which may evoke behaviors scored in the formalin test.[29] Furthermore, to delineate the antinociceptive activity of EAFOg observed in the neurogenic phase of formalin test, the hot plate model was used. The hot plate test is considered to be selective for centrally acting analgesic compounds, like morphine, while peripheral analgesics are known to be inactive on this kind of thermal stimulus. EAFOg (25, 50 and 100 mg/kg) demonstrated a significant dose dependent maximal possible effect by increasing the latency to respond to thermal pain (Figure 3A). The antinociceptive activity of EAFOg (100 mg/kg) was not significantly reversed by naloxone (1 mg/kg). Morphine demonstrated a higher magnitude of effect but which was reversed by naloxone (Figure 3B).

Table 1: Antinociceptive effect of extracts of *O. gratissimum* leaves in acetic acid –induced writhing in mice

Treatment	Dose (mg/kg)	Latency (min)	No of Writhing	% inhibition
Control (1% Tween 80)	10 ml/kg	3.53 ± 0.26	40.20 ± 1.93	-
HEO*g*	400	4.17 ± 0.85	24.20 ± 2.13*	40
CEO*g*	400	3.16 ± 0.38	23.80 ± 2.70*	41
MEO*g*	400	3.85 ± 0.33	19.40 ± 2.92*	52
Indomethacin	10	4.57 ± 0.66	20.00 ± 2.61*	50

Each value is the mean ± SEM of five mice (n = 5), * $p < 0.05$ relative to control (1% Tween 80).

Table 2: Antinociceptive effect of extracts of *O. gratissimum* leaves in Formalin –induced nociception in mice.

Experimental groups	Score of Pain[a]				Licking time (s)[a]			
	Early Phase (0 -10 mins)	% inhibition	Late Phase (15 - 40)	% inhibition	Early Phase (0 -10 mins)	% inhibition	Late Phase (15 - 40)	% inhibition
Control	2.40 ± 0.19	-	2.84 ± 0.13*	-	120.60 ± 9.66	-	38.76 ± 13.66	-
HEO*g* (400 mg/kg)	2.16 ± 0.27	10	2.40 ± 0.50*	15	94.40 ± 6.24	22	23.96 ±10.14*	38
CEO*g* (400 mg/kg)	2.08 ± 0.23	13	1.84 ± 0.54*	35	88.60 ± 14.33	27	17.00 ±11.26*	56
MEO*g* (400 mg/kg)	2.08 ± 0.29	13	1.64 ± 0.62*	42	78.00 ± 16.87	35	14.76 ± 9.27*	62
Indomethacin (10 mg/kg)	1.24 ± 0.26	48	2.00 ± 0.57*	30	72.20 ± 9.80	40	22.00 ±10.36*	43

[a] Each value is the mean ± SEM of five mice (n = 5), * Each value is significant at $p < 0.05$, compared with control.

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The effect of EAFOg in the hot plate test is suggestive that the EAFOg does not only act through the CNS pathway, thus on blockage by naloxone, all the dose was directed to other pain pathway. Morphine which acts through the opioid receptor in the CNS pathway produced the greatest effect which was reversed by pretreatment with naloxone, an opioid antagonist. Furthermore, the antinociceptive effect of EAFOg on neurogenic and inflammatory phase was demonstrated in the formalin test. EAFOg (100 mg/kg) significantly reduced paw licking time in the first phase which is the neurogenic phase. However, EAFOg (25, 50 and 100 mg/kg) demonstrated a dose-dependent and significant inhibition of paw licking in the second phase of the formalin test similarly to 10 mg/kg indomethacin (Figure 4). This indicates that EAFOg has a more potent anti-inflammatory effects as previously reported.[18]

The acetic-induced writhing model characterized by stereotypical behaviour of abdominal contractions, involves different nociceptive mechanisms. Release of biogenic amines (e.g., histamine and serotonin), bradykinin, prostaglandin E2 and PGF2α caused activation of visceral nociceptors.[30] Non-steroidal anti-inflammatory drugs have shown activity in the acetic acid-induced due to their ability to block the release of prostaglandin by inhibiting the cyclooxygenase enzyme. The antinociceptive activity demonstrated by the flavonoid-rich ethylacetate fraction of *O. gratissimum* may due to its inhibitory activity against cyclooxygenase enzymes as it was previously shown to inhibit cyclooxygenase-2 preferentially to cyclooxygenase-1 *in vitro*.[18]

In order to elucidate the possible antinociceptive mechanism of EAFOg, adrenergic, muscarinic as well as NO pathways involved in the modulation of pain perception were evaluated. In the interaction experiment (Figure 5B), the antinociceptive effect of EAFO*g* (100 mg/kg) was reversed significantly by L-NAME (10 mg/kg) and yohimbine (2 mg/kg) but not with atropine (2 mg/kg). Pain transmission is a complex mechanism that involves interaction of peripheral and central structures and different modulatory pathways.[31] Yohimbine blocks α_2-adrenoceptor, whose stimulation is associated with analgesia, bradykardia, and sympatholysis.[32] Yohimbine reversed the antinociceptive effect of EAFOg significantly, implying that the extracts might be acting via supraspinal nociceptive pathway partly activating α_2-adrenoceptor. Activation of α_2-adrenoceptor has been shown to inhibit the release of sympathetic noradrenaline thereby terminating the propagation of pain signals.[32] Similarly, effect of EAFOg was reversed by L-NAME. L - NAME selectively inhibits neuronal nitric oxide synthase (nNOS), as over-expression of it is associated with neurodegenerative disorders and neuropathic pain.[33] Nitric oxide has been shown to modulate the nociceptive pathway.[34] Inhibition of the NOS by L-NAME is associated with a dose dependent antinociceptive activity by reducing the NO level as previously reported.[34] However, in our experiment, L-NAME reversed antinociceptive activity of EAFOg, the plausible explanation for this observation was not clearly understood. EAFOg due to the presence of multiple flavonoids might have triggered antinociceptive mechanisms through other pathway other than the NO-mediated pathway. EAFOg has been previously shown to reduce LPS-induced NO release from activated macrophages and peritonitis in mice.[18]

Atropine as a muscarinic antagonist, blocks the muscuranic receptors especially M_2 and M_4 that is associated with supraspinal and spinal anti-nociception.[35] The result showed that pretreatment with atropine did not significantly reversed the antinociceptive effect of EAFOg, suggesting it might not be acting through the pathway. The inhibition of writhing response in our study clearly indicated the peripheral antinociceptive effect of EAFOg in addition to its central effect.

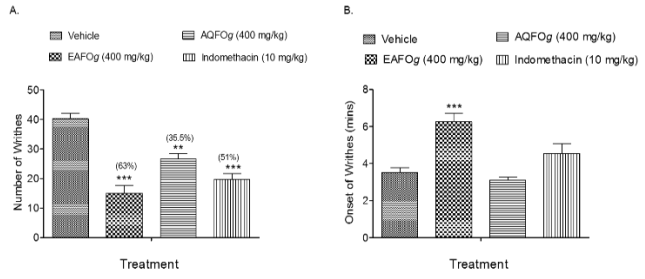

Figure 1: Anti-nociceptive effects in acetic acid –induced writhing. **(a)** No of writhes **(b)** onset of writhes (min). Data represent Mean ± SEM of five rats, values in parenthesis are percentages of inhibition. ***p < 0.001, by 1-way ANOVA followed by Newman-Keuls post hoc test compared to vehicle group (pretreated orally with 1% tween 80).

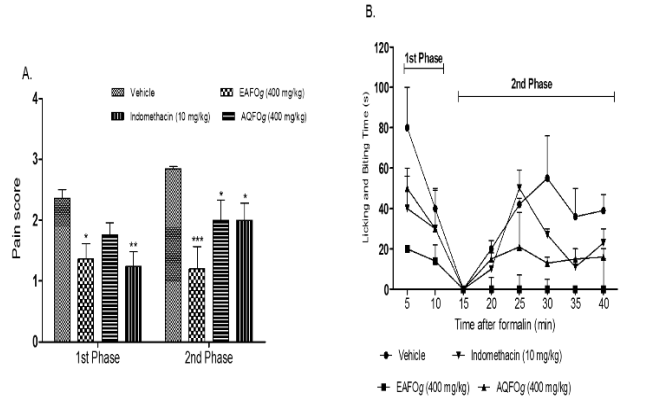

Figure 2: Anti-nociceptive effects in formalin –induced pain in mice. **(a)** Pain score **(b)** Licking and biting time (sec). Data represent Mean ± SEM of five rats. *p < 0.05, **p <0.01 ***p < 0.001, by 1-way ANOVA followed by Newman-Keuls post hoc test compared to vehicle group (pretreated orally with 1% tween 80)

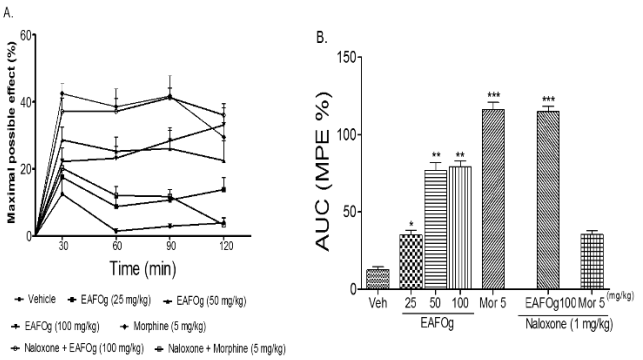

Figure 3: Antinociceptive effect of EAFOg in hot plate (A) percent maximal possible effect. (B) Area under the curve. Data represent Mean ± SEM of five mice. * p < 0.05, **p < 0.01, *** p < 0.001 by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

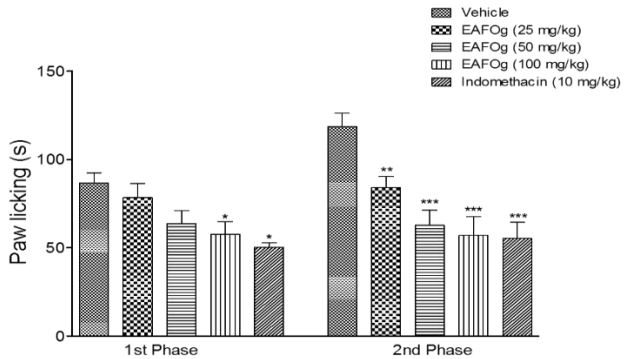

Figure 4: (A) Antinociceptive activity of EAFOg (25, 50 and 100 mg/kg) in formalin-induced paw licking in mice. Data represent Mean ± SEM of five mice. ***p< 0.001 vs vehicle using 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

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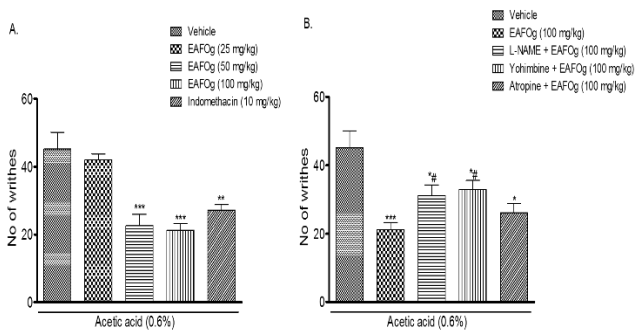

Figure 5 (A) Antinociceptive activity of EAFOg (25, 50 and 100 mg/kg), and (B) probable mechanism of antinociceptive activity, in acetic acid-induced nociception in mice. Data represent Mean ± SEM of five mice, values in parenthesis are percentages of inhibition.***p< 0.001 vs vehicle, . # p < 0.05 when compared to EAFOg using 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

Conclusion

The constituents in *O gratissimum* leaves are soluble in different solvents. Superior efficacy of the ethylacetate fraction might be due to the presence of flavonoid constituents acting synergistically to improve the antinociceptive efficacy of the fraction.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. AJTCAM. 2013; 10(5):210-229.
2. Smith-Hall C, Larsen HO, Pouliot M. People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption. J Ethnobiol Ethnomed. 2012; 8(1):43.
3. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacol. 2014; 4:177.
4. Newman DJ and Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012; 23:311-335.
5. Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol. 2015; 4(1):27.
6. Gupta LM and Raina R. Side effects of some medicinal plants. Current Sci. 1998; 75(9):897-900.
7. Mahdi JG. Medicinal potential of willow: A chemical perspective of aspirin discovery. J Saudi Chem Soc. 2010; 14(3):317-322.
8. Orafidiya LO, Agbani EO, Iwalewa EO, Adelusola KA, Oyedapo OO. Studies on the acute and sub-chronic toxicity of the essential oil of *Ocimum gratissimum* L. leaf. Phytomed. 2004; 11(1):71-76
9. Iwu MM. Handbook of African Medicinal Plants. CRC Press, Boca Raton, Florida 1993. 183-184 p.
10. Onajobi FD. Smooth Muscle contracting lipid-soluble principles in chromatographic fractions of *Ocimum gratissimum*. J Ethnopharmacol. 1986; 18(1):3-11.
11. Orafidiya OO, Elujoba AA, Iwalewa EO and Okeke IN. Evaluation of anti-diarrhoeal properties of *Ocimum gratissimum* volatile oils and its activity against enteroaggregative *Escherichia coli*. Pharm Pharmacol Lett. 2000; 10:9-12.
12. Faria TDJ, Ferreira RS, Yassumoto L, Souza JRPD, Ishikawa NK, Barbosa ADM. Antifungal activity of essential oil isolated from *Ocimum gratissimum* L.(eugenol chemotype) against phytopathogenic fungi. Braz Arch Biol Tech. 2006; 49(6):867-871.
13. Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L.(Labiatae) in mice. Braz J Med Bio Res. 2003; 36(4):521-524.
14. Paula-Freire LIG, Andersen ML, Molska GR, Köhn DO, Carlini ELA. Evaluation of the Antinociceptive Activity of *Ocimum gratissimum* L. (Lamiaceae) Essential Oil and its isolated Active Principles in Mice. Phytother Res. 2013; 27: 1220-1224
15. Ajayi AM, Tanayen JK, Ezeonwumelu JOC, Dare S, Okwanachi A, Adzu B, Ademowo OG. Anti-inflammatory, anti-nociceptive and total polyphenolic content of hydroethanolic extract of *Ocimum gratissimum* L. leaves. Afr J Med Med Sci. 2014; 43(Suppl 1): 215-224.
16. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 2017; 6(4):42.
17. Ajayi AM, Ologe MO, Ben-Azu B, Okhale SE, Adzu B, Ademowo OG. *Ocimum gratissimum* Linn. Leaf extract inhibits free radical generation and suppressed inflammation in carrageenan-induced inflammation models in rats. J Basic Clin Physiol Pharmacol. 2017a; 28(6):531-541.
18. Ajayi AM, de Oliveira Martins DT, Balogun SO, de Oliveira RG, Ascêncio SD, Soares IM, Ademowo OG. *Ocimum gratissimum* L. leaf flavonoid-rich fraction suppress LPS-induced inflammatory response in RAW 264.7 macrophages and peritonitis in mice. J Ethnopharmacol. 2017b; 204:169-178.
19. Koster R, Anderson M, DeBeer EJ. Acetic acid for analgesic screening. Fed Pro. 1959; 18:412.
20. Hunskaar S and Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987; 30(1):103-114.
21. Dubuisson D and Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977; 4:161-174.
22. Eddv NB and Leimbach D. Svnthesis analgesics. II. Dithienylbutenyl and dithienylbhtylamides. J Pharmacol Exp Therap. 1953; 107:385-393.
23. Parcha V, Dobhal Y, Dhasmana DC. Isolation, characterization and cardioprotective potential of gratissinol from chloroform extract of *Ocimum gratissimum* (Linn.) Leaves. J Bio Active Products from Nat. 2019; 9(4):260-268.
24. Bansal A, Chhabra V, Rawal RK, Sharma S. Chemometrics: A new scenario in herbal drug standardization. J Pharm Anal. 2014; 4(4):223-233.
25. Deraedt R, Jouquey S, Delevallée F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Euro J Pharmacol. 1980; 61(1):17-24.

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26. Bentley GA, Newton SH, Starr J. Studies on the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms. Br J Pharmacol. 1983; 79(1):125-134.
27. Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995; 60(1):91-102.
28. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992; 51(1):5-17.
29. Lee IO and Jeong YS. Effects of different concentrations of formalin on paw edema and pain behaviors in rats. J Korean Med Sci. 2002; 17(1):81.
30. Duarte ID, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res. 1988; 21(2):341-343.
31. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. J Clin Invest. 2010; 120(11):3779-3787.
32. Ma D, Rajakumaraswamy N, Maze M. α_2-Adrenoceptor agonists: shedding light on neuroprotection. Br Med Bull. 2005; 71(1):77-92.
33. Rahim A, Hafiz M, Zakaria ZA, Sani M, Hijaz M, Omar MH, Abdul Kadir A. Methanolic extract of *Clinacanthus nutan*s exerts antinociceptive activity via the opioid/nitric oxide-mediated, but cGMP-independent, pathways. Evid-Based Compl Altern Med. 2016
34. Abacıoğlu N, Tunçtan, B, Akbulut E, Çakıcı İ. Participation of the components of L-arginine/nitric oxide/cGMP cascade by chemically-induced abdominal constriction in the mouse. Life Sci. 2000; 67(10):1127-1137.
35. Cai YQ, Chen SR, Han HD, Sood AK, Lopez-Berestein G, Pan HL. Role of M2, M3, and M4 muscarinic receptor subtypes in the spinal cholinergic control of nociception revealed using siRNA in rats. J Neurochem. 2009; 111(4):1000-1010.