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Original Research Article

Pain-Relieving Property of the Aqueous Extract of Scent Leaf (*Ocinum gratissimum* L.) Grown in Jos

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ABSTRACT
Pain is a common ailment to all humans irrespective of gender or race. Previous reports have shown that *Ocimum gratissimum* aqueous extracts possess bioactive compounds with analgesic property This study explored the pain-relieving property of the aqueous extract of *O. gratissimum* using rat models. The extract was characterized with FTIR spectroscopy analysis while the phytochemical screening was done by chemical analysis; toxicology was perfumed using *Drosophila melanogaster* model. The qualitative photochemistry revealed the presence of alkaloids(+), flavonoid(++), saponins(+), steroids(+++), terpenoids(++), glycoside(+), tannins(+) and phenolics (+) in low to high concentrations while quantitative analysis showed that the extract contains alkaloids (0.012 ± 0.001), flavonoids (0.361 ± 0.002), saponins (5.169 ± 0.001), steroids (0.173 ± 0.001), terpenoids (0.234 ± 0.001), glycosides(0.565 ± 0.002), tannins (0.253 ± 0.001), phenolic (2.163 ± 0.003) all in g / 100 g. The FTIR spectrum revealed some functional groups (OH, NH) consistent with the obtained phytochemical compounds. The high LC_{50} (2181 mg/10g) obtained from the toxicology suggests a low toxicity of the plant's aqueous extract. The plant extract orally administered to the acetic acid-induced-writhing-rats significantly decreased the writhes counts in 40 minutes, which could be associated to the extract's analgesic activity. In low dosage the extract exhibited 44.49% as against the standard drug of 50.93 writhing inhibitions suggesting an impressive activity of the extract of the plant. The presence of alkaloids, saponins, phenolics and others may be responsible for the pain-relieving activity observed. Therefore, this study suggests that the plant may be considered as a potential candidate for analgesic and pain-killer formulations. Further studies are required to establish its activity on menstrual cramps and exploration of the extracts on mechanical and thermal pain-induced models.

CITATION
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INTRODUCTION

Ocimum gratissimum, a perennial herb belonging to the *Lamiaceae* family is widely distributed in Africa and has found application in traditional use for the treatment of a range of ailments such as diarhea, respiratory conditions and pile. Its leaves are rich in bioactive compounds such as flavonoids, alkaloids, tannins, and essential oils (Ashokkumar *et al.,* 2021; Banibrata *et al.,* 2013). Various in vivo and in vitro studies have shown that *O. gratissimum* and its bioactive constituents possess pharmacological properties such as anti-inflammatory, wound healing, antimicrobial, antioxidant, diaphoretic, antipyretic, antiseptic, antitussive and antispasmodic activities (Borgi *et al.,* 2008; Bernstein *et al.,* 2018; Achuenu *et al.,* 2026).

Chronic pain, which often accompanies inflammatory conditions, presents a significant burden on individuals and healthcare systems globally. The search for safer and cost-effective alternatives have intensified interest in herbal remedies, particularly in low- and middle-income countries where access to conventional analgesics is limited (Cubie *et al*., 2020). Furthermore, the exploration of natural products like *O. gratissimum* aligns with the global trend toward sustainable healthcare solutions and integrating scientific research with traditional knowledge can help bridge gaps in healthcare, particularly in regions where reliance on herbal medicine is deeply rooted in cultural practices.

Despite advancements in pharmacological management, the efficiency and safety of painkillers remain skeptical to many patients. This is due to factors such as drug resistance and adverse effects in addition to the high costs associated with conventional analgesics like opioids and non-steroidal anti-inflammatory drugs (NSAIDs) (Wehling, 2014), These limitations underscore the need for alternative affordable, non-toxic and plant-based remedies in treating pains. *O. gratissimum* possesses bioactive potentials for pain-relief. The essential oils of the plant have been reported to contain eugenol and thymol (Ugbogu *et al*., 2021). These compounds have been reported to modulate inflammatory pathways and neurotransmitter systems involved in pain perception and alleviation (Rocha *et al.,* 2024).

Animal models have long been employed to evaluate the analgesic efficacy of natural products due to their ability to mimic pain conditions found in humans (Walker *et al.,* 1999). Such models include thermal (hot plate and tail-flick tests), chemical (acetic acid-induced writhing), and mechanical pain models using albino rats, which provide insights into both peripheral and central mechanisms of action (Pushpendra *et al.,* 2016). Studies on medicinal plants using these models have identified numerous plant-based compounds with significant analgesic potential (Bernstein *et al.,* 2018). However, despite these promising findings, studies directly targeting the pain-relieving properties of *O. gratissimum* aqueous extracts using validated animal models have not been reported to our knowledge. A recent report in our group demonstrated 58.46% inhibition of writhing counts compared to the standard drug diclofenac potassium (10 mg/kg; 52.31%) (Achuenu *et al*., 2026). Plate 1 shows the picture of *O. gratissimum* cultiv*ated* in Jos. This research aims to explore a locally grown plant or vegetable with relatively less toxic, easily accessible and cheap alternatives to conventional analgesic medicines.

Plate 1: The leaf of *O. gratissimum* cultivated in Jos

MATERIALS AND METHODS

Sample Collection

Plant Material Collection and Extraction

Fresh leaves of *Ocimum gratissimum* (plate 1) was harvested directly from the farm at Old Legislative Quarters, Anguwan Rukuba in Jos, Nigeria. The collected sample was Identified and authenticated by a herbarium with a voucher number FHJ-847, in the Federal College of Forestry, Jos. The plant was washed thoroughly over running water, the washed sample was crushed using a

mortar and pestle, and then it was macerated with distilled water for 24hours. It was then filtered using a clean muslin cloth and concentrated. The obtained extract was appropriately kept for further analyses.

Animal Collection

Twenty (20) Swiss female albino rats were purchased from the animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. An ethical clearance certificate was obtained from the same Department with a reference number: F17-00379 for the approval of the experiment. The rats were fed for two weeks with pelletized feed purchased from Vital Feeds Ltd., PLC, Jos, Nigeria. The average body weight of the rats weighs between 92.5 – 130.9 g before they were transferred into the metabolic cages in order to have them acclimatized with the environment for twenty-four hours (24 h) before the experiment. The rats were grouped (n=4) into five different groups (I, II, III, IV, V).

Drosophila Melanogaster Strain Assay and Culture

D. melanogaster Harwich strain was obtained from the Africa Centre of Excellence in Phytomedicine Research and Development University of Jos. Species Stocked were maintained at a constant temperature and a humidity of (23 ± 2 °C; 60 % relative humidity) under 12 hours cycles. The flies were fed on standard *D. melanogaster* medium composed of yellow corn meal medium mixed with brewer's yeast (1 % w/v), standard agar (1 % w/v) and Methylparaben (0.08 % w/v).

FT-IR Spectroscopy Analysis

The Fourier transform infrared (FT-IR) spectroscopic analysis was performed with prinks Elmer spectrum 65 FT-IR spectrometer having a scanning range of 4000 – 1000, the ATR was used in its clean form. The functional groups were determined with the aid of infrared table.

Phytochemical Screening

The aqueous extract used for the pharmacological screening was tested for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and terpenoids according to the standard procedure (Sofowora, 1993; Trease and Evans, 1997).

Drug Preparation

Declofenac-K (10 mg/kg) was purchased from Lamed Pharmacy Shop in Jos, Nigeria. All drugs were freshly prepared to the desire concentration with distilled water before use. The extract was also freshly prepared using distilled water.

Acetic Acid-induced Writhing Test

The response to *i.p.* injection of an acetic acid solution is a contraction of the abdominal muscle and stretching of the hind limbs; induced according to the method described by (Winter *et al.,* 1963). Rats were randomly divided into five groups (n=4) and were administered 1.0 mL of 1 % acetic acid *i.p*. After 30 min of administration and treatment of the rats with standard drug (Diclofenac K) (0.3 mL of 1.008 mg) and the crude extract at different concentrations of low dose (128 mg), medium dose (287 mg) and high dose (513 mg) were administered. The rats were then placed in an observation box and the number of writhings were counted on different time intervals, which are, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min and after 40 min. The responses of the various groups of the rats to different concentrations of the extracts and that of Diclofenac Potassium were recorded and analyzed using a method described by Musa *et al*. (2008) with some modifications. The rats were later euthanized using chloroform after 24 h.

Dosage Calculation

The administered dosages were calculated using Shorofi, (2018) method with slide modifications based on the average body weights of the rats in each group using the standard formula:

$$\text{Dosage (mg)} = \frac{\text{Dosage (mg/kg)} \times \text{Body weight (g)}}{1000}$$

For the standard drug: $x = \frac{10 \times 100.8}{1000}$ = 1.008 mg

For the extract-treated groups:

Group III: $\frac{1360\ x\ 94}{1000}$ = 128 mg

Group IV: $\frac{2730\ x\ 105}{1000}$ = 287 mg

Group V: $\frac{4100\ x\ 125}{1000}$ = 513 mg

RESULTS AND DISCUSSION

The FTIR spectroscopy analysis (Figure 1) reveals different functional groups. The broad O-H and N-H stretch (3304.3 cm^{-1}) suggests the presence of phenolic and amine containing compounds, known to modulate pain pathways. These obtained functionalities are consistent with the results of the phytochemical screening in Table 1.

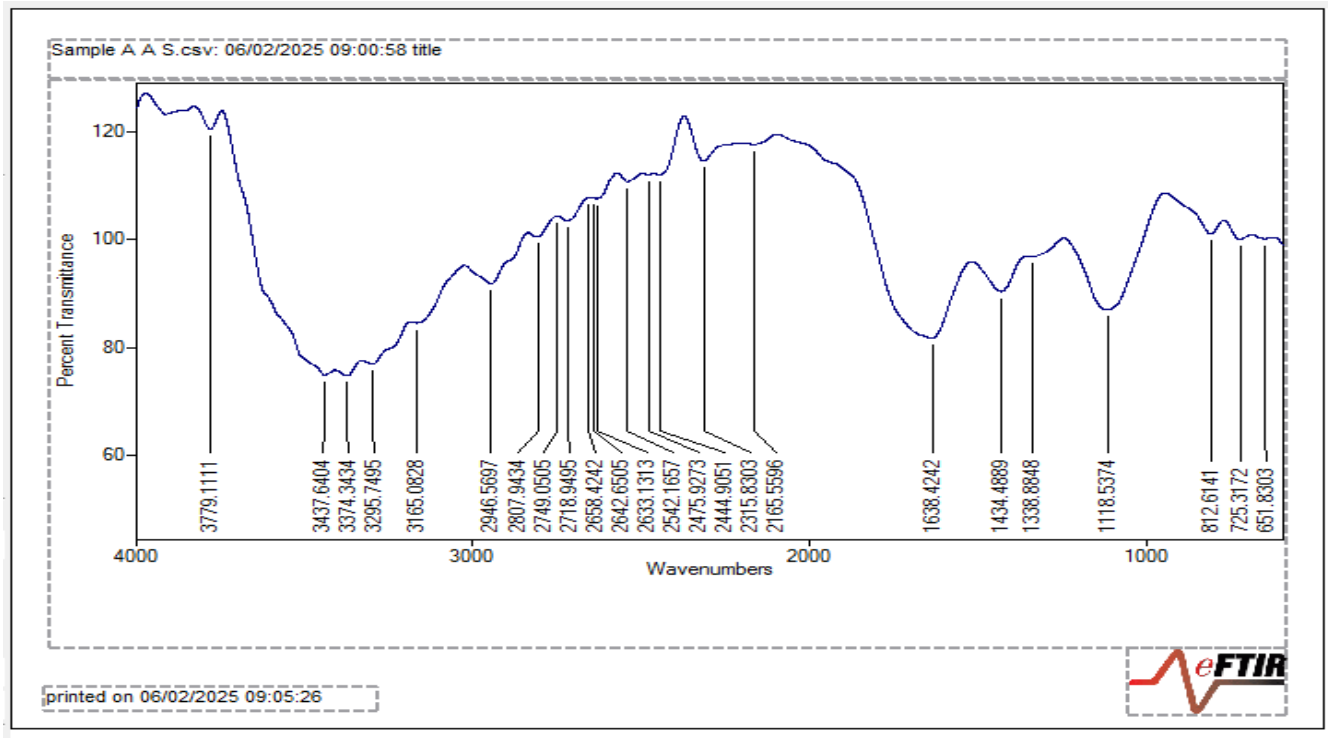

Figure 1: FTIR Spectrum of the Extract

The phytochemical screening of the plant (Table 1) revealed significant concentrations of saponins (5.169 g/100 g) and phenolics (2.163 g/100 g), both known for anti-inflammatory and analgesic effects, suggesting their potential role in pain-relieving activity of the plant. Flavonoids (0.361 g/100 g) and terpenoids (0.234 g/100 g), contribute to pain modulation through antioxidant and anti-nociceptive pathways (Borgi *et al.,* 2008). The presence of alkaloids (0.012±0.001) and steroids (0.173±0.001) could play crucial role in the synergistic effects of the bioactive compounds against pains.

Table 1: Qualitative and Quantitative Phytochemical Screening

Phytochemical	Observation	Concentration (g/100g)
Alkaloids (Atropine Eqv)	+	0.012±0.001
Flavanoid (Quarceting Eqv)	++	0.361±0.002
Saponins (Ginsenosides Eqv)	+	5.169±0.001
Steroids (Cholesterol Eqv)	+++	0.173±0.001
Terpenoids (Fernesol Eqv)	++	0.234±0.001
Glycosides (Diosgenin Eqv)	+	0.565±0.002
Tannins (Tannic Acid Eqv)	+	0.253±0.001
Phenolic (Gallic Acid Eqv)	+	2.163±0.003

Key: + = Low, ++ = Moderate, +++ = High, Mean ± SEM

The results (Tables 2–5) demonstrate the pain-relieving property of the aqueous extract of the plant against acetic acid-induced writhing in albino rats. The acetic acid writhing model is commonly employed in evaluating peripheral analgesic activity because it induces pain through the release of endogenous mediators such as prostaglandins, histamine, serotonin, and bradykinin. The groups (III-V) of rats administered with 0.4 mL of varying concentrations of the extract, were observed to respond differently to writhings compared to the positive and negative control groups. Expectedly, the positive control group recorded the lowest average writhing count (1.00), while the negative control group exhibited the highest writhing count (3.69), confirming the drug efficacy. Among the groups treated with the aqueous extract, Group III produced the lowest average writhing count (1.12), closely comparable to the standard drug(I), indicating more activity at lower concentration. The percentage inhibition data further supported this result (Table 3), with Group III (low dose) showing 44.49% inhibition, whereas Groups IV (medium dose) and V (high dose) showed lower

percentage inhibition of 19.75% and -6.15%, respectively (Table 3).

The reduced activity observed at high dosage suggests a possible dose-dependent response. The poor performance observed in Group V (high dose), which recorded the highest total writhing count among the treated groups, may be attributed to receptor saturation, antagonistic interaction among phytochemical constituents. The analgesic activity observed in this study may be attributed to a number of the phytochemical constituents present in *O. gratissimum*, particularly flavonoids, alkaloids, saponins, tannins, and terpenoids, which have been reported to possess anti-inflammatory and antinociceptive properties. Flavonoids, for instance, inhibit prostaglandin synthesis by blocking cyclooxygenase pathways, while alkaloids and terpenoids may act through central and peripheral pain inhibition mechanisms (Banibrata *et al.,* 2013). Similar findings have been reported in studies involving medicinal plants rich in related bioactive compounds that significantly inhibited acetic acid-induced writhing in rodents.

The statistical analyses further validated the obtained results of the aqueous extract. The one-way ANOVA (Table 4) result showed a statistically significant difference among the treatment groups ($F = 3.42$, $p = 0.031$), indicating that the extract significantly influenced the writhing response of the rats. The Tukey HSD post-hoc analysis revealed significant differences between the positive (I) and negative control (II) groups, as well as between the negative control (II) and Group III (low dose), demonstrate that the low-dose extract exhibited more activity. Overall, the findings of this study provide scientific support for the traditional use of scent leaf in pain management and suggest that the aqueous extract of *O. gratissimum* possesses pain-relieving properties, particularly at lower concentrations. The obtained result is comparable to our previous report on the ethanol extract of the plant which produced about 58% writhing inhibition (Achuenu *et al*., 2026).

Table 2: Pain-relieving assay with albino rat model in aqueous extract

Group/Time	5 m	10 m	15 m	20 m	25 m	30 m	35 m	m	>40 m	Av.	Rat Weight (g)
I(+ve control)	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.08	2.50 ± 0.12	2.50 ± 0.12	1.00 ± 0.06	0.50 ± 0.04	0.25 ± 0.03	0.00 ± 0.00	1.00	100.8 ± 1.20
II (-ve control)	0.00 ± 0.00	1.25 ± 0.09	2.25 ± 0.11	2.25 ± 0.10	3.00 ± 0.15	4.50 ± 0.25	2.25 ± 0.14	0.75 ± 0.07	0.00 ± 0.00	3.69	100.5 ± 1.35
III	0.00 ± 0.00	0.33 ± 0.03	1.00 ± 0.06	1.67 ± 0.09	2.00 ± 0.11	2.33 ± 0.13	1.33 ± 0.08	0.33 ± 0.03	0.00 ± 0.00	1.12	106.4 ± 1.18
IV	0.00 ± 0.00	0.75 ± 0.05	2.25 ± 0.12	2.27 ± 0.13	3.00 ± 0.15	2.27 ± 0.13	1.75 ± 0.10	0.75 ± 0.05	0.00 ± 0.00	1.63	110.7 ± 1.25
V	1.00 ± 0.06	1.25 ± 0.08	2.25 ± 0.12	3.00 ± 0.15	3.00 ± 0.15	4.00 ± 0.22	2.25 ± 0.12	0.50 ± 0.04	0.00 ± 0.00	2.16	124.6 ± 1.50

n = 4, Av. Rat weight (g) is the average weight of rats in a group in grams

Table 3: Effect of Extract on Acetic Acid–Induced Writhing Response in Rats

Group	Treatment	Total Writhes (Mean)	% Inhibition
I	Positive control (Standard drug)	8.00	50.93
II	Negative control (Vehicle)	16.25	0.00
III	Extract (low dose)	9.02	44.49
IV	Extract (medium dose)	13.04	19.75
V	Extract (high dose)	17.25	-6.15

Table 4: One-Way ANOVA Summary for Writhing Response

Source of Variation	F-value	p-value	Remark
Between Groups	3.42	0.031	Significant ($p < 0.05$)

Table 5: Post-hoc Test (Tukey HSD) Pairwise Comparisons of Groups

Comparison	Mean Difference	p-value	Significance
I vs II	-8.25	< 0.05	Significant
I vs III	-1.02	> 0.05	Not significant
I vs IV	-5.04	> 0.05	Not significant
I vs V	-9.25	< 0.05	Significant
II vs III	7.23	< 0.05	Significant
II vs IV	3.21	> 0.05	Not significant
II vs V	-1.00	> 0.05	Not significant
III vs IV	-4.02	> 0.05	Not significant
III vs V	-8.23	< 0.05	Significant
IV vs V	-4.21	> 0.05	Not significant

Table 6 shows notable reductions in both urine and feces output post-treatment across all groups (I, II, III, IV, V). Remarkably, there was a significant general drop in the quantity of both urine and feaces across the groups. The marked drop in fecal output in group IV (from 65.4 g to 11.4 g) and reduced urine volume (26 cc to 1 cc) suggest decreased physiological stress and improved comfort as potential indicators of pain relief. The positive control group showed the most significant drop in urine (from 9 cc to 0 cc), supporting prior studies that associated exposure to toxicants with renal suppression (Kataria *et al.,* 2015). Interestingly, group (IV) had the highest feces weight post-experiment (18.3 g) which is in line with the observed pain-relieving activity in this group.

Table 6: Feaces and Urine Collection

Group	Urine(cc)		Feaces(g)	
	B	**A**	**B**	**A**
I (Positive) control)	9	0	80.4	15
II (Negative control)	28	6	75.1	22.5
III (Low dose)	30	2	70.4	27.4
IV (Medium dose)	26	1	65.4	11.4
V (High dose)	27	3	87.9	18.3

Key: B = before experiment, A = after experiment, cc=

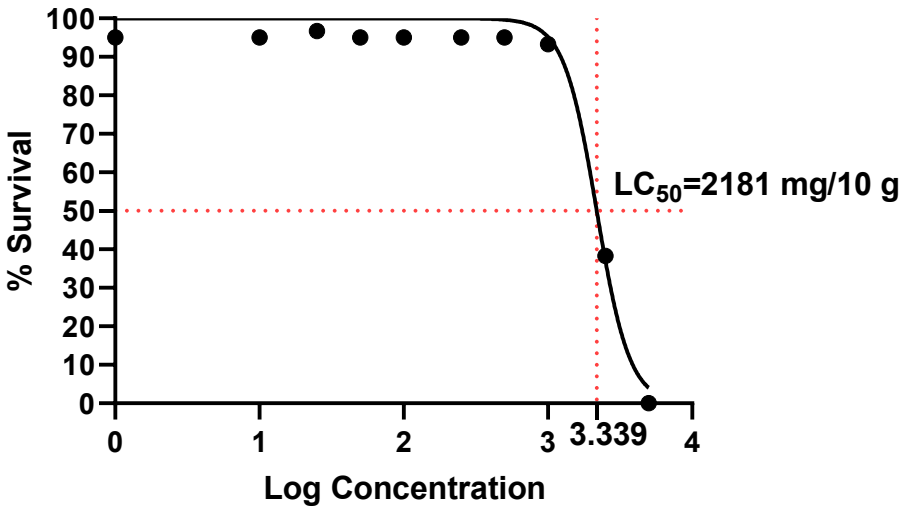

Figure 2: LC_{50} of Aqueous Extract of *Ocimum. grattissimum* from *D. melanogaster* model assay (7-day LC_{50} =2181 mg/10g)

From the toxicological study on the plant extract, an LC_{50} of 2181 mg/10g (Figure 2) was obtained using *Drosophila melanogaster* model. This demonstrates a significantly low toxicity of the aqueous extract of the plant. Which suggests that even at high concentration of the extract, it can be said to be considerable safe? As it may require 2181 mg/10g to cause 50% mortality in organisms. The implication of the obtained results is that the aqueous extract of *O. gratissimum* cultivated in Jos has an

interesting potential as a pain-relieving candidate and could be considered for further research.

CONCLUSION

This study provided an unprecedented information on the underexplored aspect of this locally grown plant, *Ocimum gratissimum*. This research work established that at low dose (III), the aqueous extract alleviates pains(44.49%). Phytochemical screening revealed the presence of very valuable biological and pharmacological constituents that have been reportedly associated to the pain-relieving property of medicinal plants; they include flavonoids, phenolics, saponins, alkaloids and terpenoids. The result of the FTIR spectroscopy analysis showed some functional groups (NH, OH and C=C) which are consistent with the bioactive compounds obtained from the phytochemical analysis. The toxicology result (LC_{50}=2181 mg/10 g) of the plant showed a significant low toxicity, validating the safe use of the aqueous extract of the plant. The findings have shown that the aqueous extract of this plant can be considered for use as a potential natural source of analgesic or pain-reliever. Further study will be necessary to establish its efficacy on menstrual cramps.

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ETHICAL CONSIDERATIONS

This study was conducted in compliance with ethical guidelines for the use of invertebrates in research. Since *Drosophila melanogaster* is not classified as a protected species, no institutional animal ethics approval was required. However, humane treatment was ensured by maintaining optimal living conditions and minimizing stress during handling.

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