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ANALGESIC ACTIVITY, PHYTOCHEMICAL PROFILING AND TOXICITY ASSESSMENT OF ETHANOL EXTRACT FROM *OCIMUM GRATISSIMUM* LEAVES CULTIVATED IN JOS, NIGERIA

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ABSTRACT

Pain is an excruciating and discomforting challenge to humans. The search for safer, cost-effective, plant-based analgesic agents from locally cultivated sources has sparked interest in medicinal chemistry. *Ocimum gratissimum* is a widely consumed plant in Africa for its medicinal properties. This study explored the analgesic potential, phytochemical composition, acute toxicity, and FTIR spectral characteristics of the ethanolic extract of the plant. Analgesic activity was assessed using the acetic acid-induced writhing assay in albino rats. The number of writhes was counted at 5-minute intervals for a total observation period of 40 minutes following intraperitoneal administration of acetic acid, and cumulative writhing counts were used for analysis. The result revealed a dose-dependent inhibition of writhes, with 62.27 mg/kg producing 58.46% inhibition compared to the negative control, which was comparable to the standard drug diclofenac potassium (10 mg/kg; 52.31%). Lower doses (24.44 mg/kg and 43.8 mg/kg) produced minimal inhibition (4.62 % and 4.49 %, respectively) and differences were not statistically significant ($p > 0.05$). The doses used in this study were selected based on preliminary experimental considerations. Acute toxicity assessment using *Drosophila melanogaster* indicated an LC_{50} of 919.5 mg/10 g diet after 7 days, determined using Probit analysis, suggesting relatively low toxicity of the extract. Phytochemical screening revealed the presence of flavonoids (1.372 ± 0.004 g/100g), saponins (3.630 ± 0.005 g/100g), tannins (0.213 ± 0.001 g/100g), terpenoids (0.486 ± 0.002 g/100g), phenolics (3.801 ± 0.005 g/100g), alkaloids (0.121 ± 0.001 g/100g), and cardiac glycosides (0.165 ± 0.002 g/100g), with phenolics and saponins present in high concentrations. FTIR spectral analysis showed the presence of functional groups consistent with the component of the observed phytochemicals, such as O–H and N–H stretching (3486, 3436, and 3255 cm^{-1}), C–H aliphatic stretching (2949.29 cm^{-1}), C=C aromatic stretching (1645.38 cm^{-1}), and C–N vibrations (1421.76 cm^{-1}).

Keywords: *O. gratissimum*, Analgesic Activity, Phytochemicals, Acute Toxicity, *D. melanogaster*

INTRODUCTION

Medicinal plants remain a rich and invaluable resources for the development of therapeutic agents, nutraceuticals, and pharmaceutical intermediates, with historical use tracing back to the earliest civilizations (Napagoda and Wijesundara, 2022, Manisha *et al.,* 2025). *Ocimum gratissimum*.L, commonly known as Scent leaf or Basil Fever plant, is a perennial aromatic herb widely distributed across tropical Africa (Ugbogu *et al.,* 2021), including Jos, Nigeria, and is used traditionally for the management of pain, fever, diarrhea, and other ailments (Ojewumi *et al.,* 2024; Idris et al., 2011). Its leaves are rich in bioactive phytochemicals such as flavonoids, alkaloids, terpenoids, saponins, tannins, glycosides, and phenolic compounds (Priyanka *et al.,* 2018), which are known for antioxidant, anti-inflammatory, and analgesic properties (Shina, 2020; Bhavani *et al.,* 2019; Rugalabamu, 2012). These compounds act through diverse mechanisms, including modulation of cyclooxygenase and lipoxygenase enzymes, inhibition of pro-inflammatory cytokines, and interaction with central and peripheral pain pathways, supporting the plant's traditional analgesic use (Aly *et al.,* 2025; Nworu and Akah, 2015).

Despite its widespread application in traditional medicine, to our knowledge there is a lack of analgesic studies on the fresh leaves of the plant specifically cultivated in Jos. In addition, correlative study combining quantitative phytochemistry, FTIR, and *D. melanogaster* toxicity model have not been reported in Jos using animal model. Limiting the available information to speculative claims.

This study aims to bridge this scientific setback by evaluating the pain-relieving potential of the ethanolic extract of *O. gratissimum* leaves cultivated in Jos and its phytochemical components and toxicity. Establishing a scientific basis for its analgesic activity will provide empirical support for traditional claims and contribute to the development of affordable, plant-based analgesics for pain management. Therefore, the objectives of the study were to obtain the crude extract from the plant by maceration using ethanol (50 % v/v), determine the analgesic activity of the crude extract using rat models, determine the toxicity of the crude extract of the plant using *Drosophila melanogaster* and to characterize the phytoconstituents of the plant extracts by FTIR spectroscopy

MATERIALS AND METHODS

Sample Collection

i. ***Plant Material:*** Fresh leaves of the plant were collected from Farin gada market in Jos north Local government area of Plateau state. The plant was identified and authenticated by a taxonomist at the herbarium unit in the Federal College of Forestry, Jos, Plateau State. The voucher number is FHJ-847.
ii. ***Drosophila Melanogaste*:** *D. melanogaster* (fruit flies) of the wild-type strain were obtained from African centre of excellence in phytomedicine research and development, Jos, Plateau state. Flies were maintained 25 ± 2°C, 12-hour light/dark cycle in glass vials containing a cornmeal-yeast-agar medium.
iii. ***Rats:*** Twenty (20) Swiss female albino rats were purchased from the animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria with a latitude 9.95101° N and a longitude: 8.89069° E. The female rats were preferred

because they exhibit lower pain thresholds and are more sensitive to experimental noxious stimuli, allowing for better identification of analgesic effects (Cook *et al.,* 2000). An ethical clearance certificate was obtained from the same Department with a reference number: F17-00379 for the approval of the rats for the experiment. The rats were fed for two weeks in their cages with pelletized feed purchased from Vital Feeds Ltd., PLC, Jos, Nigeria. The average obtained body weight range of the rats weighs between 110 – 131 g before they were transferred into the metabolic cages in order to have them acclimatized with the environment for twenty-four hours (24 h) prior to the experiment

Sample Preparation and Extraction

The fresh plant material was washed and blended using a blender. The blended leaf was macerated with ethanol (50% v/v) for 24 h. The mixture was decanted and filtered using a filter cloth. The filtrate was evaporated to dryness using open air drying under ambient temperature for 48 hours to obtain the extract. The obtained extract (30.08 %) was appropriately stored in a fridge at 4 ^{0}C for further analyses.

Acetic Acid -Induced Writhing Assay

The analgesic activity of the extract was evaluated using the acetic acid–induced writhing method as described by Winter *et al*., (1963), with slight modification. Albino Wistar rats were randomly divided into five groups (n = 6).

Animals in each group were pre-treated as follows:

Group I (Positive control): Diclofenac potassium (10 mg/kg body weight)
Group II (Negative control): Distilled water
Group III: Extract (24.44 mg/kg body weight)
Group IV: Extract (43.8 mg/kg body weight)
Group V: Extract (62.27 mg/kg body weight)

The extract dosages were prepared by dissolving the required quantities in 10 mL of distilled water, and 3 mL of the respective solutions was administered orally to each rat. Dose values were obtained by multiplying the selected mg/kg doses by the average body weight of the rats, ensuring accurate weight-based dosing. After a latency period of 30 minutes, all animals were administered 0.06% acetic acid (v/v) intraperitoneally to induce abdominal writhing. Writhing (characterized by abdominal constrictions, stretching, and elongation of the body) was counted starting from 5 minutes after acetic acid injection and recorded at 5 minute intervals (5, 10, 15, 20, 25, 30, 35, 40, and >40 minutes).

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: $\frac{260.8 \times 93.73}{1000} = 24.44$ mg

Group IV: $\frac{417.9 \times 104.8}{1000} = 43.8$ mg

Group V: $\frac{499.8 \times 124.6}{1000} = 62.27$ mg

Toxicity Assay Using Drosophila Melanogaster

The *drosophila melanogaster* method used by Pompa *et al.* (2011) was adopted with slight modifications as given below.

Preparation of Experimental Diets

The plant extract was incorporated into the standard *D. melanogaster* diet at final concentrations of 0.1%, 0.5%, 1%, 2.5%, and 5% (w/w of the diet). To achieve this, the appropriate amount of extract was thoroughly mixed with the diet before solidification. A control group was maintained on the standard diet without extract.

Exposure of the Flies

Synchronized adult flies (10 days old) were used. For each concentration and control, 20 flies were placed in a single vial containing the treated diet. Each treatment was performed in triplicate to ensure reproducibility.

Observation and Mortality Assessment

Flies were observed every 24 hours for 7 days. Mortality was defined as flies showing no movement upon gentle tapping of the vial. Behavioral changes, including locomotion activity and feeding patterns, were also recorded.

Data Analysis

Mortality data were used to calculate the median lethal concentration (LC_{50}) using Probit analysis. This allowed the determination of extract concentration causing 50% mortality under the experimental conditions.

Qualitative Phytochemical Screening

i. Test for Flavonoids

About 2 mL of the extract was treated with 2 mL of aqueous NaOH, followed by the addition of dilute HCl. The appearance of a yellow-orange coloration that changed to colorless upon addition of the acid indicated the presence of flavonoids.

ii. Test for Saponins

The presence of saponins was determined using the froth test. 2 g of the powdered sample were boiled with 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously to produce a stable, persistent froth. Three drops of olive oil were added and the mixture was shaken again. The formation of an emulsion indicated the presence of saponins.

iii. Test for Phenols

Phenolic compounds were tested using the Liebermann test. 1 mL of the extract was heated with $NaNO_3$ and H_2SO_4, then diluted with water. Excess dil. NaOH was added, and the appearance of a deep red, green, or blue color indicated the presence of phenols.

iv. Test for Tannins

The presence of tannins was determined using the ferric chloride test. A few drops of 0.1 % ferric chloride solution were added to the extract, the appearance of a brownish-green or blue-black coloration indicated the positive result for tannins.

v. Test for Glycosides

The Keller–Killiani test was used to detect the presence of glycosides as described by Evans *et al.*, (2002). 2 mL of the extract was mixed with 1 mL of glacial acetic acid containing a drop of ferric chloride, after which 1 mL of concentrated sulfuric acid was carefully added down the side of the test tube. The formation of a brown ring at the interface indicated the presence of deoxy-sugar characteristic of cardiac glycosides.

vi. Test for Alkaloids

Alkaloid was detected using Mayer's test. 2 mL of the extract was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution). The formation of a cream or pale-yellow precipitate indicated the presence of alkaloids.

vii. Test for Terpenoids

The Salkowski test was performed to detect terpenoids. 2 mL of the extract was mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid down the side of the test tube to form a layer. A reddish-brown coloration at the interface indicated a positive result for terpenoids.

Quantitative Phytochemical Analysis

Quantitative phytochemical screening was done using Ezeonu and Ejikeme, (2016) method, with slight modifications as described below:

i. Alkaloid

Alkaloid content was determined using the Dragendorff's reagent method. Briefly, the extract was acidified with 1% HCl and filtered. An aliquot of the filtrate was treated with Dragendorff's reagent to form an orange-colored complex. The mixture was incubated for 10 minutes at room temperature, and the absorbance was measured at 415 nm using a UV–Visible spectrophotometer. Alkaloid concentration was quantified using a calibration curve prepared with atropine as the standard and expressed as mg atropine equivalents per gram of extract (mg AE/g).

ii. Flavonoid

Total flavonoid content was determined using the aluminum chloride colorimetric method. The extract was mixed with 2% aluminum chloride solution and incubated at room temperature for 15 minutes to allow formation of a yellow complex. The absorbance was measured at 420 nm using a UV–Visible spectrophotometer. Quantification was carried out using a quercetin standard curve and expressed as mg quercetin equivalents per gram of extract (mg QE/g).

iii. Saponins

Saponin content was determined using the foam method. The extract was diluted with distilled water and vigorously shaken for 30 seconds, then allowed to stand for 15 minutes. The height of the persistent foam was measured and compared with a standard curve prepared using a standard saponin solution. Results were expressed as mg saponin equivalents per gram of extract (mg SE/g).

iv. Terpenoids

Total terpenoid content was determined using the sulfuric acid method. The extract was mixed with chloroform, followed by the careful addition of concentrated sulfuric acid to form a reddish-brown interface. The mixture was incubated for 10 minutes, and the intensity of the color was measured spectrophotometrically at 538 nm. Terpenoid concentration was estimated using a linalool standard curve and expressed as mg linalool equivalents per gram of extract (mg LE/g).

v. Phenolics

Total phenolic content was determined using the Folin–Ciocalteu method. The extract was mixed with Folin–Ciocalteu reagent and allowed to react for 5 minutes, followed by the addition of 7.5% sodium carbonate solution. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Total phenolic content was calculated using a gallic acid standard curve and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

vi. Tannins

Tannin content was determined using the ferric chloride method. The extract was mixed with ferric chloride solution to produce a blue-black complex, followed by the addition of potassium ferrocyanide. The absorbance of the resulting solution was measured at 700 nm using a UV–Visible spectrophotometer. Tannin concentration was calculated using a tannic acid standard curve and expressed as mg tannic acid equivalents per gram of extract (mg TAE/g).

viii. Glycosides

Cardiac glycosides were determined using Baljet's reagent method. The extract was reacted with freshly prepared Baljet's reagent (picric acid and sodium hydroxide) to produce an orange-colored complex. The reaction mixture was incubated for 1 hour at room temperature, and absorbance was measured at 495 nm using a UV–Visible spectrophotometer. Glycoside content was quantified using a digitoxin standard curve and expressed as g digitoxin equivalents per 100 g of extract (g/100 g).

Ftir Spectroscopy

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.

RESULTS AND DISCUSSION

Acetic -Acid Induced Writhing Test

The acetic acid-induced writhing model in rats was considered in this study because is a sensitive method for assessing peripherally mediated analgesic activity, as it involves the release of endogenous mediators such as prostaglandins (particularly PGE_2 and $PGF_2\alpha$), bradykinin, and substance P, which stimulate nociceptive neurons in the peritoneum (Dray, 1995).

In this study, the extract exhibited a dose-dependent analgesic effect, although not strictly linear across all doses (Tables 1 and 2). The highest dose (62.27 mg/kg) produced 58.46% inhibition of writhing, which was slightly higher than that of the standard drug diclofenac potassium (52.31%). In contrast, the lower and medium doses (24.44 mg/kg and 43.8 mg/kg) produced minimal inhibition (4.62% and 4.49%, respectively), suggesting that analgesic efficacy becomes appreciable only at higher concentrations of the extract. This pattern indicates a threshold-dependent pharmacological response, where sufficient bioactive compounds must accumulate to exert a measurable effect. Despite the apparent reduction in writhing at the highest dose, statistical analysis revealed that the differences among groups were not significant ($p > 0.05$). This lack of statistical significance may be attributed to variability in responses, small sample size, or insufficient dosing intervals. Post-hoc analysis using Tukey's HSD test showed no significant pairwise differences between groups ($p > 0.05$), confirming the ANOVA result (Table 3 and 4).

The analgesic effect observed may be linked to the phytochemical constituents identified in the extract, particularly phenolics, flavonoids, terpenoids, and alkaloids (Patel *et al.*, 2025). These compounds reportedly have analgesic properties through multiple mechanisms. Flavonoids and phenolic compounds have been reported to

inhibit cyclooxygenase (COX) and lipoxygenase pathways (Mukhopadhyay *et al.,* 2023), thereby reducing prostaglandin synthesis and attenuating inflammation-induced pain. Terpenoids may contribute by modulating inflammatory signaling pathways and reducing the release of pro-inflammatory mediators. Alkaloids, on the other hand, interact with peripheral nociceptors or central pain pathways to produce analgesia (Tiwari, 2026).

Comparatively, previous studies have reported similar findings for *O. gratissimum* and related plant extracts. Methanol extract of *O. gratissimum* leaves has been reported to produce approximately 60–70% inhibition of writhing at doses of 100–200 mg/kg (Ojewumi *et al.*, 2025), while other plant-derived flavonoid-rich extracts have shown 50–65% inhibition at comparable or higher doses (Aly *et al.*, 2026). In this context, the 58.46% inhibition observed at 62.27 mg/kg in the present study suggests consistency with prior reports.

Table 1: Result of The Acetic Acid-Induced Writhes Inhibition

Group/Time	5 m	10 m	15 m	20 m	25 m	30 m	35 m	40 m	>40m	Av. Rat weight (g)
I (+ve control)	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.08	2.50 ± 0.12	2.25 ± 0.10	1.00 ± 0.06	0.50 ± 0.04	0.25 ± 0.03	0.00 ± 0.00	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	100.5 ± 1.35
III	0.00 ± 0.00	1.75 ± 0.10	2.50 ± 0.13	3.25 ± 0.18	3.50 ± 0.20	2.50 ± 0.14	1.75 ± 0.11	0.25 ± 0.04	0.00 ± 0.00	93.73 ± 1.10
IV	0.50 ± 0.05	1.25 ± 0.08	2.25 ± 0.12	2.27 ± 0.13	3.25 ± 0.18	3.00 ± 0.16	1.75 ± 0.10	1.25 ± 0.09	0.00 ± 0.00	104.8 ± 1.40
V	0.00 ± 0.00	0.50 ± 0.05	0.50 ± 0.05	2.25 ± 0.11	1.50 ± 0.08	1.25 ± 0.07	0.75 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	124.6 ± 1.50

n = 6, Av. Rat weight (g) is the average weight of rats in a group ingrams

Table 2: Effect of Extract on Acetic Acid–Induced Writhing Response in Rats

Group	Treatment	Total Writhes (Mean)	% Inhibition
I	Positive control (Standard drug)	7.75	52.31
II	Negative control (Vehicle)	16.25	0.00
III	Extract (Low dose)	15.50	4.62
IV	Extract (Medium dose)	15.52	4.49
V	Extract (High dose)	6.75	58.46

Table 3: One-Way ANOVA Summary for Writhing Response

Source of Variation	F-value	p-value	Remark
Between Groups	1.81	0.146	Not significant (p > 0.05)

P > 0.05

Table 4: Post-hoc Test (Tukey HSD) Pairwise Comparisons of Groups

Comparison	Mean Difference	p-value	Significance
I vs II	-8.50	> 0.05	Not significant
I vs III	-7.75	> 0.05	Not significant
I vs IV	-7.77	> 0.05	Not significant
I vs V	1.00	> 0.05	Not significant
II vs III	0.75	> 0.05	Not significant
II vs IV	0.73	> 0.05	Not significant
II vs V	9.50	> 0.05	Not significant
III vs IV	-0.02	> 0.05	Not significant
III vs V	8.75	> 0.05	Not significant
IV vs V	8.77	> 0.05	Not significant

Acute Toxicity Assay Result

The ethanol extract of the plant showed an LC_{50} of 919.5 mg/10 **g** in *Drosophila melanogaster* after 7 days, indicating low toxicity and a wide safety margin (Figure 1). A high LC_{50} value implies that large doses are may be required to cause mortality, suggesting a low toxicity of the plant extract. The dose-dependent decline in survival at higher concentrations, however, indicates potential toxicity at elevated levels. This finding is consistent with reports from other models, where the extracts demonstrated high safety profiles, with LD_{50} values greater than 2000–5000 mg/kg in rodents, showing minimal toxicity (Bbosa *et al.,* 2025; Inimfon *et al.,* 2026). The high LC_{50} supports the relative safety of the extract suggesting the need for controlled dosing at higher concentrations.

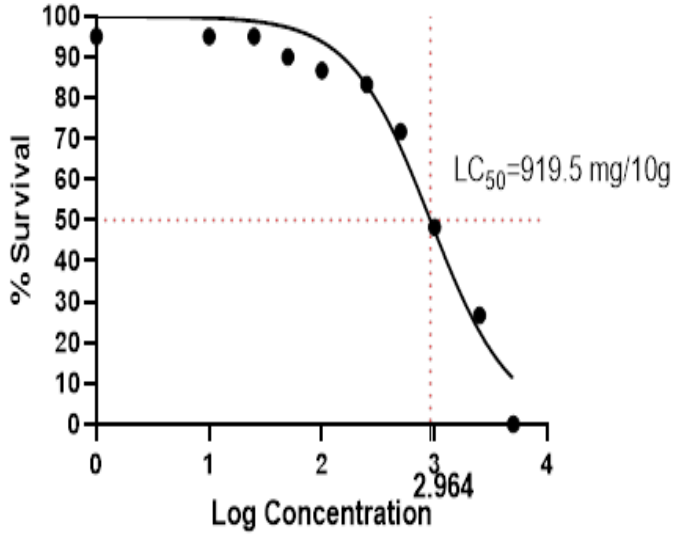

Figure 1: LC_{50} of the Extract of the Plant using *D. Melanogaster* (7-day LC_{50} =919.5 mg/10g)

Phytochemical Results

The qualitative phytochemical result of the extract revealed the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, phenols, and glycosides (Table 5) in low to high concentrations. These bioactive compounds are known to contribute to various biological effects, including analgesic and anti-inflammatory activities (Rubió *et al*., 2013). Phenolics (Table 5) recorded the highest concentration (3.801 ± 0.005 g/100g). Phenolic compounds such as rosmarinic acid reported in *O. gratissimum* are known to inhibit cyclooxygenase enzymes (Oliveira, *et al.*, 2024)., thereby contributing to analgesic activity. This was followed closely by saponins (3.630 ± 0.005 g/100g). These compounds reportedly possess anti-pain and anti-inflammation properties (Wijesekara *et al.*, 2024). Flavonoids (1.372 ± 0.004 g/100g), are recognized for their ability to modulate key cellular enzymes and reduce oxidative stress, quercetin and kaempferol are well-documented for inhibiting enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing inflammation and oxidative damage (Derbassi, 2021). Terpenoid**s** (0.486 ± 0.002 g/100g) and tannins (0.213 ± 0.001 g/100g) were moderately abundant; these class of compounds have demonstrated both analgesic and pain-relieving properties. Lower concentrations were observed for cardiac glycosides (0.165 ± 0.002 g/100g) and alkaloids (0.121 ± 0.001 g/100g). The quantitative values obtained in this study are relatively higher than those reported in previous studies on *O. gratissimum*. Amos, (2025) demonstrated phenolic (203.40 mg/g) and flavonoid (292.40 mg/g) in relatively lower concentrations than those of the phenolics (3.801 g/100g) and flavonoids (1.372 g/100g) observed in this study. In a similar study on the plant flavonoid and phenolic contents were reported in the range of 16.67–22.78 mg/100g and 21.71–23.74 mg/100g, respectively (Soola *et al.*, 2021, which are relatively lower than the results obtained in the present study (Table 5).

Table 5: Qualitative and Quantitative Phytochemical Results

Phytochemical	Observation	Concentration (g/100g)
Alkaloids (Atropine Eqv)	+	0.121 ±0.001
Flav**o**noid (Quarceting Eqv)	++	1.372 ±0.004
Cardiac glycosides (Digitoxin Eqv)	+	0.165±0.002
Saponins (Ginsenosides Eqv)	+++	3.630±0.005
Tannins (Tannic acid Eqv)	+	0.213±0.001
Terpenoids (Fernesol Eqv)	+	0.486±0.002
Phenolics (Gallic acid Eqv)	+++	3.801±0.005

Key: Low (+), Moderate (++), High (+++), Mean ± SD, n = 3

Ftir Spectrascopy Analysis

The FTIR spectral analysis of the extract showed absorption peaks of functional groups that are usually present in common phytochemical compounds (Figure 2). The frequency peaks observed at 3486, 3436 and 3255 cm^{-1} are characteristic vibration wavenumbers of O–H (alcohol or COOH functional groups) and N-H (amine functional group). These peaks are consistent with the presence of compounds containing hydroxyl group, such as phenols, terpenoids, tannins, saponins, glycosides, flavonoids and nitrogen containing compounds like alkaloids. A sharp peak at 2949.29 cm^{-1} is indicative of C–H stretching in aliphatic chains. A strong absorption at 1645.38 cm^{-1} is associated with C=C stretching in aromatic rings (Figure 2).

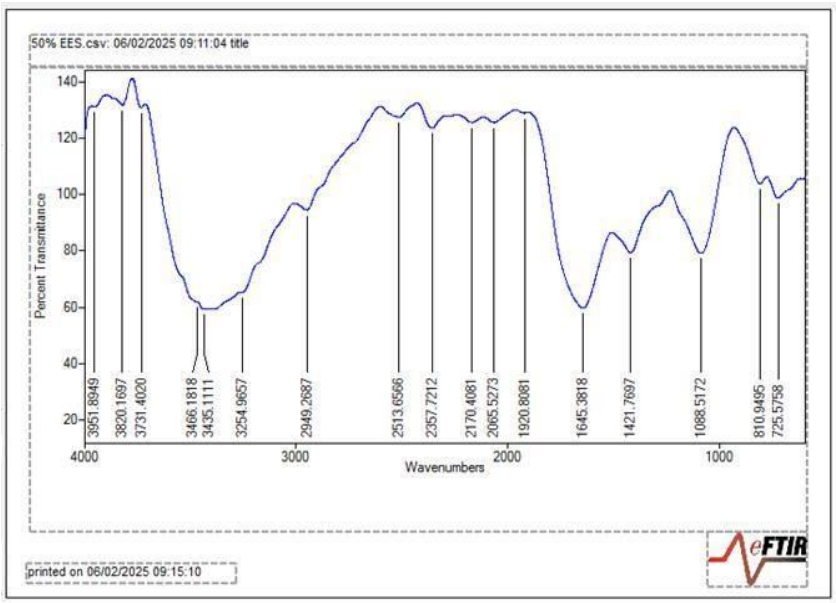

Figure 2: FTIR Spectrum

CONCLUSION

The ethanol extract of *O. gratissimum* demonstrated dose-dependent analgesic activity, with the highest dose (62.27 mg/kg) producing 58.46% inhibition of writhing, although this effect was not statistically significant ($p > 0.05$), the result is quite remarkable in analogy to prior literatures. The extract was found to be rich in bioactive phytochemicals, particularly phenolics and flavonoids, which are known to inhibit cyclooxygenase pathways and contribute to analgesic and anti-inflammatory effects. Additionally, toxicology revealed a high LC_{50} (919.5 mg/10 g) in *D. melanogaster*, indicating low acute toxicity and a safety margin. Therefore, the finding of this research work is in agreement with the traditional use of *O. gratissimum* in pain management and suggests that its ethanol extract contains phytochemicals with analgesic properties. The plant can be considered for further investigation as a potential locally grown plant-based alternative anti- analgesic source. The study was limited to the crude extract of the plant, the mechanism of alleviation of pains was also not elucidated. Furthermore, the study recommends that further studies should be done on isolation of active compounds *in vivo* anti-inflammatory models such as carrageenan-induced paw edema, formal rodent acute toxicity (LD_{50}), and mechanistic studies.

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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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