World Journal of Environmental Biosciences
Available Online at: **www.environmentaljournals.org**

Volume 13, Issue 3: 16-22

https://doi.org/10.51847/b2QEA1oG8o

ISSN 2277- 8047

Evaluation of Anti-Inflammatory Activity of a Topical Cream Containing *Ocimum gratissimum* and *Cucurbita* Seed Oil

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ABSTRACT

Molecules derived from natural products (NPs) have many applications in health and disease; due to their versatility, safety, and cost-effectiveness. This study compared the anti-inflammatory properties of a topical cream made using Ocimum gratissimum extract both alone and in combination with Cucurbita 'pumpkin' seed oil (PSO) with a commercially available product. Wistar rats, comprising males and non-pregnant females weighing between 250 and 300 g, were divided into four groups (A to D), with 3 rats in each group. They received four different cream batches, 1 to 4, respectively (after sub-acute inflammation was induced). The creams from batches '1' and '2' contained NPs, while batches '3' and '4' contained excipients devoid of NPs and the commercial product, respectively. Batch '1' contained O. gratissimum and PSO, but batch '2' contained only O. gratissimum. The extract obtained from O. gratissimum was also subjected to gas chromatograph flame ionization detector (GC-FID) analysis. The result after the treatments showed that the cream from batch '1' achieved 100% inflammation inhibition on the fourth day, while the commercial product 'batch 4' achieved the same feat on the eighth day of application. The GC-FID analysis revealed components with recognized anti-inflammatory properties, such as quercetin, naringenin, steroids, etc.; and the physical stability parameters for the cream batches didn't significantly change during an assessment period of sixty days. Conclusively; topical creams formulated with O. gratissimum and PSO are effective for skin inflammations, are stable, and outperformed the commercially available products to which they were compared.

Keywords: *Inflammation, Natural products, Ocimum gratissimum, Pumpkin seed oil*

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Received: 27 February 2024
Accepted: 20 June 2024

INTRODUCTION

Intense pain and swelling causing discomfort; is the hallmark of most inflammations (Enyedi *et al*., 2016; Muley *et al*., 2016; Hannoodee & Nasuruddin, 2020). Inflammation begins when the body senses "danger", in the form of infective, traumatic, or ischemic attacks. When the body experiences any of these listed stressors; inflammation is triggered (Bennett *et al*., 2018); and the inflammatory response is also a crucial aspect of the tissues' responses to deleterious inflammogens (Abdulkhaleq *et al*., 2018). Any unfavorable experience resulting from inflammations can affect the psychic condition of the patient as the skin is well affected. The skin is attributed to three well-known functions – as the first line of defense against external factors (e.g., pathogens, chemicals, or physical interactions), water loss prevention, and temperature maintenance (Kabashima *et al*., 2019). A deeper knowledge of the interaction between immune cells, non-immune cells, and even skin microbiome is also important for a clear understanding of the basic mechanisms of cutaneous immune reactions, allowing for the development of novel treatments for skin disorders (Fernandes *et al*., 2023).

Over the last few decades, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have been the drugs of choice for treating numerous inflammatory diseases including rheumatoid arthritis. The NSAIDs produce anti-inflammatory activity via inhibiting cyclooxygenase enzyme, responsible for the conversion of arachidonic acid to prostaglandins. Likewise, cyclooxygenase-2 inhibitors (COX-2) selectively inhibit the COX-2 enzyme and produce significant anti-inflammatory, analgesic, and anti-pyretic activity without producing COX-1-associated gastrointestinal and renal side effects (Dunaway *et al*., 2018). In the last two decades, numerous selective COX-2 inhibitors (COXIBs) have been developed and approved for various inflammatory conditions. However, data from clinical trials have suggested that the prolonged use of both NSAIDs and COX-2 inhibitors is associated with life-threatening cardiovascular side effects including ischemic heart failure and myocardial infarction. In this scenario, secondary metabolites from natural products can offer great hope for the development of novel anti-inflammatory compounds (Dunaway *et al*., 2018).

Natural products (NPs) derived from the plant stem, leaves, roots, fruits, or seeds offer a great deal of source of metabolites with pharmacological relevance in medicine (Fernandes *et al*., 2023). Numerous studies have demonstrated NPs' efficacy against a variety of skin diseases. The hunt for novel strategies to combat antibiotic resistance has also intensified research on NPs due to the growing need for novel compounds with

antibacterial and anti-inflammatory properties (Yousefi *et al*., 2021; Mahdi *et al*., 2022). NPs offer different solutions as possible treatments for several skin conditions. It is shown that they can have notable anti-inflammatory and antioxidant properties, as well as the capacity to alter skin immunological responses. Different kinds of naturally-derived chemicals are recognized by several membrane-bound immune receptors in the skin which can trigger various immune responses that can help with skin disorders (Fernandes *et al*., 2023).

Ocimum gratissimum (*O. gratissimum*) commonly known as clove basil or African basil, is a plant renowned for its diverse chemical constituents, which contribute to its medicinal and aromatic properties (Akinmoladun *et al*., 2007). *O. gratissimum's* purported main component - eugenol, has been associated with anti-inflammatory properties. It is known for its analgesic and anti-inflammatory effects; and is effective against several diseases such as reproductive disorders, nervous system disorders, blood glucose and cholesterol irregularities, microbial infections, tumorigenesis, hypertension, inflammations, and digestive complications (Nisar *et al*., 2021). This plant has been shown to possess anti-inflammatory properties akin to drugs such as aspirin and ibuprofen but has less toxicity to the inner lining of the stomach. A decoction made from leaves of *O. gratissimum* is useful for healing menstrual pain, stomach ache, earache, and fever (Ajayi *et al*., 2014). Anti-inflammatory effects were also reported for the *Ocimum* extracts and their bioactive fractions in cells and animal models by effectively inhibiting cytokine secretion (Anusmitha *et al*., 2022). Okoye *et al*. (2014) researched the chemical composition and anti-inflammatory activity of essential oils from the leaves of *Ocimum basilicum L.* and *O. gratissimum L. (Lamiaceae).* Their findings support the potential use of volatile oils from *O. gratissimum* as topical agents for managing inflammatory mycoses. The two plant species exhibited notable topical anti-inflammatory effects in a mouse ear edema model induced by xylene. The observed anti-inflammatory activity varied and was dependent on the method of essential oil extraction, influencing the chemical compositions.

Cucurbita sp. (Pumpkin) seeds also have anti-inflammatory properties due to their fatty acid composition, particularly fatty acyl esters of hydroxyl fatty acids (FAHFAs), which reduce inflammation in adipose tissue by inhibiting cytokine expression (Dong *et al*., 2021). Yahya (2020), in showing the role of anti-inflammatory and analgesic properties of Iraqi pumpkin seed oil (PSO) explained that the oil seed (25-100 mg/kg) was investigated using various experimental models for analgesic and anti-inflammatory benefits. Acetic acid and thermal-induced models of pain were used to examine the anti-nociceptive property. Models of edema, induced by carrageenan were used to evaluate anti-inflammation and results reported from the studies showed that the extract prepared from Iraqi pumpkin seeds possessed anti-inflammatory and analgesic activity when compared with a standard drug - Diclofenac. Essential oils like PSO are a complex mixture of aromatic substances whose pharmacological actions, including antimicrobial, antioxidant, anticancer, and anti-inflammatory activities have been widely reported (Sosa *et al*., 2023). PSO has also demonstrated the ability to modify inflammatory response by influencing cellular and molecular mediators associated with inflammatory pathways triggered by inflammatory agents. Moreover, it effectively resolved a persistent inflammatory lesion comparable to dexamethasone, without causing any skin alterations commonly associated with corticosteroids. This therapeutic effect is likely linked to the well-balanced presence of omega-6 and omega-9 unsaturated fatty acids in PSO, indicating its potential as an alternative treatment for inflammatory skin conditions (de Oliveira *et al*., 2013). Despite the demonstrated individual anti-inflammatory capabilities of both *O. gratissimum* and PSO, there is no reported literature on a commercial formulation incorporating both for the management of inflammations.

The goal of this research is to formulate a topical cream containing *O. gratissimum* and PSO, both with inherent anti-inflammatory properties and compare its effectiveness to an existing commercial product. Given the benefits of the two NPs, combining them in a formulation creates a unique and innovative strategy for combating skin inflammations with a potential for a multi-action effect.

MATERIALS AND METHODS

Materials

O. gratissimum leaves (purchased in Awka, Nigeria), fresh pumpkin seeds (purchased in Awka, Nigeria), emulsifying wax (Lobechem, India), white paraffin (Griflin and George, India), Citric acid (Griflin and George, India), Glycerin (Lobechem, India), etc.

Methodology

Extraction of O. gratissimum using cold maceration method

The *O. gratissimum* plant after being sourced, was authenticated by a botanist and deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria with the voucher number of the plant obtained - PCG/474/L/034. The leaves of the plant were plucked manually from the stalk after which they were gently rinsed under running cold water to remove any loose dirt or debris before immersing inside a bowl of water to help loosen any stubborn dirt. Before extraction, the plant material was dried indoors in the dark and subsequently crushed to increase the surface area for extraction. 84 g of the chopped *O. gratissimum* leaves were weighed with a weighing balance and then put into a suitable container with a tight covering after which about 504 ml of ethanol was measured with a measuring cylinder and poured into the container containing the leaves. It was ensured that the ethanol fully covered the plant material after which the container was tightly covered to prevent evaporation of the ethanol. The container was left in a cool and dark environment for 3 days to obtain the desired extract. After 3 days, the mixture was filtered with filter paper to separate the liquid ethanol extract from the solid plant material. The liquid obtained was further placed in a water bath maintained at a temperature of about 39 ± 2 °C to allow evaporation of the remaining ethanol thus leaving behind a concentrated extract. The extract was then put in a suitable container and stored in a cool and dark place. The percentage yield for the whole extract was also calculated and recorded appropriately (Onyebuchi & Kavaz, 2020).

Extraction of PSO

Fresh pumpkin (*Cucurbita*) seeds were carefully peeled and then dried under a tree shade (out of direct sunlight) for 3 days. The dried seeds were then ground with a household blender with a mill (AKAI BD038A-1031 Japan). The milled material was placed in a column within the collecting chamber of a soxhlet extractor (B54R37RL4J Eisco Labs, U.S.A). Petroleum ether (analytical grade) was added to the collection chamber and utilized to extract the PSO. After extraction, the oil was placed in flat stainless steel containers and stored in a dark location until the solvent scent was no longer detectable.

Formulation of cream

Table 1. Formula and constituents for the cream batches.

Excipients	Batch 1	Batch 2	Batch 3
PSO	12.5 ml	-	-
***O. gratissimum* extract**	10 ml	10 ml	-
Emulsifying wax	7 g	7 g	7 g
White paraffin	10 g	10 g	10 g
Citric acid	3.75 g	3.75 g	3.75 g
Glycerin	2.5 ml	2.5 ml	2.5 ml

The required quantities of excipients as presented **(Table 1)**, were all weighed out appropriately and used. Two batches of cream were formed – batches '1' and '2'. Batch '1' contained *O. gratissimum* extract and PSO with other excipients while Batch '2' contained only *O. gratissimum* extract without PSO plus other excipients as also contained in Batch '1'.

For batch 1; the oily excipients - emulsifying wax, white paraffin, and PSO were melted in a crucible placed in a water bath maintained at a temperature of about 39 ± 2 °C. The other aquaphilic excipients – *O. gratissimum* extract, citric acid, and glycerin were also mixed in another crucible and placed in a water bath maintained at the same temperature of about 39 ± 2 °C. The melted mixture of emulsifying wax, pumpkin seed oil, and white paraffin was gradually poured into the crucible containing the *O. gratissimum* extract, citric acid, and glycerin with continuous stirring. The stirring continued until a cream was formed. Finally, the resulting cream was poured into a suitable container and allowed to cool and set. For the batch 2 cream, the same quantity of excipients and the same procedure as in batch '1' above were used but without the addition of PSO (Simoes *et al*., 2018).

Anti-inflammatory study using Wistar rats

A total of twelve (12) Wistar rats comprising males and non-pregnant females weighing between 250 and 300 g were grouped into four - labeled A to D, with 3 rats in each group. The rats were first acclimatized at laboratory room temperature in Nigeria (33 ± 2 °C) for two weeks with free access to chicken grower mash and drinking water. The effect of the product (cream) on subacute-induced inflammation was studied using a modified formaldehyde-induced arthritis model in rats as described by Manan *et al*. (2022). The cream batches 1, 2, and 3 were applied to animals in groups A, B, and C respectively. While a commercial cream – Funbact A®- was applied to group D animals.

One hour post-application; all the animals received sub-plantar injection of 0.1 ml of 2.5% (v/v) formaldehyde in the right hind paw (day zero). The paw volume was then measured (basal) once every day for 10 days. Meanwhile on day '3', the paw of the animals was re-inflammed with formaldehyde. The volume of water displaced by the inflamed paw was used as a measure of edema. An increase in paw volume is quantified as inflammation (Zaringhalam *et al*., 2016; Paniagua-Pérez *et al*., 2017).

Percentage inhibition of edema was calculated using the formula;

$$= 100\left[1 - \left(a - \frac{x}{b} - y\right)\right] \quad (1)$$

Where 'a' = mean paw volume of treated rat at various days after formaldehyde injection

'x' = mean paw volume of treated rats before formaldehyde injection

'b' = mean paw volume of control rats at various days after formaldehyde injection

'y' = mean paw volume of control rats before formaldehyde injection.

Physical stability testing of the cream batches

- *Organoleptic properties:* The physical appearance of the cream batches was checked visually to detect any changes in color over time. Possible changes in odor were also checked.
- *Spreadability potential*: The spreadability studies were carried out using 1g of the creams on butter paper that was then placed between two parallel tiles and left for 60 secs. The standard weight applied to the upper plate was 25 g. The average diameter of the circle formed after the spreading of the creams was determined and recorded appropriately (Lakshmi *et al*., 2022).
- *pH measurement:* The pH of the cream batches was determined using a pH meter (PHS-3C Searchtech India). Using the modified method by Chen *et al*. (2016), 1g of the creams was dispersed in 25 ml of deionized water. The electrode was washed with double distilled water, and dried with tissue paper and the pH meter was calibrated with standard buffer solutions (at pH 4, 7, and 10) before each use. Measurements were done in triplicate and average values were obtained and recorded appropriately.
- *Density:* The density of all the batches of cream was determined based on the principle of 'mass divided by volume occupied by the product'. A pycnometer (ELISCO D369) was used to determine their densities using the formula below; 0.25g of the cream batches was used each time for assessment.

$$Density = \frac{M2 - MI}{flask\ volume} \quad (2)$$

Where M_1 is the weight of the glass pycnometer without the cream, and M_2 is the weight of the glass pycnometer and the cream together.

The above physical properties were all assessed over sixty days – at days 0, 7, 14, 30, and 60; and done in triplicate, and their mean values were used.

Gas chromatograph flame ionization detection (GC-FID) analysis of the O. gratissimum extract

A 1g quantity of extracted sample was weighed and transferred into a test tube and 15 ml ethanol and 10 ml of 50% potassium hydroxide were added. The test tube was allowed to react in a water bath at 60 °C for 30 mins. After the reaction time, the reaction product contained in the test tube was transferred to a separator funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water, and 3 ml of hexane, which was all transferred to the separating funnel. These extracts were combined and washed three times with 10 ml of ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 10 ml of pyridine and transferred to a vial for analysis with GC-FID.

The analysis for the phytochemical content was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m x 250 um x 0.15 µm) was used. The injector temperature was 280 °C with a splitless injection of 2 ul of sample and a linear velocity of 30 cms^{-1}, Helium 5.0 Pa. was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200 °C before it was then heated to 330 °C at a rate of 3 °C min^{-1} and was kept at this temperature for 5 min. The detector operated at a temperature of 320 °C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in µg/ml.

RESULTS AND DISCUSSION

Percentage yield of extracts

The percentage yield of the *O. gratissimum* extract was 71.43% while that of the pumpkin seed oil was 75.84%.

Physical stability results

Table 2. Density and PH values.

Product	Day	Density (g/ml)	pH
Batch 1	0	0.09 ± 0.00	2.58 ± 0.04
	7	0.10 ± 0.05	2.58 ± 0.02
	14	0.09 ± 0.00	2.60 ± 0.02
	30	0.10 ± 0.05	2.62 ± 0.04
	60	0.10 ± 0.05	2.62 ± 0.04
Batch 2	0	0.10 ± 0.05	2.38 ± 0.02
	7	0.09 ± 0.00	2.38 ± 0.02
	14	0.09 ± 0.00	2.38 ± 0.04
	30	0.09 ± 0.00	2.42 ± 0.04
	60	0.09 ± 0.00	2.42 ± 0.04
Batch 3	0	0.12 ± 0.05	2.14 ± 0.04
	7	0.12 ± 0.05	2.14 ± 0.04
	14	0.12 ± 0.05	2.14 ± 0.04
	30	0.12 ± 0.05	2.10 ± 0.04
	60	0.12 ± 0.05	2.10 ± 0.04
Batch 4	0	0.10 ± 0.00	6.50 ± 0.02
	7	0.11 ± 0.05	6.50 ± 0.02
	14	0.10 ± 0.00	6.50 ± 0.02
	30	0.10 ± 0.00	6.48 ± 0.04
	60	0.10 ± 0.00	6.50 ± 0.04

The negative control 'batch 3' (without the NPs) had a higher density than the other batches which indicates a thicker formulation with heavier texture and thus was difficult to apply **(Table 2)**. All the formulations (batches 1 to 3) also had a low pH and can tend to stimulate the skin as it reported that some commercial products maintain low pH but tend to increase on the skin on application and that acidic products can stimulate the skin to produce the key substances it needs to look smooth, supple, and hydrated while also looking radiant (Sebamed, 2023). The optimum pH value of the skin is between 4 and 6 denoting a weakly acidic surface. The values obtained with this work show more research has to be done to ensure the values don't drop further below the readings, as with very low pH/acidic products it can take the skin a long time to get back to normal and can expose it to external vulnerable factors (Proksch, 2018). Extended stability study, formula optimization, and more research are recommended to understand the underlying cause. Throughout the physical stability evaluation, there were no observable changes in both color and odor for all the batches, indicating remarkable stability in the organoleptic attributes over the 60 days. Batches 1 & 2 creams containing the NPs also demonstrated higher spreadability compared to other batches suggesting a smoother application and thus a potential for enhanced user experience with them **(Table 3)**.

Table 3. Organoleptic and Spreadability Properties.

Product	Day	Color	Odor	Spreadability (mm)
Batch 1	0	Light green	Odorless	32
	7	Light green	Odorless	32
	14	Light green	Odorless	34
	30	Light green	Odorless	34
	60	Light green	Odorless	34
Batch 2	0	Light green	Odorless	30
	7	Light green	Odorless	30
	14	Light green	Odorless	30
	30	Light green	Odorless	32
	60	Light green	Odorless	32
Batch 3	0	Pale white	Odorless	15
	7	Pale white	Odorless	15
	14	Pale white	Odorless	15
	30	Pale white	Odorless	15
	60	Pale white	Odorless	15
Batch 4	0	White	Odorless	24
	7	White	Odorless	22
	14	White	Odorless	24
	30	White	Odorless	24
	60	White	Odorless	24

Anti- Anti-inflammatory study results

The Batch 1 cream (containing *O. gratissimum* and PSO) administered to the experimental group 'A' achieved 100% inflammation inhibition on the day '4' of use while the commercial product 'Funbact A®' (batch 4) administered to the positive control group 'D' achieved 100% inflammation inhibition on the day '8' of use. The batch 2 cream containing only one of the natural products (*O. gratissimum*) however achieved the same feat but in a lesser capacity than the commercial product, on day '9' of use. The negative control

group that received batch '3' cream product containing neither of the NPs didn't achieve any reduction in the mean paw size of the animals (inflammation inhibition) throughout the 10-day assessment period **(Figure 1)**.

The animal study result shows a fast response to the treatment batch containing both *O. gratissimum* and the PSO and thus was better than the commercial product (for the positive control group). This result demonstrates the importance of NPs and the potential of combining 2 or more of them for a better response. The reduction in the inflammation with the combination of *O. gratissimum* and PSO **(Figure 1)**, is quite insightful and portrays a great potential for utilization in inflammatory conditions.

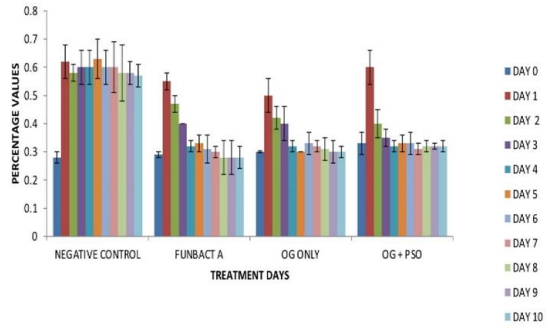

Figure 1. Bar Chart comparison of batches for the Percentage Inhibition Values Against Treatment
Key: OG – *O. gratissimum*, PSO – pumpkin seed oil.

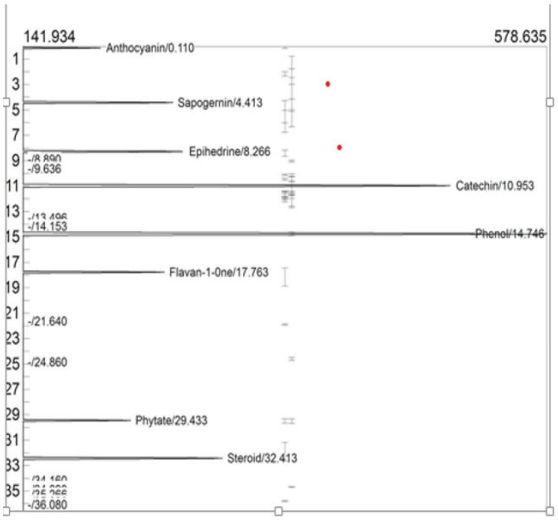

Figure 2. GC-FID spectra (1) of the *O. Gratissimum.*

GC-FID analysis result

Table 4. Phytochemical components of *O. gratissimum* extract from GC-FID analysis.

Components	Concentration (ug/ml)	% Concentration
Anthocyanin	12.4712	7.0432
Sapogenin	10.5694	5.9691
Epihedrine	16.2088	9.1541
Catechin	23.2587	13.1356
Phenol	27.1980	15.3603
Flavon-1-0ne	12.6926	7.1682
Phytate	13.7646	7.7737
Steroids	8.8555	5.0012
Flavones	6.9376	3.9181
Naringenin	38.2796	21.6188
Quercetin	6.8299	3.8572
Total	177.0660	

The phytochemical analysis result showed the different constituents of *O. gratissimum* extract with naringenin at the highest concentration of about 21.6% **(Table 4)**. The components have been purported in literature to have anti-inflammatory properties thereby surely contributing to the anti-inflammatory effect of *O. gratissimum.* Ajayi *et al*. (2014), and Okoye *et al*. (2014) have all suggested that *O. gratissimum* extract contains a diverse range of chemical compounds including flavonoids, phenolics, alkaloids, and steroids that all possess anti-inflammatory properties. For the GC-FID spectra of the same phytochemicals **(Figures 2 and 3)**, each compound's retention time indicates how long it takes for that specific compound to move through the chromatographic system. The height of each component's peak in the chromatogram signifies the intensity or concentration of that particular compound in the extract. The height of the peak directly correlates with the amount of the compound present in the sample. A taller peak indicates a higher concentration of the compound, while a shorter peak suggests a lower concentration (Novák, 2021). Components with a height of 400 and above in the result are ephedrine, steroid, narigenin, and quercetin; signifying higher concentrations in the extract. Additionally, ephedrine provides respiratory relief; steroids offer immunomodulation; naringenin confers antioxidant protection; and quercetin shows neuroprotective potential (Liu *et al*., 2016; Samini, 2019; Gad *et al*., 2021; Strzelec *et al*., 2023). Harnessing their collective and many other benefits holds promise for advancing therapeutic interventions and enhancing overall health, with the use of *O. gratissimum*.

This study opens avenues for additional investigations such as clinical trials and optimization of the formulation. These steps would be essential in advancing the potential therapeutic application of the *O. gratissimum* and PSO-based creams, thus ultimately contributing to the broader landscape of anti-inflammatory treatments.

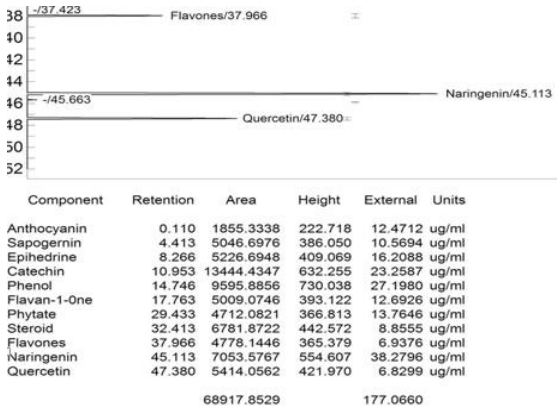

Figure 3. GC-FID spectra (2) of the *O. gratissimum* showing naringenin and other components.

CONCLUSION

The findings of this study collectively suggest that a topical cream formulated with the two natural products (NPs) – *O. gratissimum* and pumpkin seed oil- is an effective and stable anti-inflammatory formulation that is comparable to available commercial products not containing NP. Creams formulated with the two NPs give a faster anti-inflammatory effect compared to creams formulated with *O. gratissimum* alone.

ACKNOWLEDGMENTS: None.

CONFLICT OF INTEREST: None.

FINANCIAL SUPPORT: None.

ETHICS STATEMENT: Animal experiments were conducted after obtaining ethical approval from the animal research ethics committee of Nnamdi Azikiwe University, Awka, Nigeria with approval number NAU/AREC/2024/0027.

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