Original Article

Antinociceptive and anti-inflammatory activities of the methanol extract of *Ferula elaeochytris* Korovin in a rat model

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How to cite this article: **Jadgalradeh A, Iqbal M. Antinociceptive and anti-inflammatory activities of the methanol extract of *Ferula elaeochytris* Korovin in a rat model. Archives of Razi Institute Journal. 2024;79(3):645-650.** DOI: 10.32592/ARI.2024.79.3.645

ABSTRACT

Today, the current chemical agents used for the management of pain cause numerous complications. They are associated with the occurrence of disorders in the digestive system, damage to the kidney, or addiction, which has prompted individuals to seek novel drugs that, apart from removing the side effects, are cost-effective and available. The present *in vivo* survey aimed to assess the antinociceptive and anti-inflammatory activity of *Ferula elaeochytris* Korovin methanolic extract (FEME) in male Swiss mice. After obtaining the methanolic extract through the maceration process, the antinociceptive efficacy of FEME at doses of 25 to 100 mg/kg was assessed by the tail-flick, hot-plate, and formalin tests. Moreover, anti-inflammatory evaluation was performed using the Carrageenan-induced paw edema model. It was found that in the tail-flick and hot plate test, FEME, mainly at the dose of 100 mg/kg, significantly reduced the latency time and increased the time of the observance of licking or jumping, in comparison to normal saline (P<0.001). We reported that FEME at 50 and 100 mg/kg significantly decreased pain behaviors in acute and chronic phases in comparison to normal saline (P<0.001). FEME significantly declined paw edema in a dose- and time-dependent response (P<0.05); therefore, a significant difference was observed in paw edema, followed by treatment with FEME at 50 and 100 mg/kg (P<0.001). To conclude, this study reported the potent analgesic and inflammatory effects of FEME in controlling peripheral and central pain. Nonetheless, additional experiments are mandatory to clarify the accurate mechanisms of action of this plant.

Keywords: Analgesic, Inflammation, *In vivo*, Natural products, Pain

Article Info:
Received: 6 October 2023
Accepted: 16 November 2023
Published: 30 June 2024

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1. Introduction

Pain is one of the body's defense mechanisms operating when the tissue is damaged, causing the person to react and remove the generative stimulus (1); therefore, it is considered one of the critical physiological issues (1). Inflammatory activities are strongly correlated with pain since the chemicals released through the inflammatory pathway may further stimulate pain receptors and lead to inflammatory pain (2, 3). Today, the current chemical agents (e.g., aspirin, diclofenac, and indomethacin) used for pain control cause numerous complications and are associated with the occurrence of disorders in the digestive system, kidney damage, or addiction, which has caused people to find newer drugs that, apart from removing the side effects, are cheap and available (4, 5). Nowadays, natural products and their bioactive compounds still stand out as valuable sources for the development of new therapeutic agents (6). The *Ferula* genus plants from the Apiaceae family broadly grow in various parts of the world, especially in Central Asia and Mediterranean regions (7). In folk medicine, the *Ferula* species are applied as laxative, tonic, anticonvulsant, anti-infective, and antispasmodic for controlling various diseases (8). *Ferula elaeochytris* Korovin is one of the species of the *Ferula* genus (8). Although there are limited studies on the pharmacological effects of this plant, recent research has demonstrated that this plant displays some properties, such as antioxidant, anticholinesterase, and anti-tyrosinase activities (9, 10). In light of the aforementioned issues, the current *in vivo* study aimed to estimate the antinociceptive and anti-inflammatory efficacy of *F. elaeochytris* methanolic extract (FEME) in mice.

2. Materials and Methods

2.1. Plant material

The aerial portions of the plant were provided from an herb market in Heydarabad, Pakistan, in June 2021 and were recognized by a botanist from the Faculty of Pharmacy, University of Sindh Jamshoro, Pakistan. Voucher specimens (10,701) were maintained in the herbarium of the Faculty of Pharmacy, University of Sindh, Heydarabad, Pakistan.

2.2. Preparing the methanolic extract

A volume of 300 g of dried and powdered plant materials was extracted using maceration with methanol (70%) for 72 h. Thereafter, the combination was separated and concentrated in a vacuum at 50°C using a rotary evaporator (Hei-VAP, Heidolph, Germany) (11, 12).

2.3. Phytochemical tests

Phytochemical analysis of the methanolic extract was performed according to the previous studies to determine the compounds (e.g., flavonoids, tannins, and saponins with the ability to produce suds) (13).

2.4. Animal

A total of 250 male Swiss mice (7-week-old) weighing 25-30 g were used in this study. Mice were kept in a 12/12 h light/dark cycle at 22±1°C. For each experiment, five groups of 10 mice orally received the extract at 25, 50, and 100 mg/kg, the control drug (morphine, 1 mg/kg), and the solvent (normal saline), respectively (15).

2.5. Antinociceptive effects of *Ferula elaeochytris* methanolic extract

2.5.1. Tail flick test

The tail-flick assay was performed based on the previous study using the Tail Flick apparatus (7360, Ugo Basile, Italy), and the latency was recorded in seconds from the emission of infrared radiant heat to the tail to the point at which the mice responded by moving its tail (14).

2.5.2. Hot plate test

The hot plate assay was carried out according to the method described elsewhere (15) using a plexiglass wall (model LE710, Lsi LETICA, Spain) at a temperature of 55 0.1°C to record pain sensation in mice. Meanwhile, paw licking or jumping was determined as the response time to thermal pain, with a maximum cutoff point of 60 s.

2.5.3. Formalin test

The formalin test was also performed based on the technique explained in the previous study (16) using a 30×30×30 cm glass chamber with mirror walls. After subcutaneous injection of formalin (50 µL, 1.5%) into the hind paw of mice, animals were put in the glass chamber and checked by a video camera. The time periods of 0-3 min and 10-40 min were regarded as acute and chronic phases, respectively (16).

2.6. Anti-inflammatory evaluation by the carrageenan-induced paw edema model

Five groups of mice orally received the extract at the three aforementioned doses of indomethacin (20 mg/kg) and normal saline, respectively. One hour later, 20 µl of carrageenan (2.0%) and sterile normal saline (control) were injected into the right and left hind paws of rats, respectively (17). The mice's paw volume was then assessed using a plethysmometer (PanLab LE 7500, Spain) at 1-4 h after carrageenan inoculation.

2.7. Rotarod test

To study the motor coordination of mice, five groups of mice orally received the extract at 25-100 mg/kg, the control drug, and normal saline, respectively. Thereafter, the assay was performed based on the method described elsewhere (18) using a 3 cm-diameter rolling rod. Animals were put on the rod rotating at 8 rpm, and the number of falls during 3 min was noted.

2.8. Statistical analysis

The data were analyzed in SPSS software (version 26.0) using the one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Phytochemical analysis of the extract

Phytochemical analysis displayed the presence of saponins, flavonoids, terpenoids, and phenols in FEME and the lack of alkaloids (Table 1).

Table 1. The primary phytochemical analysis of the *Ferula elaeochytris* methanolic extract.

Phytochemical	Test	Presence
Flavonoids	Ammonia test, Alkaline reagent test	++
Alkaloids	Dragendorffs test	-
Saponins	Frothing test	+
Terpenoids	Salkowski test	+++
Tannins	Gelatin test	+

3.2. Tail flick test

The time latency to painful motivation after receiving FEME is illustrated in figure 1. FEME increased the mean latency time in comparison to normal saline (P<0.001) in a dose-dependent manner. In contrast, a significant change was observed in the mean latency time of FEME at 100 mg/kg compared to morphine (P<0.05).

3.3. Hot plate test

As depicted in figure 2, FEME increased the time of the observance of licking or jumping in a dose-dependent response (P<0.001); nonetheless, FEME at 100 mg/kg did not exhibit a significant change compared to morphine.

3.4. Formalin test

The effect of FEME on formalin-induced pain reactions in tested mice is illustrated in figure 3. FEME at 50 and 100 mg/kg significantly decreased pain behaviors in both acute and chronic phases of the assay in comparison to the control mice (P<0.001).

3.5. Carrageenan-induced paw edema

As displayed in figure 4, in the carrageenan-induced hind paw edema model, FEME significantly reduced paw edema in dose- and time-dependent responses (P<0.05). In this regard, a significant difference was observed in paw edema, followed by treatment with FEME at 50 and 100 mg/kg, compared to the control mice (P<0.001).

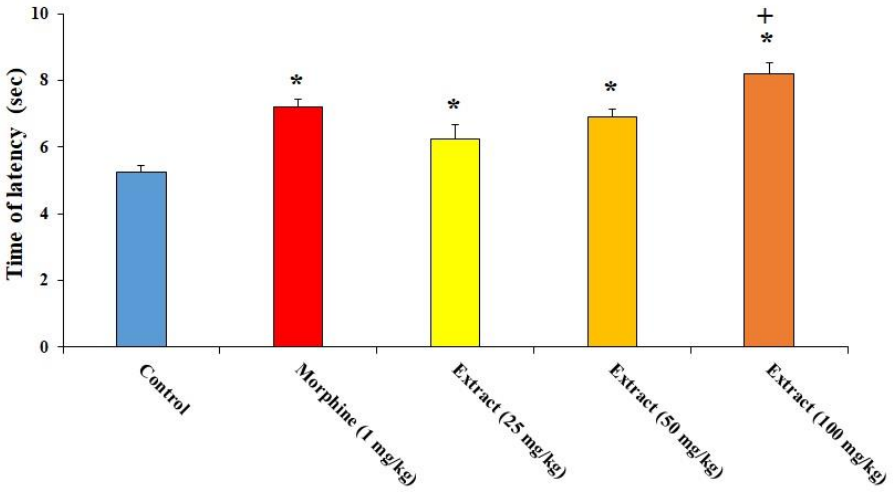

Figure 1. The effect of *Ferula elaeochytris* methanolic extract (FEME) on the latency time in the Tail flick test. *p<0.001 compared to the control (normal saline). +p<0.01 compared to the morphine.

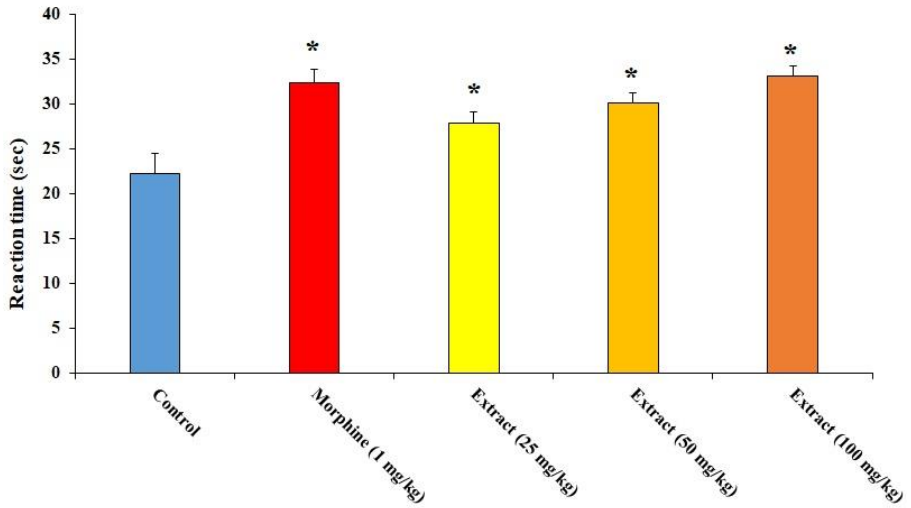

Figure 2. The effect of *Ferula elaeochytris* methanolic extract (FEME) on the response time of the mice in the hot plate assay. *p<0.001 compared to the normal saline.

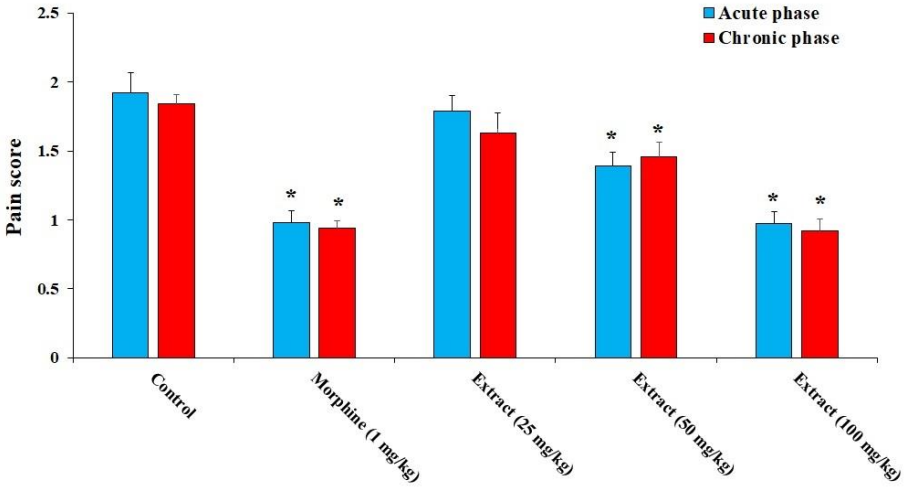

Figure 3. The effect of *Ferula elaeochytris* methanolic extract on pain reactions after formalin inoculation. *p<0.001 compared to the normal saline.

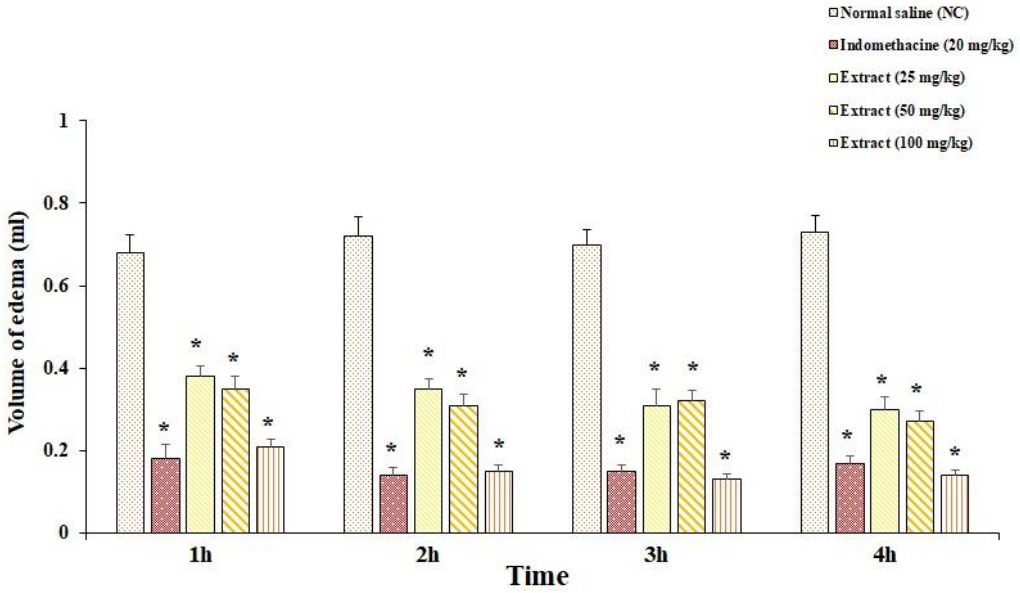

Figure 4. Anti-inflammatory effect of *Ferula elaeochytris* methanolic extract (FEME) on paw edema produced by carrageenan in mice in comparison with normal saline and indomethacin (20 mg/kg). *p<0.001 compared to the control mice.

3.6. Rotarod test

No significant difference was observed in the sensorv-motor trial after the treatment of the tested mice with FEME at 25, 50, and 100 mg/kg.

4. Discussion

Nowadays, the current chemical agents (e.g., aspirin, diclofenac, and indomethacin) utilized for pain relief come with numerous complications and are associated with the occurrence of disorders in the digestive system, damage to the kidney, or addiction. This has prompted people to seek novel medications that, apart from removing side effects, are cost-effective and available (4, 5). The present study assessed the *in vivo* antinociceptive and anti-inflammatory activity of *F. elaeochytris* methanolic extract in mice. It was revealed that in the tail-flick and hot plate test, FEME, mainly at 100 mg/kg, significantly reduced the latency time compared to the control mice. The tail flick assay is widely applied to assess the central analgesic activity of new agents (19). The hot plate assay is considered a proper model of acute supraspinal pain generally applied to investigate the analgesic activity of new drugs. In contrast, this examination has the capability to prepare a direct and precise investigation of animals' reactions to agents (20, 21). The formalin test demonstrated that FEME 50 and 100 mg/kg significantly reduced pain responses in both acute and chronic phases. Regarding the anti-inflammatory

effects of FEME, it was found that FEME significantly decreased paw edema in dose- and time-dependent responses (P<0.05). In this regard, a significant difference was observed in paw edema, followed by treatment with FEME at 50 and 100 mg/kg. For the last several decades, formalin tests have been utilized to investigate the pain-triggering mechanism of new analgesic agents (22). In this assay, the early phase was initiated right after formalin infusion due to the direct motivation of type C sensory filaments and was maintained for 3-5 min. On the other hand, the late phase, as an inflammatory response, commenced about 15 min after formalin injection and lasted for 60 min (22). It has been proven that anti-inflammatory agents, such as indomethacin, hydrocortisone, and dexamethasone, reduce pain in the chronic phase of the formalin assay, indicating that inflammatory procedures exhibit a critical role in the late phase (23). We reported the presence of some phytochemical compounds, such as saponins, phenols, and mainly high amounts of terpenoids and flavonoids, in FEME. Studies reported that terpenoids exhibited their antinociceptive activity by affecting K+ efflux and cell membrane hyperpolarization, reducing the impulsiveness of peripheral neurons, stimulating the production of spinal cord neurotransmitters, and triggering opioid receptors (24). On the other hand, flavonoid compounds showed their analgesic and inflammatory effects by inhibiting the production of some cytokines (e.g., interleukin-1 beta and prostaglandin) and prompting nitric oxide release and endogenous opioid-dependent mechanisms (25). Consequently, it seems that terpenoids and flavonoid compounds in FEME are principal factors in controlling pain and inflammation in mice. The results of this study reported the potent analgesic and inflammatory effects of *F. elaeochytris* methanolic extract in controlling both peripheral and central pain. Nevertheless, additional experiments are mandatory to clarify the accurate mechanisms of action of this plant. The most notable limitation of this study was that the exact analgesic and anti-inflammatory mechanisms of this extract remained unknown.

Acknowledgment
The authors would like to thank the staff of the Faculty of Pharmacy, University of Sindh Jamshoro, Pakistan.

Authors' Contribution
AJ designed the survey, AJ did the tests and data analysis, and MI supervised and critically reviewed the manuscript.

Ethics
This survey was approved by the Ethics Committee of the Faculty of Pharmacy, University of Sindh Jamshoro, Pakistan (No. 2020-10701).

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

Funding resources
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References
1. Lamont LA, Tranquilli WJ, Grimm KA. Physiology of pain. Vet Clin North Am Small Anim Pract. 2000; 30(4): 703–728.
2. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MN. The crucial roles of inflammatory mediators in inflammation: A review. Veterinary world. 2018;11(5):627.
3. Ahmed AU. An overview of inflammation: mechanism and consequences. Frontiers in Biology. 2011;6(4):274-81.
4. Khodabakhshi Rad, A., Kazemi Mehrjerdi, H., Pedram, M. S., Azizzadeh, M., Amanollahi, S. Clinical Evaluation of the Effect of Methylprednisolone Sodium Succinate and Meloxicam in Experimental Acute Spinal Cord Injury. Iranian Journal of Veterinary Medicine, 2023;17(2):129-138.
5. Nikjooy, N., Asghari, A., Hassanpour, S., Arfaee, F. Study of Antinociceptive Role of the Manna of Hedysarum and the Neurotransmitter Systems Involved in Mice. Iranian Journal of Veterinary Medicine, 2022;16(3):265-273.
6. Gagnier JJ, van Tulder MW, Berman B, Bombardier C. Herbal medicine for low back pain: a Cochrane review. Spine. 2007;32(1):82–92.
7. Mohammadhosseini M, Venditti A, Sarker SD, Nahar L, Akbarzadeh A. The genus Ferula: Ethnobotany, phytochemistry and bioactivities–A review. Industrial crops and products. 2019;129:350-94.
8. Salehi M, Naghavi MR, Bahmankar M. A review of Ferula species: Biochemical characteristics, pharmaceutical and industrial applications, and suggestions for biotechnologists. Industrial Crops and Products. 2019;139:111511.
9. Sahebkar A, Iranshahi M. Volatile constituents of the genus Ferula (Apiaceae): A review. Journal of Essential Oil Bearing Plants. 2011;14(5):504-31.
10. Bagheri SM, Shieh A, Ghalenoei JA, Yadegari M, Alborzi N. Review of potential spermatogenic and aphrodisiac effects of the Ferula genus. Clinical and Experimental Reproductive Medicine. 2023;50(3):143.

11. Mahmoudvand H, Sepahvand P, Jahanbakhsh S, Azadpour M. Evaluation of the antileishmanial and cytotoxic effects of various extracts of garlic (Allium sativum) on Leishmania tropica. Journal of Parasitic Diseases. 2016;40:423-6.
12. Jahanbakhsh S, Azadpour M, Tavakoli Kareshk A, Keyhani A, Mahmoudvand H. Zataria multiflora Bioss: lethal effects of methanolic extract against protoscoleces of Echinococcus granulosus. Journal of parasitic diseases. 2016;40:1289-92.
13. Evans WC. Trease and Evans pharmacognosy. 14th ed. London: WB Saunders Company Limited, 1972.
14. Mahmoudvand H, Khaksarian M, Ebrahimi K, Shiravand S, Jahanbakhsh S, Niazi M, Nadri S. Antinociceptive effects of green synthesized copper nanoparticles alone or in combination with morphine. Annals of Medicine and Surgery. 2020;51:31-6.
15. Ghasemian Yadegari J, Fazeli Moghadam E, Golmohammadi H, Dastyarhaghighi S, Ghoulami M, Mahmoudvand H. Effect of oral administration of Astragalus ecbatanus chloroform extract on acute and chronic pain in Balb/C mice. Research Journal of Pharmacognosy. 2023.
16. Mahmoudvand H, Ziaali N, Ghazvini H, Shojaee S, Keshavarz H, Esmaeilpour K, Sheibani V. Toxoplasma gondii infection promotes neuroinflammation through cytokine networks and induced hyperalgesia in BALB/c mice. Inflammation. 2016;39:405-12.
17. Medeiros MA, Gama e Silva M, de Menezes Barbosa J, Martins de Lavor É, Ribeiro TF, Macedo CA, de Souza Duarte-Filho LA, Feitosa TA, de Jesus Silva J, Fokoue HH, Araújo CR. Antinociceptive and anti-inflammatory effects of hydrazone derivatives and their possible mechanism of action in mice. Plos one. 2021;16(11):e0258094.
18. Nadri S, Mahmoudvand H, Mahmoudvand H, Rashnoo M, Khaksarian M. Chemical composition, antinociceptive and acute toxicity of Pistacia atlantica fruit extract. Entomol Appl Sci Letters. 2018;5(3):8-12.
19. Carlisson KH, Jurna I. Depression by flupirtine; a novel analgesic agent of motor and sensory response of nociceptive system in the rat spinal cord. Eur J Pharmacol. 1987;143(1): 87–99.
20. Dubisson D, Dennis SG. The formalin test a quantivie study of the analgesic effects of morphin, meperidne and brainstem stimulation in rat and cats. Pain. 1997;4(2):161–174.
21. Chen CL, Broom DC, Liu Y, Denooji JC, Li Z, Cen C, Abdel Samad O, Jessell TM, Woolf CJ, Ma Q. Run x1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. Neuron. 2006; 49(3):365–377.
22. Tgolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain. 1991; 51(1):17–25.
23. Manabu S, Tsuyako O, Hiroshi T, Reizo I. Modified formalin test: characteristic biphasic pain response. Pain. 1989;38(3):347–352.
24. Souza MA, Scapinello J, Guzatti JG, Scatolin M, Martello R, Schindler MS, Calisto JF, Alves B, Morgan LV, Oliveira JV, Dal Magro J. Antinociceptive effect and mechanism of supercritical carbon dioxide extract of Aloysia gratissima leaves in mice. biomedical journal. 2021;44(6):S63-72.
25. Verri Jr WA, Vicentini FT, Baracat MM, Georgetti SR, Cardoso RD, Cunha TM, Ferreira SH, Cunha FQ, Fonseca MJ, Casagrande R. Flavonoids as anti-inflammatory and analgesic drugs: mechanisms of action and perspectives in the development of pharmaceutical forms. Studies in natural products chemistry. 2012;36:297-330.