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PHARMACOLOGICAL EVALUATION OF THE ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF THE HYDROETHANOLIC EXTRACT OF THE LEAVES OF *RAPHANUS SATIVUS* (L.) VAR. *OLEIFERA* METZG IN MICE

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ABSTRACT

To evaluate the pharmacological potential and to perform the phytochemical characterization of the dry hydroethanolic extract of the leaves of *Raphanus sativus* (nabo forrageiro). The leaves of this species are used worldwide because of its medicinal properties. Material and Methods: *In vivo* anti-inflammatory and antinociceptive effects of dry hydroethanolic extract of the leaves were evaluated through lipopolysaccharide (LPS)-induced peritonitis and carrageenan-induced paw edema tests and by the formalin test, respectively. In this study we used adult male Swiss mice. To elucidate the compounds present in the extract, a phytochemical analysis was performed using high performance liquid chromatography (HPLC). Results and Conclusions: The extract induced anti-inflammatory activity as determined by the LPS-induced peritonitis and carrageenan-induced paw edema tests. The extract also showed activity in the formalin test. Additionally, on the basis of HPLC data, the structures of three flavonoids, kaempferol, rutin, and quercetin, which can be associated with the biological activity of *R. sativus*, were identified.

Keywords: Anti-inflammatory, Phenolics, Flavonoids, *Raphanus sativus*.

INTRODUCTION

Medicinal plants are of great interest because they have a broad spectrum of biological activities, and thus offer the potential for new drug discovery. They are commonly used to assist the healing process. However, studies providing a better understanding of the chemical constitution and biological activity of plants are required because they have the potential to poison as well as heal [1].

Raphanus sativus L. var. *oleifera* Metzg is a plant popularly known in Brazil as "nabo forrageiro," belonging to the family Brassicaceae (Cruciferae) [2]. *R. sativus* has been used as a herbal drug in traditional Chinese medicine for over 1400 years; it was recorded in the first Chinese Pharmacopoeia [3]. The leaves and roots of this species are used in different parts of the world for its medicinal properties [4]. Antioxidant, antimicrobial, and chemopreventive activities have been reported for this plant [5-7]. In addition, phenolic compounds have been isolated and are described to be responsible for these activities [8].

Considering the long standing popular use of *Raphanus sativus* L. as an anti-inflammatory traditional medicine [9], the aim of this study was to evaluate the pharmacological potential of the dry hydroethanolic extract from the leaves of *R. sativus* in animal models of inflammation and nociception. In addition, phytochemical characterization was performed to elucidate the compounds present in the extract.

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MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Raphanus sativus* L. var. *oleifera* Metzg were collected on August 2, 2012 at Avenida Afonso Pena, Jardim Boa Esperança, Alfenas, state of Minas Gerais. Coordinates: 21°25′22.87″S; 45°57′29.42″O. Dr. Geraldo Alves da Silva performed the taxonomic identification of *R. sativus*. A sample was deposited in the Herbarium of the Federal University of Alfenas (voucher n. 2279).

Preparation of the dry hydroethanolic extract and drugs

The leaves of *R. Sativus* were dried in a circulating air oven and ground with mechanical mill knives to obtain a powdered drug. The hydroethanolic extract was obtained using an extraction method of exhaustive percolation of the drug. Subsequently, the hydroalcoholic extract was taken into a rotary vaporizer under reduced pressure and a temperature of 45°C, in order to concentrate the extract. Next, the extract was frozen in an Ultrafreezer at −70°C, and lyophilized in a lyophilizer unit [10].

Analysis of the dry hydroethanolic extracts using High Performance Liquid Chromatography (HPLC)

HPLC analysis of the dry hydroethanolic extract was performed using a Shimadzu UFLC 20A CLC-ODS (250–4.6 mm) C18 column with a 5 µm particle size. Mobile phases were composed of (A) 0.5 % aqueous acetic acid and (B) 0.5% acetic acid in methanol. The gradient of the mobile phases (A:B) used for separation were a linear gradient of 0 to 60 min (90:10 to 0:100) with a solvent flow rate of 1.0 ml/min, an injection volume of 25µl at a concentration of 1 mg/ml. The eluent was detected with a photodiode array detector (DAD) with UV light at 268 nm. LC solution software (Shimadzu) was used for data collection.

Experimental procedures

Animals

Adult male Swiss mice weighing 30 to 45 g (n = 8 animals per group) were obtained from the Central Animal Facility of the Federal University of Alfenas (Alfenas, Minas Gerais, Brazil) and housed in a controlled 12 h light–dark room (lights on at 07:00 am) at 23 ºC with *ad libitum* water and food. All experiments were conducted in accordance with Helsinki's Declaration of Welfare for experimental animals, and approved by the local Research Ethics Committee of the Federal University of Alfenas (protocol n. #490/2013).

Lipopolysaccharide (LPS)-induced peritonitis

The LPS-induced peritonitis test assessed the effect of the dry hydroethanolic extract from *R. sativus* in the leukocytes migration into the peritoneal cavity. The mice (n = 8per group) were orally pretreated with: (1) Indomethacin (10 mg/kg); (2) vehicle (CMC; 10 ml/kg); and (3) dry hydroethanolic extract at doses of 30, 100 and 300 mg/kg, respectively. One hour after treatment, the inflammatory agent (lipopolysaccharide from *E. coli* serotype 026: B6 Sigma, 100 mg/kg) was intraperitoneally administered. The animals were sacrificed by isoflurane inhalation 4 h after LPS injection procedure. The peritoneum of each animal was washed with 3ml of phosphate buffer (PBS, pH 7.4). The peritoneum was adequately massaged and the cell suspension removed. An aliquote of 10µl of exudate was used to determine the total leukocyte count in a hemocytometer [10].

Paw edema induced by carrageenan

The paw edema test was commenced by injection with carrageenan (2% w/v in sterile saline) into the subplantar region of the right hind paw of the mice. The mice (n = 8 per group) were orally pretreated with: (1) Indomethacin (10 mg/kg); (2) vehicle (CMC; 10 ml/kg); and (3) dry hydroethanolic extract from *R. sativus*, at doses of 30, 100, and 300 mg/kg, respectively. The basal volume of the right hind paw was determined before administration of any drug. One hour post-treatment, paw edema was induced by injection of carrageenan, and applied to the plantar region of the right hind paw of the mice. As a control, sterile saline was used. The paw volume was measured 1, 2, 3, and 4 h after the injection of the inflammatory stimulus. The edema was measured using the plethysmograph. The results are presented as the paw volume (µl) variation in relation to the basal values (Δ) [11, 12].

Formalin test

The mice were orally pretreated under fasting conditions (n = 8 per group) in the following groups: (1) Indomethacin (10 mg/kg); (2) morphine (1 mg/kg); (3) vehicle (CMC; 10 ml/kg); and (4) the dry hydroethanolic extract from *R. sativus* at doses of 30, 100, and 300 mg/kg, respectively. One hour post-treatment (excluding group 2, 30 min), 20 µl of formalin (5%) were injected into the plantar region of the right hind paw of the animals. The time spent licking the affected paw was rated during two time intervals after the injection: 0 to 5 min (first phase or neurogenic pain) and 20 to 30 min (second phase or inflammatory pain) [12-14].

Acute toxicity

The acute toxicity in mice (n = 10) was performed with an oral dose of 5g/kg of the dry hydroethanolic extract from *R. sativus*. The following parameters were assessed: hyperactivity, sedation, changes in stool characteristics (consistency), and food and water intake. The parameters were evaluated during a seven-day period with adequate acclimatization (21ºC). Food and water were given *ad libitum*.

Fig 1. Structures of the flavonoids rutin (1), quercetin (2), and kaempferol (3)

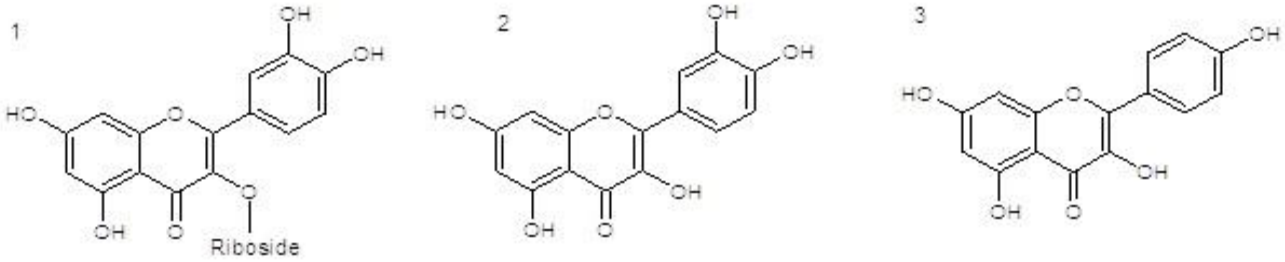


Fig 2. Chromatogram of the dry hydroethanolic extract using High Performance Liquid Chromatography (HPLC). Retention times for standards of rutin (1), quercetin (2), and kaempferol (3) are shown in the table..

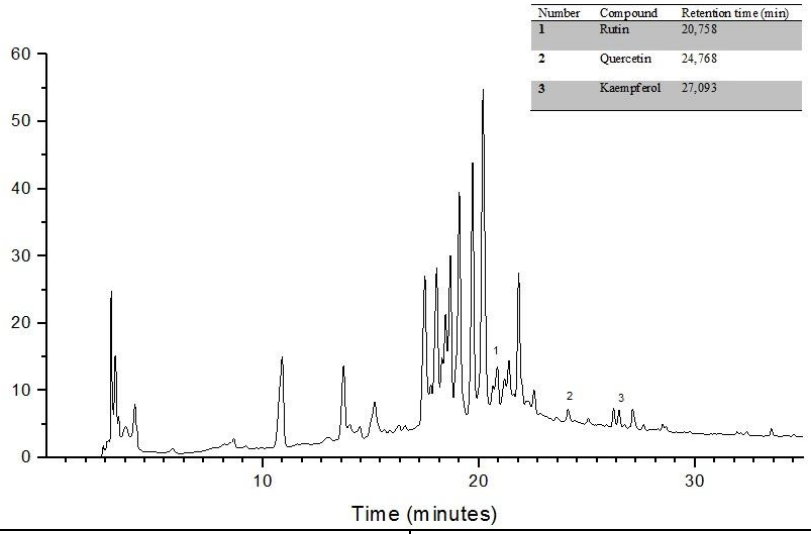


Fig 3. Effects of the administration of the dry hydroethanolic extract of *R. sativus* leaves (30, 100, and 300mg/kg) or indomethacin (INDO, 10 mg/kg) on the lipopolysaccharide-induced recruitment of leukocytes to the peritoneal cavity of mice. Each column represents the mean±SEM of eight animals. ###*p*<0.001 compared with the saline+vehicle group. ***p< 0.001 compared with LPS + vehicle group

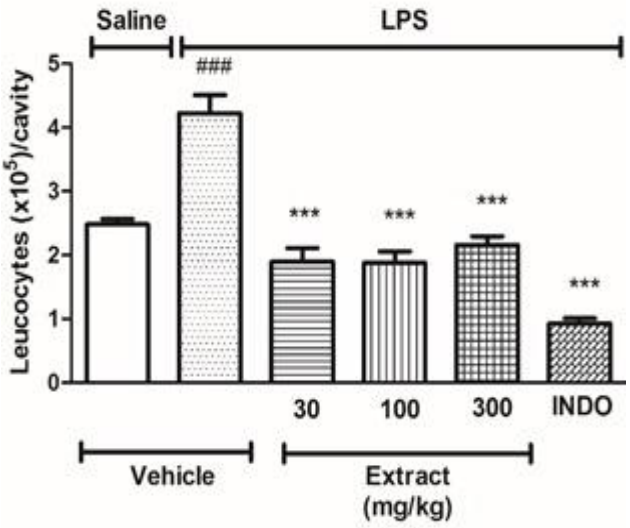


Fig 4. Effects of the administration of the dry hydroethanolic extract of *R. sativus* leaves (30, 100, and 300mg/kg) or indomethacin (INDO, 10mg/kg) on mice paw edema induced by intraplantar (i.pl.) carrageenan injection (2% w/v). Each point represents the mean±SEM of eight animals. The asterisks denote the significance levels when compared with the control group: *p<0.05, **p<0.01, ***p<0.001.

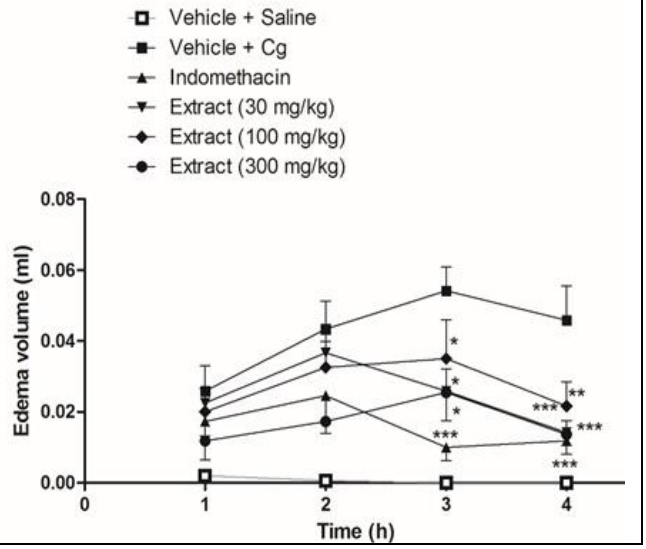
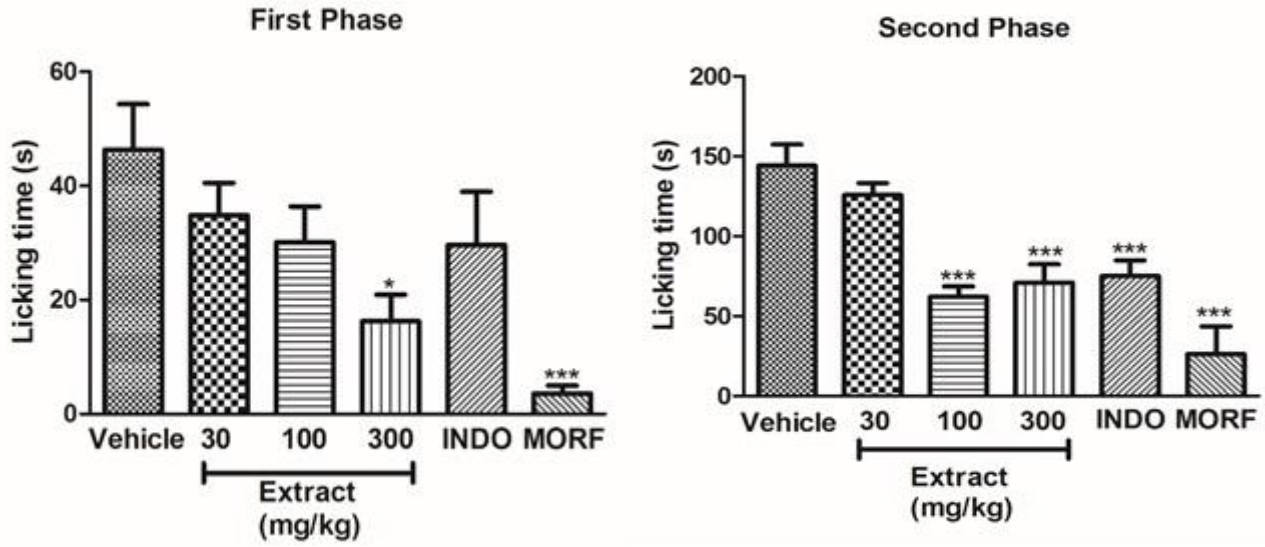


Fig 5. Effects of the dry hydroethanolic extract from the *R. sativus* leaves on the formalin test in mice. Animals were pretreated with the vehicle, Extract (30, 100, and 300mg/kg), indomethacin (INDO; 10mg/kg) or morphine (MORF; 1mg/kg) prior to formalin. The total time spent licking the hind paw was measured in the first and second phases after intraplantar injection of formalin. Each column represents the mean with S.E.M. for eight mice. The asterisks denote the significance levels when compared with the control group: *p<0.05; ***p<0.001.



Statistical analysis

All data was analyzed using GraphPad statistical software Version 5.0 and expressed as mean ±standard error. Statistically significant differences between groups were investigated using analysis of variance (ANOVA) followed by a Newman–Keuls *post hoc* test for multiple comparisons of means (p-values of less than 0.05 were considered statistically significant).

RESULTS

Phytochemical analysis using high performance liquid chromatography (HPLC) of the dry hydroethanolic extract of *R. sativus*

The chromatogram of the extract revealed the presence of phenolic compounds between the 13th and 25th minutes of the chromatographic run (Figure 1). The flavonoids kaempferol (R_t = 27.093 min), quercetin (R_t = 24.768 min), and rutin (R_t = 20.758 min) were identified by running the standards of these compounds, determining their retention times, and comparing with the dry extract (Figure 2).

Lipopolysaccharide (LPS)-induced peritonitis

The LPS-induced peritonitis was followed by a significant increase (p< 0.001) in the number of leukocytes in the peritoneal cavity of mice compared with the group treated with saline. However, indomethacin reduced the number of leukocytes compared with the group treated with LPS (p< 0.001). The pretreatment with dry extract obtained from *R. sativus* (30, 100, and 300mg/kg) reduced the number of exudate leukocytes from the peritoneal cavity (p< 0.001) compared with the group of animals treated with LPS (Figure 3).

Paw edema induced by carrageenan

In the test of carrageenan-induced paw edema, an increase in the edema volume was monitored over the course of 4 h has shown in Figure 4. The group that received saline did not demonstrate changes in the volume of edema.

Three hours after the carrageenan injection, a decrease in the edema in animals pretreated with the dry extract was observed (p< 0.05) at doses of 30, 100, and 300 mg/kg compared with the group treated with carrageenan. A decrease in edema volume was also observed in the fourth hour in these groups. Indomethacin (10mg/kg) inhibited edema formation in the third and fourth hours (p< 0.001).

Formalin test

The formalin test verified the time in which the mice licked the right hind paws in the first and second phases (Figure 5). In the first phase of the formalin test, the dry hydroethanolic extract induced a significant antinociceptive effect at dose of 300mg/kg (p< 0.05) compared with the control group. Additionally, in the second phase, the extract reduced the time of licking at doses of 100 and 300 mg/kg (p< 0.001). Indomethacin suppressed only the second phase of the formalin test, whereas morphine inhibited both phases of the test.

Acute toxicity

No significant differences in acute toxicity were observed in the habits of mice that received the dry hydroethanolic extract from the leaves of *R. sativus* at a dose of 5g/kg during the seven days. Food and water intake did not change. The production and consistency of feces did not change. No deaths occurred in the mice post-treatment. Thus, the dry extract shows no toxic effects *in vivo*.

DISCUSSION

The leaves and roots of *R. sativus* are used for its medicinal properties [4]. This specie has long been used as an anti-inflammatory traditional medicine [9]. However, its pharmacological actions have not been thoroughly investigated. This study evaluated the pharmacological potential of dry hydroethanolic extract from the leaves of *R. sativus* in animal models of nociception and inflammation. The extract demonstrated antinociceptive and anti-inflammatory properties. It did not produce mortality in mice in a dose of 5g/kg, and may thus be considered relatively safe. Furthermore, phytochemical characterization was performed in order to isolate the compounds present in the extract. The chemical structures of kaempferol, quercetin were identified.

The anti-inflammatory activity of the dry hydroethanolic extract from the leaves of *R. sativus* was evaluated using carrageenan-induced paw edema and LPS-induced peritonitis tests. The carrageenan-induced inflammation is important to detect the anti-inflammatory activity of herbal drugs. Therefore, it has a significant predictive value for anti-inflammatory compounds that inhibit mediators release in acute inflammatory response [12, 15]. The carrageenan-induced paw edema produces a biphasic event. In the initial phase (1–2 h), chemical mediators such as histamine, serotonin, and bradykinin are involved, while prostaglandins are involved in the late phase, between the third and fifth hour, after the injection of carrageenan [16]. The LPS- induced peritonitis model resembles a bacterial infection, and represents a model for the study of peritoneal acute inflammation [10, 17, 18]. The recruitment of cells during inflammation depends upon the local release of the mediators, which are responsible for changes in tissues and immune cell recruitment [19, 20].

The dry hydroethanolic extract from *R. sativus* inhibited inflammation in the late phase of the carrageenan-induced paw edema test. In the LPS-induced peritonitis test, the extract significantly reduced the number of cells recruited at the doses tested. We suggest that these effects are attributable to the inhibition of release and/or action of the mediators of acute inflammatory response, mainly prostaglandins.

The formalin test aims to evaluate nociception in two phases. The first phase, neurogenic or acute pain, is a result of the direct effect of formalin on sensory C fibers, which are modulated by the action of centrally-acting analgesics, such as opioids (e.g., morphine) [21, 22]. The second phase occurs by the action of inflammatory mediators, and is specially inhibited by non-steroidal anti-inflammatory drugs such as indomethacin, a non-selective cyclo-oxygenase inhibitor [23]. This delayed reaction is due to the action of inflammatory mediators such as serotonin, histamine, bradykinin, and prostaglandin, which may cause sensitization of nociceptive neurons [24, 25]. The effect of the dry hydroethanolic extract during the second phase suggests an anti-inflammatory action.

The formalin test demonstrated the significant effect of the dry hydroethanolic extract at a dose of 300 mg/kg in both phases, whereas the dose of 100mg/kg was effective only in the second phase. These results are consistent with the anti-inflammatory activity of the extract observed in previous tests, because the second phase of the formalin test occurs via the action of the inflammatory mediators.

Phenols are widely distributed in the plant kingdom. There is a vast literature establishing the importance of these compounds, especially flavonoids, which are known for their anti-inflammatory properties [11, 15, 22, 26]. To flavonoids was reported inhibitory activity of blockade of the lipooxygenase and cyclooxygenase pathways, inhibiting the production of inflammatory mediators such as leukotrienes and prostaglandins [27]. Antinociceptive and antiedematogenic effects were demonstrated for the triterpenes in the formalin test, and for steroid in edema induced by carrageenan [28-31].

In this study, the structures of three common flavonoids, quercetin, rutin, and kaempferol, were confirmed using HPLC. Zhou et al. (2015) [32] demonstrated the inhibitory effect of kaempferol on the inflammatory response of lipopolysaccharide (LPS)-stimulated HMC-1 mast cells. In this study, kaempferol significantly decreased the release of histamine, IL-6, IL-8, IL-1β, and TNF-α of activated HMC-1 mast cells. Selloum et al. (2003) [33] described the effect of rutin on inflammation. The important role of quercetin to regulate inflammatory responses in microglial cells also was demonstrated [34].

In conclusion, we suggest that the *in vivo* anti-inflammatory and antinociceptive effects observed in the present study, are associated with the compounds identified in the dry hydroethanolic extract of *R. sativus*. This work contributes to a better knowledge of the chemical constituents and pharmacological potential of *R. sativus* in treating inflammatory disorders.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. World Health Organization (WHO). Quality control methods for medicinal plant materials. Geneva, 1998.
2. Gutiérrez RMP, Perez RL. *Raphanus sativus* (Radish): their chemistry and biology. *ScientificWorld Journal*, 4, 2004, 811-837.
3. Feng DL, Feng B, Wang W, Chen F, Cai G, Pei Y, Wang Y. Thioglucosides from the Seeds of *Raphanus sativus* L. *Helv. Chim. Acta*, 89, 2006, 2953-2957.
4. Nadkarni KM, Nadkarni AK, Chopra RN. Popular Prakashan: Bombay. *Indian Materia. Medica*, 1, 1976, 1031–1035.
5. Barillari J, Cervellati R, Costa S, *et al*. Antioxidant and choleretic properties of *Raphanus sativus* L. sprout (Kaiware Daikon) extract. *J Agric Food Chem*, 54, 2006, 9773-9778.
6. Devaraj VC, Gopala KB, Viswanatha GL, Satya PV, Vinay BSN. Protective effect of leaves of *Raphinus sativus* Linn on experimentally induced gastric ulcers in rats. *Saudi Pharm J*, 19, 2011, 171-176.
7. Shukla S, Chatterji S, Yadav DK, Watal G. Antimicrobial Efficacy of *Raphanus Sativus* Root Juice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3, 2011, 3-6.
8. Cruz SM, Nery MC, Von Pinho, EVR,Laia, ML. Molecular characterisation of radish cultivars. *Rev Ciência Agronômica*, 45, 2014, 815-822.
9. Kook SH, Choi KC, Lee YH, Cho HK, Lee JC. Raphanus sativus L. seeds prevent LPS-stimulated inflammatory response through negative regulation of the p38 MAPK-NF-κB pathway. *Int Immunopharmacol*, 23(2), 2014, 726-34.
10. Padilha MM, Vilela FC, Rocha CQ, *et al*. Antiinflammatory properties of *Morus nigra* leaves. *Phyther Res*, 24, 2010, 1496-1500.
11. Levy L. Carrageenan paw edema in the mouse. *Life Sci*, 8, 1989, 601-606.
12. Orlandi L, Vilela FC, Santa-Cecília FV, Dias DF, Alves-Da-Silva G, Giusti-Paiva A. Anti-inflammatory and antinociceptive effects of the stem bark of *Byrsonima intermedia* A. Juss. *J Ethnopharmacol*, 137, 2011, 1469-1476.
13. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain*, 4, 1977, 161-174.
14. Dickenson H, Sullivan F. Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. *Neurosci Lett*, 83, 1987, 207-211.
15. Moreira LQ, Vilela FC, Orlandi L, *et al*. Anti-inflammatory effect of extract and fractions from the leaves of *Byrsonima intermedia* A. Juss. in rats. *J Ethnopharmacol*, 138, 2011, 610-615.
16. Posadas I, Bucci M, Roviezzo F, *et al*. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. *Br J Pharmacol*, 142, 2004, 331-338.
17. Castelucci S, Paula Rogerio A, Ambrosio SR, *et al*. Anti-inflammatory activity of *Dasyphyllum brasiliensis* (Asteraceae) on acute peritonitis induced by beta-glucan from Histoplasma capsulatum. *J Ethnopharmacol*, 112, 2007, 192-198.
18. Cunha FQ, Souza GE, Souza C, Cerqueira BC, Ferreira SH. *In-vivo* blockage of neutrophil migration by LPS is mimicked by a factor released from LPS-stimulated macrophages. *Br J Exp Pathol*, 70, 1989, 1-8.
19. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*, 13, 2013, 159-175.
20. Thomazzi SM, Silva CB, Silveira DCR, *et al*. Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). *J Ethnopharmacol*, 127, 2010, 451-456.
21. Przewłocki R, Przewłocka B. Opioids in chronic pain. *Eur J Pharmacol*, 429, 2001, 79-91.
22. Calixto JB, Beirith A, Ferreira J, Santos ARS, Filho VC, Yunes R. Naturally occurring antinociceptive substances from plants. *Phyther Res*, 14, 2000, 401-418.
23. Dannhardt G, Kiefer W. Review Cyclooxygenase inhibitors – current status and future prospects. *Eur J Med Chem*, 36, 2001, 109-126.
24. Woolf CJ. Central Sensitization: Implications for the diagnosis and treatment of pain. *Pain*, 152, 2012, 1-31.
25. Jakubowski M, Levy D, Goor-Aryeh I, Collins B, Bajwa Z, Burstein R. Terminating migraine with allodynia and ongoing central sensitization using parenteral administration of COX1/COX2 inhibitors. *Headache*, 45, 2005, 850-861.
26. Leary K, De Pascual-Tereasa S, Needs PW, Bao YP, O'Brien NM, Williamson G. Effect of flavonoids and Vitamin E on cyclooxygenase-2 (COX-2) transcription. *Mutat Res - Fundam Mol Mech Mutagen*, 551, 2004, 245-254.
27. Landolfi R, Mower RL, Steiner M. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. *Biochemical Pharmacology*, 32, 1984, 1525-1530.
28. Gaertner M, Muller L, Roos JF, Can G, Santos ARS, Niero R, Calixto JB, Yunes RA, Monache FD, Cechinel-Filho V. Analgesic triterpenes from *Sebastiania schottiana*roots. *Phytomedicine*, 1, 1999, 41-44.

29. Lima FV, Malheiros A, Otuki MF, Calixto JB, Yunes RA, Filho VC, Monache FD. Three triterpenes from the resinous bark of *Protium kleinii* and their antinociceptive activity. *Journal of the Brazilian Chemical Society*, 16(3B), 2005, 578-582.
30. Mattos WM, Campos MM, Fernandes ES, Richetti GP, Niero R, Yunes RA, Calixto JB. Anti-edematogenic effects of velutinol A isolated from *Mandevilla velutina*: Evidence for a selective inhibition of kinin B1 receptor-mediated responses. *Regulatory Peptides*, 136, 2006, 98-104.
31. Veloso CC, Rodrigues VG, Ferreira RCM, Duarte LP, Klein A, Duarte ID, Romero TRL, Perez AC. Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to opioidergic activation. *Planta Medica*, 80, 2014, 1615-1621.
32. Zhou YJ, Wang H, Li L, Sui HH, Huang JJ. Inhibitory effect of kaempferol on inflammatory response of lipopolysaccharide-stimulated human mast cells. *Yao Xue Xue Bao*, 50(6), 2015, 702-7.
33. Selloum L, Bouriche H, Tigrine C and Boudoukha C. Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation. *Exp. Toxic. Pathol*, 54, 2003, 313-318.
34. Sun GY, Chen Z, Jasmer KJ, Chuang DY, Gu Z, Hannink M, Simonyi A. Quercetin Attenuates Inflammatory Responses in BV-2 Microglial Cells: Role of MAPKs on the Nrf2 Pathway and Induction of Heme Oxygenase-1. *PLoS One*, 10(10), 2015, 25.