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ORIGINAL ARTICLE

Antinociceptive and Anti-inflammatory Effects of Triterpenes from *Pluchea quitoc* DC. Aerial Parts

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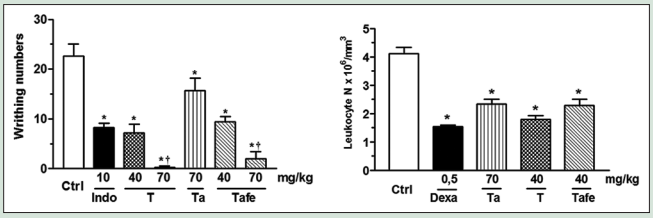
ABSTRACT

Background: *Pluchea quitoc* DC. (Asteraceae), a medicinal plant known as "quitoco," "caculucage," "tabacarana" and "madre-cravo," is indicated for inflammatory conditions such as bronchitis, arthritis, and inflammation in the uterus and digestive system. **Objective:** This study evaluated the analgesic and anti-inflammatory activities of the triterpenes compounds obtained from *P. quitoc* aerial parts. **Materials and Methods:** The triterpenes compounds β-amyrin, taraxasterol and pseudo-taraxasterol in a mixture (T); β-amyrin, taraxasterol and pseudo-taraxasterol acetates in a mixture (Ta); β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with β-amyrin, taraxasterol and pseudo-taraxasterol myristates (Tafe) were analyzed in the models of nociception and inflammation. The evaluation of antinociceptive activity was carried out by the acetic acid-induced writhing and tail-flick tests while leukocyte migration to the peritoneal cavity was used for anti-inflammatory profile. **Results:** The oral administration of T or Tafe (40 mg/kg and 70 mg/kg) and Ta (70 mg/kg) to mice reduced acetic acid-induced writhing. The tail-flick response of mice was not affected by T or Tafe (40 mg/kg). T or Tafe (40 mg/kg) and Ta (70 mg/kg) also inhibited peritoneal leukocyte infiltration following the injection of carrageenan. **Conclusion:** The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol that were decreased when these were acetylated; while the acetylated triterpenes in mixture with myristyloxy triterpenes improved this activity. These compounds seem, at least in part, to be related to the plant's reported activity.

Key words: Anti-inflammatory, antinociceptive, *Pluchea quitoc*, triterpene

SUMMARY

The mixtures of hydroxylated, acetylated, and myristate triterpenes isolated from hexanic extracts of *Pluchea quitoc* DC. were analyzed in the models of nociception and inflammation in mice. The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol. This study showed too that the activity of triterpenes may be decreased by their being acetylated, while the acetylated triterpenes in mixture with myristate triterpenes improved this activity.



Abbreviations Used: T: Triterpenes compounds β-amyrin, taraxasterol, and pseudo-taraxasterol in a mixture, Ta: Triterpenes compounds β-amyrin, taraxasterol and pseudo-taraxasterol acetates in a mixture, Tafe: Triterpenes compounds β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with β-amyrin, taraxasterol and pseudo-taraxasterol myristates, Ctrl: Control, Indo: Indomethacin, Dexa: Dexamethasone, EtOAc: Ethyl acetate, MeOH: Methanol.

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INTRODUCTION

Pluchea quitoc DC., a medicinal plant used in folk medicine, like some other plants belonging to the same genus,[1-5] is an aromatic shrub of the Asteraceae family, popularly known in Brazil as "quitoco," "madre-cravo," "caculucage," or "tabacarana." It is used in traditional medicine for the treatment of inflammation as well as of digestive and respiratory diseases in the North and Central West of Brazil.[6,7]

The phytochemical analysis of the less polar fractions of the hexane extract of this species afforded stigmasterol, a mixture of β-amyrin, taraxasterol and pseudo-taraxasterol, and six eudesmane derivatives such as cuauthemone[8] while more polar fractions yielded the such as cuauthemones and epoxycuauthemones.[9,10] Casticin, a flavone, was isolated from the hexane extract of this species and has shown *in vitro* activity against the epimastigote forms of *Trypanosoma cruzi*.[11] The ethanolic extract from aerial parts of *P. quitoc* modulated the hematopoietic response during bacterial infection[12] and counteracted the tumor-induced myelopoietic suppression in Ehrlich ascites tumor-bearing mice.[13]

Experimental studies have demonstrated anti-inflammatory and antinociceptive activities of triterpenes β-amyrin and taraxasterol. It was determined the *in vivo* anti-arthritic effect of taraxasterol on arthritis induced by Freund's complete adjuvant in rats.[14] *In vitro* study demonstrated that taraxasterol inhibited IL-1 β-induced NO and PGE2

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production, as well as MMP-1, MMP-3, MMP-13, iNOS, and COX-2 expression in chondrocytes and exhibited anti-inflammatory effects in IL-1 β-stimulated chondrocytes through the inhibition of NF-κB activation.[15] The triterpenes *α* and *β*-amyrin produced antinociceptive effect on rat model of orofacial pain induced by formalin or capsaicin.[16]

We have previously shown that the ethanolic extract of *P. quitoc* had anti-inflammatory and antinociceptive effects in mice and rats in various analgesic and inflammatory models.[17]

In this work, we evaluated the anti-inflammatory and antinociceptive effects of mixtures of hydroxylated, acetylated and myristate triterpenes isolated from hexanic extracts of *P. quitoc* in mice, to determine whether these compounds might be responsible for the plant's reported activity.

MATERIALS AND METHODS

Plant materials

P. quitoc was collected at Peixe-Boi, Pará State, Brazil and identified by the botanist Dr. João Ubiratan Santos, from Museu Paraense Emílio Goeldi (Belém, Pará, Brazil) where a voucher specimen has been deposited (No. 147609).

Isolation of the compounds

The aerial parts of *P. quitoc* (7 kg) were air dried and extracted with hexane at room temperature. Part of the crude hexane extract (20 g) was subjected to chromatography on a silica gel column and eluted with solvents of increasing polarity in the order hexane, hexane- ethyl acetate (EtOAc), EtOAc, and MeOH. The fraction containing the esterified triterpenes (2257 mg) was obtained from 1% to 3% EtOAc in hexane and was submitted to further chromatography separation on a silica gel column eluted with mixtures of EtOAc in hexane 0.5% and 1% yielding 977 mg of a mixture of *β*–amyrin acetate (16.26%), taraxasterol acetate (21.35%), pseudo-taraxasterol acetate (5.33%) in mixture with *β*–amyrin myristate (16.26%), taraxasterol myristate (27.18%), pseudo-taraxasterol myristate (13.59%) (Tafe) and 313 mg of a mixture of *β*–amyrin acetate (16.66%), taraxasterol acetate (66.66%), and pseudo-taraxasterol acetate (16.66%) (Ta, 1:4:1). Part of the fraction Tafe was submitted to hydrolysis yielding the hydroxylated triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol and myristic acid, which structures were confirmed by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and gas chromatography-mass spectroscopy spectral analysis. The acetates of the triterpenes (Ta) were identified by comparison of their ^{13}C NMR spectral data with those reported in the literature.[18,19] The hydroxylated triterpenoid fractions were obtained from 5% to 10% EtOAc in hexane, yielding 127 mg of a mixture of β-amyrin (40.0%), taraxasterol (40.0%), and pseudo-taraxasterol (20.0%) (T, 1:1:0.5).[8,9]

Animals

Swiss mice (25–30 g), obtained from the Animal House of Federal University of Maranhão, were used. Animals were maintained under environmental conditions and had free access to a standard diet and water *ad libitum*. All experimental protocols were developed in accordance with the principles of ethics and animal welfare designated by the Brazilian College of Animal Experiments (COBEA) and the ethical guidelines for the investigation of experimental pain in conscious animals.[20]

Antinociceptive activity

Acetic acid-induced writhing test

The writhing test described previously[21] was used with adaptations.[22,23] T or Tafe (40 mg/kg and 70 mg/kg) and Ta (70 mg/kg) in 4% tween 80 and water (vehicle), indomethacin (10 mg/kg) or vehicle, were administered p. o. in mice (n = 5–9), 60 min before acetic acid (0.8% v/v, 0.1 ml/10 g). The response to intraperitoneal injection of acid was cumulatively counted for 20 min.

Tail-flick test

Experiments were carried out according to previously described methodology,[24] with modifications. Male and female Swiss mice (n = 5) were placed on the tail-flick unit (Ugo Basile) so that the tail occluded a slit over a photocell. The source of heat was applied by a 70-W lamp mounted in a reflector and adjusted to 55°C ± 2°C. When the animal moved its tail away from the slit light fell on the photocell, and the timer was automatically stopped. The apparatus was previously calibrated to produce tail-flick latencies of approximately 3–5 s in control animals. The mice were treated with vehicle, T or Tafe (40 mg/kg, p. o.), or morphine (20 mg/kg, s. c.). All mice were observed in control conditions (60 and 30 min before) and 30, 60, 90, 120, and 150 min after drug administration.

Anti-inflammatory activity

Carrageenan-induced peritonitis test

The acute carrageenan-induced inflammatory reaction was induced by modification of the technique previously described.[25] Male and female Swiss mice (n = 5) were pretreated with T or Tafe (40 mg/kg), Ta (70 mg/kg), dexamethasone (0.5 mg/kg) or vehicle, p. o., 30 min before the injection of carrageenan (0.25 ml, 1% w/v in saline), into the peritoneal cavity. Four hours after the application of the irritant agent, the mice were sacrificed by cervical dislocation. Ca^{++} and Mg^{++} free heparinized (10 IU/ml) phosphate-buffered saline (2 ml) was injected into the peritoneal cavity and after a gentle massage, peritoneal fluids were removed; total leukocytes were determined in a Neubauer chamber.

Statistical analysis

The data are expressed as means ± standard error of the mean for 5–9 animals per group. The statistical analysis between treatment groups was done by one-way analysis of variance followed by Newman–Keuls test. Comparison between individual groups was analyzed with Student's t-test. Results were considered statistically significant when $P < 0.05$.

RESULTS

The triterpenes caused dose-dependent inhibition of the acetic acid-induced writhing response in mice [Figure 1]. In control animals treated with vehicle, the total number of writhing movements determined over 20 min was 22.6 ± 2.4 (n = 8). Pretreatment with T or Tafe (40 mg/kg and 70 mg/kg) inhibited the number of writhing by 68.2% and 98.9% or 58.5% and 91.2%, respectively, when compared with the control ($P < 0.05$). In comparison, indomethacin (10 mg/kg) reduced writhing movements by 63.8%. However, Ta (70 m/kg) returned an inhibition of only 30.8%. The tail-flick test demonstrated that the mice treated with T or Tafe (40 mg/kg, p. o.) did not present a longer latency than the control animals. In the same conditions, the tail-flick latency of mice treated with morphine (20 mg/kg, s. c.) was increased 92.3% after 30 min. In the experiment involving carrageenan-induced inflammation in the peritoneal cavity, the leukocyte migration in control mice treated with the vehicle was $4.11 \times 10^{6}/mm^{3} \pm 0.23$ [Figure 2]. Previous treatment p. o. with T or Tafe (40 mg/kg) or Ta (70 mg/kg) reduced the migration by 56.5%, 44.6%, and 43.1%, respectively ($P < 0.05$). The animals treated with dexamethasone, a steroidal agent used as a positive control, exhibited an inhibition by 63.3%.

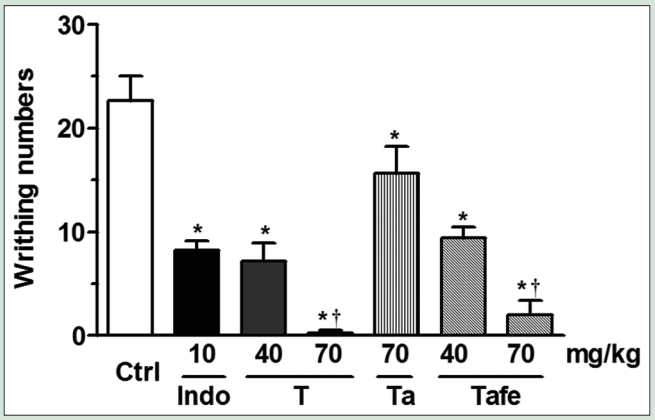

Figure 1: Effects of oral treatment of mice with the vehicle (Ctrl), triterpene compounds-β-amyrin, taraxasterol, pseudo-taraxasterol in a mixture (T), β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture (Ta), β-amyrin, taraxasterol, pseudo-taraxasterol acetate in a mixture with β-amyrin, taraxasterol, pseudo-taraxasterol myristates (Tafe) extracted from *Pluchea quitoc* DC. aerial parts or indomethacin (Indo) on acetic acid-induced writhing. The vertical bars indicate the mean ± standard error of the mean. Of numbers of writhing movements. *P < 0.05 versus control (analysis of variance-Newman–Keuls test). †P < 0.05 versus 40 mg/kg (Student's *t*-test)

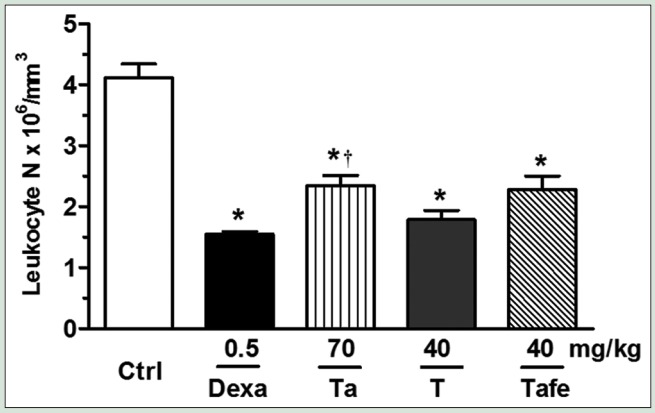

Figure 2: Effects of oral treatment of mice with the vehicle (Ctrl), triterpene compounds-β-amyrin, taraxasterol, pseudo-taraxasterol in a mixture (T), β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture (Ta), β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with β-amyrin, taraxasterol, pseudo-taraxasterol myristates (Tafe) extracted from *Pluchea quitoc* DC. aerial parts or dexamethasone (Dexa) on carrageenan-induced peritonitis. The vertical bars indicate the mean ± Standard Error of the Mean. of numbers of total leukocytes (leukocyte N × 10^6/mm^3) of peritoneal wash. *P < 0.05 versus control (analysis of variance-Newman–Keuls test). †P < 0.05 versus T group (Student's *t*-test)

DISCUSSION

This study evaluated the antinociceptive and anti-inflammatory effects of a mixture of triterpenes from *P. quitoc* using different stimuli, including chemical agents (acetic acid, carrageenan) and heat (tail flick). The triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol in mixture, administered p. o. showed a significant and dose-dependent activity against the acetic acid-induced writhing response in mice and exhibited higher activity than the acetylated triterpenes (β-amyrin, taraxasterol, and pseudo-taraxasterol acetates); at the same dose (70 mg/kg), the effect of the former was 63 times higher. Nevertheless, the mixture of acetylated triterpenes with β-amyrin, taraxasterol and pseudo-taraxasterol myristates presented an effect comparable with that of the triterpenes. The hydroxylated triterpenes and acetylated triterpene with myristate triterpene were inactive in the mouse tail-flick model of analgesia, suggesting that these compounds have no central analgesic properties. In contrast, when mice were treated with the ethanolic extract of *P. quitoc* the response time was significantly prolonged by 44.1% after 30 min.[17] The ability of the triterpenes to reduce the number of writhing and to not prolong the time of response of the tail-flick reflex indicates that a peripheral mechanism is involved. The writhing response is thought to involve, in part, local peritoneal receptors while the tail-flick test response is essentially a spinal reflex.[26] It is possible that other compounds of the plant are responsible for this effect. The migration of leukocytes to the site of inflammation is a fundamental aspect of the inflammatory process. Cell migration is the result of many different processes including adhesion and cell mobility.[27] Intraperitoneal injection of carrageenan led to the recruitment of leukocytes to the inflammatory site. In this study, we compared the effect on cell migration of vehicle, dexamethasone, and the mixture of triterpenes. It has been reported that triterpenoids, a fraction containing cycloartenol as the main component, inhibited peritoneal leukocyte infiltration.[27] Here, similar to what was shown in the writhing test the acetoxyl group decreased the effect of triterpenes on leukocytes migration. The treatment of animals with Tafe (myristyloxy triterpenes with acetyloxy triterpene, 40 mg/kg), did not show a significant difference compared to treatment with T at the same dose. However, the treatment of animals with Ta (acetyloxy triterpene) at a dose 1.75 times higher (70 mg/kg) reduced the migration by 13.4% less than T (hydroxylated triterpenes, 40 mg/kg, P < 0.05), indicating higher hydroxylated triterpene activity. These results raise the possibility that the esterified triterpenes possess lower antinociceptive and anti-inflammatory properties, similar to the finding that naturally occurring palmitate lupeol decreased the anti-inflammatory activity comparatively less than triterpene lupeol.[28] *In vitro* studies investigating the effect of triterpenes isolated from *Pluchea lanceolata* under neuroinflammation conditions also demonstrated a higher effect of triterpene compared to acetyloxy triterpene.[29] This study showed the anti-inflammatory and peripheral antinociceptive activity of triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol, β-amyrin, taraxasterol and pseudo-taraxasterol acetates and β-amyrin, taraxasterol and pseudo-taraxasterol myristates in misture. This study showed too that the activity of triterpenes may be decreased by their being acetylated, while acetoxyled triterpenes in mixture with myristate triterpenes improved this activity.

CONCLUSION

The results demonstrate the anti-inflammatory and peripheral antinociceptive activities of the mixture of the triterpenes β-amirina, taraxasterol and pseudo-taraxasterol, that these activities were diminished in the mixture of triterpenes acetate, whereas these in mixture with triterpenos miristato improved these activities. These compounds appear, at least in part, to relate to the reported activity of the plant.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Martino VS, Debenedetti SL, Coussio JD. Caffeoyl quinic acids from *Pterocaulon virgatum* and *Pluchea sagittalis*. Phytochemistry 1979;18:2052.
2. Sen T, Nag Chaudhuri AK. Antiinflammatory evaluation of a *Pluchea indica* root extract. J Ethnopharmacol 1991;33:135-41.
3. Scholz E, Heinrich M, Hunkler D. Caffeoylquinic acids and some biological activities of *Pluchea symphytifolia*. Planta Med 1994;60:360-4.
4. Agbonon A, Eklu-Gadegbeku K, Aklikokou K, Essien K, Akpagana K, Gbeassor M, *et al*. The effect of *Mangifera indica* stem bark and *Pluchea ovalis* roots on tracheal smooth muscle *in vitro*. Fitoterapia 2002;73:619-22.
5. de Souza GC, Haas AP, von Poser GL, Schapoval EE, Elisabetsky E. Ethnopharmacological studies of antimicrobial remedies in the South of Brazil. J Ethnopharmacol 2004;90:135-43.
6. Cruz GL. Dicionário das plantas úteis do Brasil. Rio de Janeiro: Bertrand Brasil; 1995.
7. Correa MP. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Vol. 5. Rio de Janeiro: Imprensa Nacional; 1984.
8. Guilhon GM, Müller AH. Eudesmane derivatives from *Pluchea quitoc*. Phytochemistry 1996;43:417-21.
9. Guilhon GM, Müller AH. Eudesmane sesquiterpenoids from *Pluchea quitoc*. Phytochemistry 1998;47:227-9.
10. Guilhon GM, Müller AH. Eudesmanolides and epoxycuauthemones from sesquiterpenoids from *Pluchea quitoc*. Phytochemistry 1998;49:1347-51.
11. Zani CL, Alves TM, Oliveira AB, Murta SM, Ceravolo IP, Romana AJ. Trypanocidal components of *Pluchea quitoc* DC. Phytother Res 1994;8:375-7.
12. Queiroz ML, Justo GZ, Pereira-da-Silva FR, Müller AH, Guilhon GM. Stimulatory action of *Pluchea quitoc* extract on the hematopoietic response during murine listeriosis. Immunopharmacol Immunotoxicol 2000;22:721-40.
13. Queiroz ML, Justo GZ, Valadares MC, Pereira-da-Silva FR, Müller AH. Adjuvant effect of *Pluchea quitoc* extract on the resistance of tumor-bearing mice by modulation of the host hematopoietic response. Immunopharmacol Immunotoxicol 2001;23:215-28.
14. Wang S, Wang Y, Liu X, Guan L, Yu L, Zhang X, *et al*. Anti-inflammatory and anti-arthritic effects of taraxasterol on adjuvant-induced arthritis in rats. J Ethnopharmacol 2016;187:42-8.
15. Piao T, Ma Z, Li X, Liu J. Taraxasterol inhibits IL-1β-induced inflammatory response in human osteoarthritic chondrocytes. Eur J Pharmacol 2015;756:38-42.
16. Holanda Pinto SA, Pinto LM, Guedes MA, Cunha GM, Chaves MH, Santos FA, *et al*. Antinoceptive effect of triterpenoid alpha, beta-amyrin in rats on orofacial pain induced by formalin and capsaicin. Phytomedicine 2008;15:630-4.
17. Barros IM, Lopes LD, Borges MO, Borges AC, Ribeiro MN, Freire SM, *et al*. Anti-inflammatory and anti-nociceptive activities of *Pluchea quitoc* (DC.) ethanolic extract. J Ethnopharmacol 2006;106:317-20.
18. Reynolds WF, Sawyer JF, Enriquez RG, Escobar LI, Chavez MA, Shoolery JN. Total assignment of the ^{13}C spectrum of taraxasterol acetate by ^{13}C-^{13}C connectivity experiments and determination of the stereochemistry of taraxasterol by X-ray diffraction. Can J Chem 1985;63:1048-54.
19. Nehrli FW, Nishida T. The use of carbon-13 nuclear magnetic resonance spectroscopy in Natural Products Chemistry. Fortschr Chem Org Naturst 1986;36:1-229.
20. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109-10.
21. Koster R, Anderson M, Debeer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412.
22. Freire SM, Emin JA, Lapa AJ, Souccar C, Torres LM. Analgesic and anti-inflammatory properties of *Scoparia dulcis* L. extracts and glutinol. Phytother Res 1993;7:408-14.
23. Santos TC, Marques MS, Menezes IA, Dias KS, Silva AB, Mello IC, *et al*. Antinociceptive effect and acute toxicity of the *Hyptis suaveolens* leaves aqueous extract on mice. Fitoterapia 2007;78:333-6.
24. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74-9.
25. Griswold DE, Marshall PJ, Webb EF, Godfrey R, Newton J Jr., DiMartino MJ, *et al*. SK and F 86002: A structurally novel anti-inflammatory agent that inhibits lipoxygenase-and cyclooxygenase-mediated metabolism of arachidonic acid. Biochem Pharmacol 1987;36:3463-70.
26. Alexandre-Moreira MS, Piuvezam MR, Araújo CC, Thomas G. Studies on the anti-inflammatory and analgesic activity of *Curatella americana* L. J Ethnopharmacol 1999;67:171-7.
27. Ahumada C, Sáenz T, García D, De La Puerta R, Fernandez A, Martinez E, *et al*. The effects of a triterpene fraction isolated from *Crataegus monogyna* Jacq. on different acute inflammation models in rats and mice. Leucocyte migration and phospholipase A2 inhibition. J Pharm Pharmacol 1997;49:329-31.
28. Nikiéma JB, Vanhaelen-Fastré R, Vanhaelen M, Fontaine J, De Graef C, Heenen M, *et al*. Effects of antiinflammatory triterpenes isolated from *Leptadenia hastata* latex on keratinocyte proliferation. Phytother Res 2001;15:131-4.
29. Srivastava P, Mohanti S, Bawankule DU, Khan F, Shanker K. Effect of *Pluchea lanceolata* bioactives in LPS-induced neuroinflammation in C6 rat glial cells. Naunyn Schmiedebergs Arch Pharmacol 2014;387:119-27.