



ISSN: 2321-9114
 AJEONP 2018; 6(4): 13-17
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 Received: 06-08-2018
 Accepted: 10-09-2018

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Essential oil of *Cordia millenii* from Nigeria

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Abstract

Cordia millenii Bak. (Boraginaceae) is used in the south-western Nigeria to cure ache and pain disorders. Essential oil was obtained by hydrodistillation of air dried leaves of *C. millenii* in a Clevenger-type apparatus. The yield of the essential oil was 0.026% (v/w), calculated on dry weight basis. The chemical constituents of the oil were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) on HP-5 column. The major constituents of the essential oil were limonene (19.9%), diallyl disulfide (18.4%), β -caryophyllene (16.6%), linalool (13.4%) and nonanal (10.6%). The anti-nociceptive property of the essential oil was statistically non-significant ($p > 0.05$) when compared to the control for most concentrations except at 120th mins ($p < 0.05$) for 200 mg p.o. The 200-mg p.o. dose only displayed anti-inflammatory activity at the 1st h ($p < 0.01$) with others non-significant ($p > 0.05$). This is the first report of the chemical composition and biological activities of the essential oils of *C. millenii*.

Keywords: *Cordia millenii*, terpenes, anti-nociceptive activity anti-inflammatory activity

1. Introduction

From time immemorial, smokes from the medicinal plants have been in use by humans for the treatment of different diseases. It was a common practice in many cultures and among famous ancient physicians. The records, written on clay tablets (from Mesopotamia dated about 2600 BC) are still in use as references for the treatment different ailments ranging from coughs and colds to parasitic infections and inflammations [1]. Plant derived smoke has multiple uses, including air purification, flavoring, medicinal, seed germination, pest control, preservation, religious and veterinary among other historical and modern applications [2].

1.2 *Cyperus esculentus* (Tiger nut)

Cordia millenii (Bak.) is a medicinal plant belonging to Boraginaceae family. It is widely distributed in tropical Africa. The plant can grows to a height of 60 to 100 ft, bole cylindrical, but rarely straight, 30 to 40 ft. in length; trunks about 3 ft in diameter above buttresses [1]. The plant has been used in ethnomedicine for the treatment of fever, cough, stomachache, mild tonic, astringent, toothache and inflammation related disorders. Extracts from *C. millenii* have shown the antifertility [1], antimicrobial [2] and antioxidants [2] effects. In addition, the extracts have prevented lipopolysaccharide-induced neuroinflammation [3]. The phytochemical compounds previously isolated from the plant include cordiachromes A–F [4]. Until now, no information is available on essential oils of *C. millenii*. This paper describes for the first time, the chemical composition and observed anti-inflammatory actions of *C. millenii* essential oil. Recently, we have published data on the chemical constituents, anti-inflammatory and anti-nociceptivel activities of essential oils from Nigerian plants [5-7].

2. Materials and methods

2.1 Drugs and chemicals

Carrageenan drug of analytical grade was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Ibuprofen injection (May and Baker) and Diclofenac Injection (Dizpharm, Nigeria Ltd.) were purchased from Lagos State University Pharmacy.

2.2 Collection of plant

The leaves of *C. millenii* were collected from Ayetoro, Ilesha (7°37'0N 4°43'0E), Osun State in June 2017. Botanical identification was achieved by Mr. Dotanus E. of Herbarium, Department of Botany, University of Ibadan, Nigeria. A voucher specimen (UIH-22607) was

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deposited at the herbarium. The leaves were air-dried under laboratory shade (27 °C) for two weeks.

2.3 Hydrodistillation of essential oil

Two hundred and sixty grams of the air-dried and pulverized leaves of *C. milleni* was used. The essential oil was obtained by hydrodistillation which was carried out in an all-glass Clevenger-type distillation as reported previously [5-7].

2.4 Analysis of oil sample

Gas chromatography (GC) analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30 m × 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/min to 220°C. The injector and detector temperatures were maintained at 250°C; carrier gas nitrogen (2 mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 µL. The relative proportions of the oil constituents were percentages obtained by FID peak area normalization without the use of a response factor.

Gas chromatography-mass spectrometry (GC-EIMS) analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; film thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature 220°C and 240°C, respectively; oven temperature programmed from 60-240°C at 3°C/min.; carrier gas helium at a flow rate of 1mL/min.; injection volume 0.2 µL (10% *n*-hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was *m/z* 30-300 at a scan rate of 1 scan/sec.

The identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to a series of *n*-alkanes. Further identifications were also made possible by the use of a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data as described previously [5-7]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

2.5 Study Animals

Eight-week-old Wistar rats of average weight of 150 to 200 g of either sex were bought and kept in the animal house of the Department of Biochemistry, Lagos State University, Nigeria. Standard conditions of temperature (23 ± 2°C), light accessibility (12 h light and darkness cycle) with free access to standard pellet feed, tidy environment and water *ad libitum*. All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2017/LASU/BCH).

The animals were assigned at random to a group of 5 consisting of 5 animals per group: Group 1- Control group (Saline solution); Group 2- Diclofenac treated group 100 mg/kg (Standard Group); Group 3- 100 mg/kg of *C. mellini* essential oils; Group 4- 200 mg/kg of essential oil; and Group 5- 400 mg/kg of essential oil. All treatments were administered orally using the canula syringe.

2.6 Carrageenan-induced rat paw edema (Anti-inflammatory test)

Carrageenan induced rat paw edema was done according to a modification form of an established procedure [8,9]. Twenty-five Wistar rats (both sexes, 150-200 g each) divided into 5

animals in each groups used for study, induced by subcutaneous injection of a 0.1 ml of 1% freshly prepared carrageenan in saline in the right hind paw of rats and 1mL of the vehicle were administered for all doses. Paw volume of the injected rats was measured every hour for 4 h using a plethysmometer (Ugo Basile, Italy).

2.7 Hot plate test for anti-nociceptive study

The experiment was carried out according to the modified method [10]. Twenty (25) mature Wistar rats both sexes were randomly divided into 5 groups of 5 rats per group. The animals were fasted for 12 h with provision of clean water *ad libitum*. Each rat was placed upon the heated metal plate (hot plate) maintained at the temperature of about 50-55°C within the restraining glass cylinder. Group 1 rat received 10 ml/kg of saline solution and served as control. Group 2 mice received sodium salicylate (10 mg/kg (ASA) (standard control) and groups 3, 4 and 5 received 100, 200 and 400 mg/kg of *C. milleni* extract respectively per 0s (p.o.). Animal response to the heat varies and such changes includes; kicking of hind foot and jumping about, licking of foot, raising the foot, holding the foot tightly to its body or shaking of the foot. The reaction time was recorded 30, 60, 90 and 120 min after the administration of the treatments. The maximum reaction time was fixed at 30 sec to prevent any injury to the tissues of the paws. If the reading exceeds 30 sec, it would be considered as maximum analgesia.

The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{30 \text{ sec} - \text{reaction time for saline}} \times 100$$

2.8 Statistical analysis

Repeated Measures One way ANOVA Analysis using Dunnett's multiple comparisons post hoc test was performed using GraphPad Prism (version 7.02), San Diego CA, USA, www.graphPad.com) to compare activity between the control groups and rat treated with the test compounds and values were considered significant at *p* < 0.05 and above. Results were expressed as mean ± SEM [5-7, 9].

3. Results & Discussion

3.1 Chemical constituents of essential oil

The yield of the essential oil was 0.026% (v/w), calculated on a dry weight basis. Eleven compounds representing 100% of the total oil contents were identified in *C. milleni* (Table 1). The volatile compounds were displayed in Table 1, along with their percentages and retention indices calculated on HP-5 column. The major constituents of the oil were limonene (19.9%), diallyl disulfide (18.4%), β-caryophyllene (16.6%), linalool (13.4%), nonanal (10.5%) and *trans*-calamenene (8.5%). The results could not be compared since no data previously existed on the chemical constituents of *C. milleni* oils. However, the chemical compositions and biological activities of essential oils of *C. sebestina* from Nigeria have been reported. The fruit of *C. sebestina* [11] contained 4-pentenylbutanoic acid ester (12.2 %) while phytol was found in the leaf (13.7%) and flower (16.9%). However, (*E*)-9-octadecene (20.26%), (*E*)-5-octadecene (18.68%), 9-eicosene (13.99%) and nonyl cyclopropane (12.42%) were the main compounds in the stem bark oil [12].

Table 1: Chemical constituents of *C. millenii* essential oil

Compounds ^a	LRI ^a	LRI ^b	Percent composition
Limonene	1032	1031	19.9
<i>trans</i> -Linalool oxide (furanoid)	1076	1072	2.7
Diallyl disulfide	1082	1088	18.4
Linalool	1101	1101	13.4
Nonanal	1102	1104	10.5
Allyl methyl trisulfide	1142	1148	3.2
β -Caryophyllene	1420	1417	16.6
α -Humulene	1456	1454	3.0
Germacrene D	1478	1480	0.8
<i>trans</i> -Calamenene	1530	1529	8.5
Caryophyllene oxide	1581	1583	3.0
Total			100.0
Monoterpene hydrocarbons			19.9
Oxygenated monoterpenes			16.1
Sesquiterpene hydrocarbons			28.9
Oxygenated sesquiterpenes			3.0
Sulfur-containing compounds			21.6
Non-terpenes			10.5

^a Elution order on HP-5 column; ^b Linear retention indices on HP-5 column; ^c Literature retention indices;

The chemical compositions of essential oils from *Cordia* plants varied from each other. Although terpene compounds were most common, the identities of these terpenoids differed from each other. On the basis of the main classes of compounds identified in them from literature, the essential oils of *Cordia* plants may be classified into different group. There are group containing mainly monoterpene hydrocarbons common to *C. curassavica* [13], *C. globosa* [14]; those containing sesquiterpene hydrocarbons as seen in *C. verbenacea* [15], *C. leucocephala* [16], *C. leucomalloides* [17] and *C. multispicata* [18]; mixture of monoterpene and sesquiterpene hydrocarbons prominent in *C. globosa* [13], *C. curassavica* [17], *C. verbenacea* [19], *C. multispicata* [18]; oils containing oxygenated sesquiterpenes such as *C. curassavica* [17]; oils with large contents of non-terpenes dominated by *C. sebestina* [11,12] and *C. nitida* [20].

Moreover, the biological potentials of essential oils of *Cordia* plants have been published. The stem oil of *C. sebestina* possessed antioxidant activity [12]. The essential oil of *C. globosa* displayed antimicrobial activities against *Vibrio cholera* [13], while *C. verbenacea* was found to showed fungistatic activity against *Candida albicans* and *C. krusei* and antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* 27 [15]. The oil of *C. leucomalloides* [17] and *C. curassavica* [13] exhibited significant larvicidal activity against the third-instar of *Aedes aegypti* larvae. The oil *C. verbenacea* inhibited the growth of *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212 [19].

3.2 Anti-inflammatory activity of the essential oil

Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome [21,22]. In this study, we evaluated the anti-inflammatory activity of the *C. mellini* by the carrageenan-induced paw edema. The carrageenan-induced paw edema model is used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is believed to be biphasic. The first phase (1 h) involves the release of serotonin and histamine and the second phase (> 1 h) is

mediated by cyclooxygenase products characterized by release of neutrophils [23]. At the intermediate of the two phases is as a result of kinin release. Continuity between the two phases is provided by kinin [24].

The essential oil of *C. millenii* in this report at 200 mg/kg significantly (** $p < 0.01$) inhibited the edema formation in only the first phase. The anti-edematous activity of *C. millenii* in the first phase could be attributed to the possible suppression of histamine signaling by the mast cell stabilizing effect [24,25] and direct inhibition of histamine H1 receptor and serotonin.

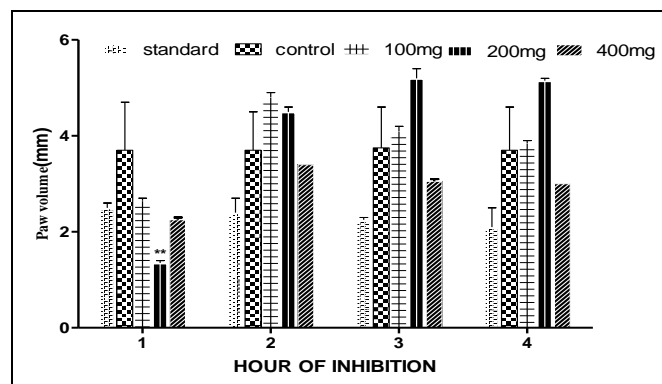


Fig 1: Effect of the essential oils of *C. millenii* leaves on Carrageenan-induced inflammation. Control, standard and *C. millenii* represent 1 mL saline solution, 100 mg/kg of dichlofenac injection and 1 mL of 100, 200 and 400 mg of *C. millenii* leaves essential oil respectively. * $p < 0.05$, ** $p > 0.01$, *** $p > 0.001$ statistically compared to the control

The other doses were non-significant during the analysis period. Limonene, diallyl disulfide, β -caryophyllene and linalool which are major constituents in the oils of *C. millenii* are terpenoids with known anti-inflammatory properties. *In-vitro* study of the anti-inflammatory effects of limonene showed that at low concentration, there is suppression of ROS (Reactive Oxygen species) for eotaxin-stimulated HL-60 clone 15 cells, while at a higher concentrations limonene inhibited some inflammation mediators such as the NF- κ B activation [8], Nitric oxide, PGE₂, IL-1 β and IL-6 [26]. In addition β -caryophyllene has also been shown to exert inhibition on pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 [27].

3.3 Anti-nociceptive activity of the oil

The results of the analgesic effect of the essential oils of *C. millenii* on hot plate method are presented in Figure 2. The results showed that there was no significant difference on the thermal stimulus in rats treated with normal saline (control) throughout the 120th min observation. In comparison to the saline treated animals, the significant increase in the reaction time to thermal pain was not detectable in both sodium salicylate (standard). However, the observation in 200 mg/kg treated animals is only noted at 120th min with $p < 0.05$. Rats treated with sodium salicylate exhibited analgesic activity at a slower interval, which began at 30 min (30.0%) and then increased to a maximum of 50% at the 60th min.

The MPA value for the essential oils did not show any analgesic effect in the first 30 min after treatment for all doses but the 200 mg/kg showed an increase activity between the 60th min (30%) to the 120th min (45%).

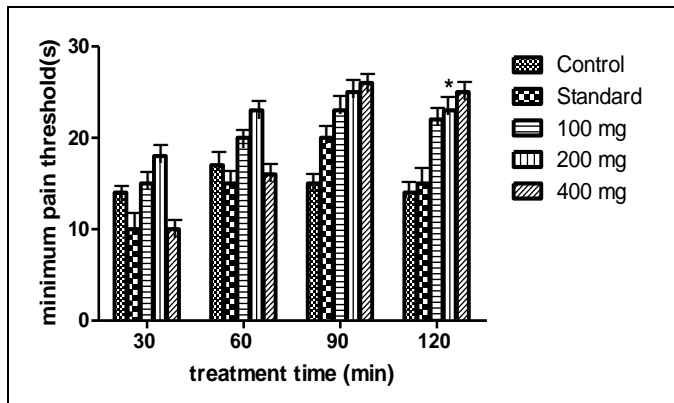


Fig 2: Effect of the essential oils of *C. millenii* leaves on hot place-induced anti-nociceptive. Control, standard and *C. millenii* represent 1mL saline solution, 100 mg/kg of aspirin injection and 1mL of 100, 200 and 400 mg of *C. millenii* leaves essential oil respectively. * $p < 0.05$, ** $p > 0.01$, *** $p > 0.001$ statistically compared to the control.

In hot plate test pain induced by thermal stimulus is exact for centrally mediated analgesia and reported to involve opioids^[28]. The hot plate test was chosen to analyze a central analgesic activity, because of its sensitivity to strong analgesics and limited tissue damage and offers a good step in analyzing pains that are supraspinally mediated. In this study, the essential oil has shown a relatively low or non-opioid property. In a related study, the anti-nociceptive effect of limonene on the hot plate test has been reported showing no significant inhibition^[29].

4. Conclusions

It could be seen that the chemical compositions of essential oils from *C. millenii* varied from those of other members in the genus. In addition, the oil of *C. millenii* exhibited moderate anti-inflammatory action while displaying no anti-nociceptive activity at reasonable concentrations when compared with standards.

5. References

- Nnadozie IJ, Olajide OC, Agbabiaye OO, Okpuzor J. Effect of *Cordia millenii* extract on reproductive hormone in cisplatin induced infertility in female albino rats. *Journal of Advances in Biomedical Studies*. 2017; 2(1):27-35.
- Nnanga NGA, Deli V, Mboug AF, Victoire N, Lazare SS, Sandrine S, *et al.* Preliminary screening of *Cordia mellenii* and their antimicrobial and antioxidant activities. *International Journal of Research Studies in Biosciences*. 2015; 3(1):39-46.
- Olatunji BP, Fasola TR, Onasanwo SA, Akinyemi AJ, Adeniyi PA, Ishola AO. Neuronal alterations and antioxidant status of lipopolysaccharide induced neuronal damage in mice: Efficacy of three medicinal plants. *Journal of Applied Pharmaceutical Science*. 2017; 7(2):156-162.
- Moir M, Thomson RH. Naturally occurring quinones. Part XXII. Terpenoid quinones in *Cordia* spp. *Journal of Chemical Society Perkin Transaction 1*, 1973; 1:1352-1357.
- Aboluwodi AS, Avoseh NO, Lawal AO, Ogunwande IA, Giwa AA. Chemical constituents and anti-inflammatory activity of essential oil of *Datura stramonium* L. *Journal of Medicinal Plant Studies*. 2017; 5(1):21-25.
- Adeosun TE, Avoseh NO, Ogunwande IA, Raji IP, Lawal AO. Composition and anti-inflammatory activity of

essential oil of *Jatropha curcas* L. *Natural Product Communications*. 2017; 12(3):439-440.

- Oluwa AA, Avoseh NO, Omikorede O, Ogunwande IA, Lawal AO. Study on the chemical constituents and anti-inflammatory activity of essential oil of *Petiveria alliacea* L. *British Journal of Pharmaceutical Research*. 2017; 15(1):1-8.
- Henriques MG, Silva PM, Martins MA, Flores CA, Cunha FQ, Assreuy-Filho J *et al.* Mouse paw edema. A new model for inflammation? *Brazilian Journal of Medical and Biological Research*. 1987; 20:243-249.
- Avoseh NO, Oyedeji OO, Aremu K, Nkeh-Chungag BN, Songca SP, Oluwafemi SO *et al.* Chemical composition and anti-inflammatory activities of the essential oils from *Acacia mearnsii* de Wild. *Natural Product Research*. 2015; 29(12):1184-1188.
- Shethy SN, Anika SM. *Laboratory Manual of Pharmacology and Toxicology*. 1st Edition. Fourth Dimension Publishers, Enugu, Nigeria. 1982; 78-81.
- Adeosun CB, Bamigbade O, Osho A, Atolani O. Volatile composition of the leaf, flower and fruit of *Cordia sebestena* (L.). *Journal of Essential Oil-Bearing Plants*. 1982; 18(4):976-981.
- Adeosun CB, Olaseinde S, Opeifa AO, Atolani O. Essential oil from the stem bark of *Cordia sebestena* scavenges free radicals. *Journal of Acute Medicine*. 2013; 3(4):138-141.
- de Oliveira JCS, da Camara CAG, Schwartz MOE. Volatile constituents of the stem and leaves of *Cordia* species from mountain forests of Pernambuco (North-eastern Brazil). *Journal of Essential Oil Research*. 2007; 19(5):444-448.
- Miguel M, García-Bores A, Meraz S, Piedra E, Ávila M, Serrano R, *et al.* Antimicrobial activity of essential oil of *Cordia globosa*. *African journal of Pharmacy and Pharmacology*. 2016; 10(11):179-182.
- Rodrigues FFR, Oliveirs LGS, Saraiva ME, Almeida SCX, Cabral MES, Campos AR *et al.* Chemical composition, antibacterial and antifungal activities of essential oil from *Cordia verbenacea* DC leaves. *Pharmacognosy Research*. 2012; 4(3):161-165.
- Diniz JC, Viana FA, de Oliveira OF, Silveira ER, Pessoa ODL. Chemical composition of the leaf essential oil of *Cordia leucocephala* Moric from Northeast of Brazil. *Journal of Essential Oil Research*. 2008; 20(6):495-496.
- Santos RP, Nunes EP, Nascimento RF, Santiago GMP, Menezes GHA, Silveira ER *et al.* Chemical composition and larvicidal activity of the essential oils of *Cordia leucomalloides* and *Cordia curassavica* from the Northeast of Brazil. *Journal of Brazilian Chemical Society*. 2006; 17(5):1027-1030.
- das Graças M, Zoghbi M, Andrade EHA, Pereira RA, Oliveira J. Volatiles of the *Cordia multispicata* Cham.: a weed medicinal Brazilian plant. *Journal of Essential Oil Research*. 2010; 22(6):543-545.
- Meccia G, Rojas LB, Velasco J, Díaz T, Usubillaga A, Arzola JC *et al.* Chemical composition and antibacterial activity of the essential oil of *Cordia verbenacea* from the Venezuelan Andes. *Natural Product Communications*. 2009; 4(8):1119-1122.
- Pino JA, Bello A, Urquiola A, Marbot R. Fruit volatiles from *Cordia nitida* Vahl. *Journal of Essential Oil Research*. 2002; 14(2):118-119.
- Vinegar R, Schreiber W, Hugo RJ. Biphasic development of carrageenan edema in rats. *Journal of Pharmacology*

- and Experimental Therapeutics. 1969; 166(1):96-103.
22. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology*. 1971; 104(1):15-29.
 23. Gilligan JP, Lovato SJ, Erion MD, Jeng AY. Modulation of carrageenan-induced hind paw edema by substance. *Inflammation*. 1994; 18(3):285-292.
 24. Salvemini D, Wang ZQ, Wyatt PS, Bourdon PM, Marino MH, Manning PT, *et al*. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *British Journal of Pharmacology*. 1996; 118(4):829-838.
 25. Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. *Indian Journal of Pharmacology*. 2015; 47(3):292-289.
 26. Hirota R, Roger NN, Nakamura H, Song HS, Sawamura M, Sukanuma N. Anti-inflammatory effects of limonene from yuzu (*Citrus junos* Tanaka) essential oil on eosinophils. *Journal of Food Science*. 2010; 75(3):H87-H92.
 27. Yoon W, Lee NH, Hyun C. Limonene suppresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2 and pro-inflammatory cytokines in RAW 264.7. macrophages. *Journal of Oleo Science*. 2010; 59(8):415-421.
 28. Besra SE, Sharma RM, Gomes A. Anti-inflammatory effect of petroleum ether extract of leaves of *Litchi chinensis* Gaertn. (Sapindaceae). *Journal of Ethnopharmacology*. 1996; 54(1):1-6.
 29. Do Amaral JF, Silva MI, Neto MR, Moura BA, de Melo CT, de Araujo FL, *et al*. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. *Biological and Pharmaceutical Bulletin*. 2007; 30(7):1217-1220.