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Full Length Research Paper

The impact of compounds isolated from *Ocimum* sp. on apoptotic activity of osteoclast

Paulo J. L. Juiz [1]*, Roberto Gambari[2], Roberta Piva[2], Letizia Penolazzi[2], Franceli Silva[1], Reinaldo J. C. Alves[3], Angélica M. Lucchese[3] and Ana Paula T. Uetanabaro[4]

[1]Center for Health Sciences, Federal University of Recôncavo da Bahia, Bahia, Brazil.
[2] University of Ferrara, Ferrara, Italy.
[3]State University of Feira de Santana, Bahia, Brazil.
[4]State University of Santa Cruz, Bahia, Brazil.

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Periodontal disease has multifactorial etiology. The immune response to the microbial challenge leads to osteoclast activation and resorption of the alveolar bone, resulting in tooth loss. Compounds isolated from *Ocimum americanum* and *Ocimum basilicum* were analyzed for apoptotic activity towards osteoclast in this study. Steam distillation was used for the extraction of essential oils (EOs) from dry leaves and flowers. The assessment of apoptosis in osteoclasts was carried out through the TUNEL assay and immunocytochemistry for the Fas receptor. The chemical profile of EOs, characterized through gas chromatography-mass spectrometry analysis, revealed methyl cinnamate (*O. americanum*), linalool, caryophyllene, 1,8-cineole (*O. basilicum*) as major components. The results showed that essential oils were not able to induce apoptosis in osteoclast; however, linalool (50 to 300 µg.ml^{-1}) induced 75% of apoptotic osteoclasts at non-toxic concentrations and the apoptotic activity was confirmed by the increasing levels of Fas receptor on osteoclasts treated with this compound. This study suggests that linalool could be used to control osteoclast activity.

Key words: Essential oil, linalool, *Ocimum*, osteoclast, periodontal disease.

INTRODUCTION

Periodontal disease is considered a common pathological condition in oral cavity, with possible systemic repercussions, particularly in adult individuals. They are of a multifactorial nature, including a broad spectrum of inflammatory and destructive responses to dental biofilm and bacterial components in a susceptible host. Pro-inflammatory mediators (IL-1, PGE_2, TNFα) produced by T lymphocytes present in the periodontal pocket promote osteoclast differentiation and activation, culminating in alveolar bone resorption and tooth loss (Ishikawa, 2007).

Osteoclasts (OCs) are specialized motile bone resorptive cells, derived from haematopoietic stem cells and they are the effector cells of alveolar bone loss in periodontal disease. In response to key factors, such as vitamin D3, parathyroid hormone (PTH) and proinflamatory cytokines (IL-1, IL-6, TNFα, PGE_2, IL-17)

*Corresponding author. E-mail: limajuiz@ufrb.edu.br.

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osteoblasts express receptor activator of nuclear factor κB ligand (RANKL), which in combination with CSF-1/M-CSF stimulates osteoclast development from peripheral blood cell precursors by binding to its receptor. Osteoprotegerin (OPG) is a decoy RANKL receptor that prevents RANKL-RANK interaction to inhibit osteoclastogenesis (Emery et al., 1998; Nakashima et al., 2011; Balvers et al., 2015).

Periodontal treatment consists of two sessions at one-week intervals of supragingival scaling and oral hygiene instructions, followed by subgingival scaling and root planing under local anesthesia performed within a period of 14 days, with or without use of antibiotics; however, systemic antibiotics do not act on osteoclasts directly to prevent or suppress bone resorption (Eguchi et al., 2008). Recently, many plants and their extracts have been recognized as useful sources for the prevention and treatment of bone-related disorders, including periodontal disease. In this context, the influence of *Stewartia koreana* extract on differentiation of osteoclasts was evaluated by Park et al. (2012). According to these authors, the extract (20 $\mu g.mL^{-1}$) of this plant was able to inhibit the differentiation of osteoclasts.

The activity of magnolol present in *Magnolia officinalis* was described by Lu et al. (2015). The administration of magnolol in mice with induced periodontal disease, was able to inhibit alveolar bone resorption and the number of osteoclasts on the bone surface, decreased expression of RANKL, MMP-1, MMP-9, NFκB and showed antimicrobial activity against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and inhibited differentiation of RAW 264.7 macrophages into osteoclasts.

In this study, the *in vitro* apoptotic activity of essential oils obtained from *O. americanum* and *O. basilicum* was assessed towards osteoclasts. The genus *Ocimum*, family Lamiaceae, comprises more than 64 herbaceous and subshrub species. This genus exhibits great diversity of species, popularly known in Brazil as "alfavacas" and "manjericões" (Blank et al., 2002; Lorenzi and Matos, 2008) and are found in tropical and subtropical regions (Paton, 1992). Due to their economic importance, the most cultivated species in the world are *Ocimum citriodorum* Vis., *Ocimum americanum* L., *Ocimum basilicum* L., *O. gratissimum* L., *Ocimum minimo* L., and *O. tenuiflorum* L. (Carović-Stanko et al., 2010).

Ocimum species have several biological properties, including anti-inflammatory, antileishmanial (Rabelo et al., 2003; Ueda-Nakamura et al., 2006), antibacterial and antifungal activities (Suppakul et al., 2003) and could be a promising medicinal species to control osteoclast activity.

MATERIALS AND METHODS

Study species

O. americanum L. of the Lamiaceae family originating from Asia and Africa has adapted to the tropical Americas, where it is found spontaneously germinating. Propagation can be done by cuttings or seeds and can be planted all year round and has adapted well to Brazilian climatic conditions. Unlike other species of basil, it is not frequently used for culinary purposes and is more often applied as a medicinal plant (Vieira et al., 2003) to treat asthma, fever, coughs, colds, bronchitis and indigestion (Agra et al., 2008; Chowdhury et al., 2008). The essential oils and extracts of this species present several biological activities, such as antioxidant (Hakkim et al., 2008), repellent (Seyoum et al., 2002), insecticide (Shadia et al., 2007) and antibacterial against *Propionibacterium acnes* (Viyoch et al., 2006).

Ocimum basilicum L. is an annual aromatic subshrub. The plant is very branched, ranging from 30 to 50 cm, aromatic underbrush, annual or perennial, depending on growing region. Propagation can be done by cuttings or seeds and can be planted all year round. It has simple leaves, membranous, opposite in shape and vary in size depending on the species, wavy edges and protruding ribs, from 4 to 7 cm long, its inflorescence is the espiciforme summit type, and its white flowers, pink or purplish, fruit achene type with small seeds, black and oblong (Couto, 2006; Lorenzi and Matos, 2008) and has adapted well to Brazilian climatic conditions and can be grown year round.

In traditional Indian medicine, *O. basilicum* is used as a sedative and for asthma and diabetes, as well as for cosmetic purposes (Lin and Kan, 1990). The Uyghurs use the species as a cardiotonic and antidiarrheal, and to relieve abdominal pain (Upur et al., 2004). *O. basilicum* is used by the pharmaceutical industry because of its spasmolytic, carminative, hepatoprotective and diuretic properties (Baritaux et al., 1992).

Scientific studies have shown that *O. basilicum* has antioxidant, antimicrobial, antifungal (Bozin et al., 2006), anticancer (Manosroi et al., 2006) and hypoglycemic activity (Vats et al., 2002). It also has the capacity to reduce platelet aggregation and thrombi in mice (Tohti et al., 2006).

Plant samples

Plants were cultivated from March to June 2010, in the Medicinal Plants Garden at the Federal University of Reconcavo of Bahia, Santo Antônio de Jesus, Bahia, Brazil, Latitude 12° 57′ 59.2”, Longitude 039° 15′ 49.4” LO. The herborization treatment was carried out according to Mori et al. (1989). The botanical material collected was deposited in the Herbarium of the State University of Feira de Santana, and it was identified by the taxonomist Teonildes Sacramento Nunes as *O. americanum* L. (Lamiaceae) - HUEFS 167947 and *O. basilicum* L.(Lamiaceae)- HUEFS 167950), according to the Cronquist system (1981).

Extraction of essential oils

Steam distillation was performed using a Clevenger-type apparatus for the extraction of essential oils from dry leaves and flowers. The chemical composition of the essential oil was determined by gas chromatography-mass spectrometry (GC/MS) in a Shimadzu GC-2010 gas chromatograph coupled to a GC/MS-QP 2010 Shimadzu mass spectrometer. The extraction process was carried out for 3 h and the components were identified by comparing the obtained mass spectra with the library of the equipment used, and by comparing the calculated Kovats indices with those found in the literature (Adams, 1995) using a homologous series of hydrocarbons. Linalool, E-methyl cinnamate and caryophyllene were purchased from Sigma-Aldrich Chemical Co. (St. Loius, MO). Yield of the essential oil (%) was calculated based on fresh and dry biomass in the moisture free basis (MFB) (Santos et al*.,* 2004) and the result was expressed in percentage.

Cell culture

Human primary osteoclasts (OCs) were obtained as described by Matsuzaki et al. (1999), with minor modifications. Briefly, peripheral blood (PB) was collected from healthy volunteers after informed consent was obtained. PB mononuclear cells (PBMC) were obtained from diluted peripheral blood (1:2 in Hanks solution), separated by Histopaque®-1077 (Sigma, St. Louis, MO, USA) and subsequently grown in DMEM High glucose (Euroclone SpA, Milan, Italy), in the presence of M-CSF (25 ng.ml^{-1}), RANKL (30 ng.ml^{-1}) at 37°C in a 5% CO_2 atmosphere for 14 days. To evaluate osteoclastogenesis, TRAP staining was carried out with Acid Phosphatase Leukocyte (TRAP) Kit no. 386 (Sigma, St. Louis, MO, USA), according to the manufacturer's protocol.

Cytotoxicity assay

Mature OCs were plated in 96-well plates and incubated for 3 days in the presence of essential oils (5, 50 and 500 µg.ml^{-1}), and the purified compounds linalool, methyl cinnammate and caryophylenne (Sigma, St. Louis, MO, USA), at 5, 50 and 300 µg.ml^{-1}. A 3% methanol/DMSO solution was used as a negative control, and to solubilize the compounds. Determinations of viable cells were performed after colorimetric assay with MTT (thiazolyl blue). The assay, based on the conversion of the yellow tetrazolium salt MTT (Sigma, St. Louis, MO, USA) to purple formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. After 72 h of treatments in triplicate, 25 µl of MTT was added to each well of cells, and the plate was incubated for 2 h at 37°C. The medium was removed, and the MTT crystals were solubilized with 50% dimethylformamide. Spectrophotometric absorbance of each sample was then measured at 570 nm (Sunrise absorbance reader, Tecan Group Ltd, Männedorf, Switzerland).

Apoptosis assay

At the end of appropriate days of treatment, cells were rinsed two times with PBS solution and fixed for 25 min in 4% paraformaldehyde at room temperature. Apoptotic cells were detected by the DeadEnd Colorimetric Apoptosis Detection System (Promega, Madison, USA) according to the manufacturer's instructions. Moreover, all cells were subjected to hematoxylin solution, showing blue stained nuclei. Cells were mounted in glycerol/PBS 9:1 and observed using a Leica microscope (Leica Microsystems GmbH, Wetzlar,Germany). Measurement of apoptosis was calculated as a percentage of apoptotic nuclei (dark brown nuclei) versus total nuclei of multinucleated TRAP positive cells, evaluated for three different experiments.

Immunocytochemistry assay

Immunocytochemistry analysis was performed using an ImPRESS Universal Reagent Kit (Vector Laboratories, Inc. Burlingame, CA, USA). OCs were seeded in 4-well chamber slides, fixed in cold 100% methanol and permeabilised with 0.2% (v/v) Triton X-100 (Sigma, St. Louis, MO, USA), in TBS (Tris-buffered saline). Cells were incubated in 0.3% H_2O_2 and the endogenous peroxidase was blocked with ready-to-use (2.5%) normal horse blocking serum (ImPRESS Reagent Kit, Vector Laboratories). After reaction with the primary antibodies (1:500 dilution), specific for the Fas receptor (C-20, rabbit anti-human), (Santa Cruz Biotechnology, Inc, Dallas, Texas, U.S.A) the material was incubated for 16 h at 4°C. After rinsing in TBS substrate–chromogen mix (ImmPACT DAB, Vector Laboratories), cells were then incubated for 30 min at room temperature with ImmPRESS reagent (ImPRESS Reagent Kit, Vector Laboratories). After washing, cells were mounted in glycerol/PBS 9:1 and observed using a Leica microscope (Leica Microsystems GmbH, Wetzlar,Germany).

Statistical analysis

The sample test for equal proportions without continuity correction data was carried out, using the program R version 2.10.1 (Copyright Foundation for Statistical Computing ISBN 3-900051-07-0). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Chromatographic profile of essential oils

The chromatographic profile of essential oils studied showed E-methyl cinnamate as the most concentrated component in *O. americanum* essential oils, constituting 45.5% of the leaf essential oil and 54.4% of the flower essential oil. The essential oil from leaves of *O. basilicum* contained 18% methyl eugenol, 12% caryophyllene, 11.2% 1,8-cineole, whereas the flower essential oil had 15.9% linalool, 13.4% 1,8-cineole and 12.5% caryophyllene (Tables 1 and 2).

The essential oil yield of *O. americanum* essential oils was 1.46% for leaves and 2.95% for flowers, and for *O. basilicum*, it was 1.81% for leaves and 1.77% for flowers.

Cytotoxicity

Compounds that allowed cell viability greater than or equal to 90% were considered non-toxic. Therefore, the following were found to be non-toxic:

1. Essential oil extracted from leaves and flowers of *O. americanum*, when lower than or equal to 50 µg.ml^{-1};
2. Essential oil extracted from leaves of *O. basilicum*, when lower than or equal to 50 µg.ml^{-1};
3. Essential oil extracted from flowers of *O. basilicum* at concentrations lower than or equal to 5 µg.ml^{-1};
4. Linalool at concentrations lower than or equal to 300 µg.ml^{-1};
5. Methyl cinnamate and caryophyllene, both with non-toxic concentrations lower than or equal to 5 µg.ml^{-1}.

Apoptotic activity in osteoclasts

Only non-toxic concentrations were used to assess the apoptotic activity. The results showed that linalool (50 to 300 µg.ml^{-1}) was the most active component ($p < 0.05$) and was able to induce 75% of apoptotic osteoclasts (Figure 1). The essential oils of *O. americanum* (50 µg.ml^{-1}) and *O. basilicum* (50 µg.ml^{-1}), as well as caryophyllene (5 µg.ml^{-1}) and methyl cinnamate (5 µg.ml^{-1}) were not able to induce apoptosis. Linalool was able to induce

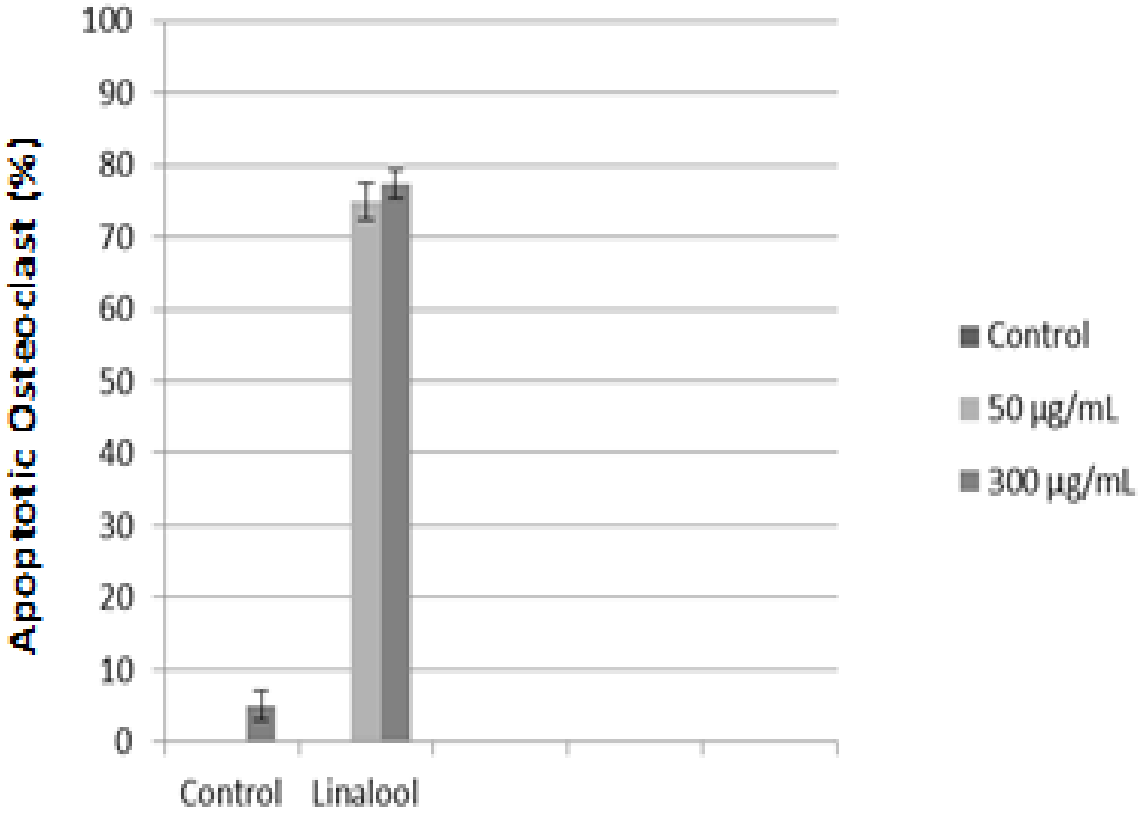


Figure 1. Linalool apoptotic activity at 50 and 300 µg.ml^{-1} on a human osteoclast culture. Percentage of apoptotic osteoclast in culture after 72 h of treatment.

Table 1. Chemical composition of essential oil extracted from *O. americanum* leaves and flowers.

Compound	KI_{lit}	KI_{calc}	Leaves (%)	Flowers (%)
α-Pinene	939	939	1.0	0.7
Sabinene	975	977	0.5	0.3
β-pinene	979	982	1.1	0.7
Myrcene	990	992	0.6	0.3
Limonene	1029	1033	1.2	0.6
1,8-Cineole	1031	1036	10.4	6.7
E-β-ocimene	1050	1051	1.6	1.3
Fenchone	1092	1086	1.2	0.5
Linalool	1096	1100	3.0	5.6
Camphor	1146	1150	2.1	2.0
Terpinen-4-ol	1177	1182	2.3	1.8
Methyl chavicol	1196	1200	9.7	6.8
Z-Methyl cinnamate	1299	1305	6.5	4.6
E-Methyl cinnamate	1378	1388	45.5	54.4
E-caryophyllene	1419	1427	0.9	1.1
Germacrene D	1485	1489	1.0	1.1
Total identified			88.6	88.5

KI, Kovats index; Ki_{lit}, Kovats index in literature; Ki_{calc}, calculated Kovats retention index.

Table 2. Chemical composition of essential oil extracted from *O. basilicum* leaves and flowers.

Compound	KI_{lit}	KI_{calc}	Flowers (%)	Leaves (%)
α-Pinene	939	939	0,9	0,5
Sabinene	975	977	0,5	0,4
β-pinene	979	982	1,8	1,3
Myrcene	990	992	0,6	0,5
Limonene	1029	1033	0,4	0,5
1,8-Cineole	1031	1036	13,4	11,2
Z-β-ocimene	1037	1044	3,1	5,1
Linalool	1096	1100	15,9	2,2
Eugenol	1359	1359	2,9	9,3
β-Elemeno	1390	1396	6,4	2,7
Methyleugenol	1403	1403	2,1	18,0
caryophyllene	1419	1427	12,5	12,0
α-humulene	1454	1461	3,5	2,6
β-selinene	1490	1492	1,7	2,2
Bicyclogermacrene	1500	1502	14,6	4,7
α-Bulneseno	1500	1502	2,8	4,1
Elimicina	1557	1558	2,1	14,6
Spathulenol	1578	1584	1,8	1,8
Total identified			87,0	93,7

KI, Kovats index; Ki_{lit}, Kovats index in literature; Ki_{calc}, calculated Kovats retention index.

either the expression of Fas receptor on osteoclasts.

DISCUSSION

In the present study, the effects of essential oils extracted from *O. americanum* and *O. basilicum* on osteoclast activity was investigated. By considering the lack of effects of essential oils of *Ocimum* species studied on OCs, the effects of purified components (linalool, caryophyllene and methyl cinnamate) on the induction of OCs apoptosis were included in the experimental plan.

Essential oils are complex mixtures of volatile constituents biosynthesized by plants and the interactions between these components may lead to antagonistic, additive or synergistic effects. Some studies have demonstrated that whole essential oils usually have higher activity than the mixtures of their major components, suggesting that the minor components are critical to the synergistic activity, though antagonistic effects have also been observed and this could explain the lack of effects of whole essential oils of *Ocimum* species towards OCs, in the present study. Antagonistic effect has been attributed to the interaction between non-oxygenated and oxygenated monoterpene hydrocarbons (Goñi et al., 2009). Linalool was considered the most active compound. The chromatographic profile of essential oils studied showed that linalool was presented in both, *O. americanum* and *O. basilicum* essential oils. According to Pandey et al. (2014), the major constituents which have been isolated from different *O. basilicum* oils include linalool, methyl chavicol, eugenol, methyl cinnamate, 1,8-cineole, bergamotene, limonene, camphor, geraniol. Oliveira et al. (2009) reported that in the Brazilian basil leaf essential oils, linalool, geraniol and 1,8-cineole are the major compounds corroborating with this study.

Linalool was able to induce apoptosis in osteoclasts and the apoptotic activity was confirmed by the increasing levels of Fas receptor on osteoclasts treated with this compound. Fas, also called APO-1 (CD95 molecules), plays a role in signal transduction in cellular apoptosis. Fas combines with Fas ligand (FasL), and then interacts with Fas-related death domain structure protein (FADD), to form the FasL-Fas-FADD death-inducing signaling complex (DISC), leading to pro-caspase-8 activation in the cytoplasm, resulting in eventual apoptosis (Zhang et al., 2016).

Understanding the mechanism of apoptosis can be applied in the development of drugs to control bone resorption by osteoclasts. Bone remodeling is a necessary process to maintain homeostasis of bone tissue; it is the result of a balanced activity between bone resorption by osteoclasts and bone apposition mediated

by osteoblasts. When there is an imbalance in favor of osteoclastic activity, the pathological resorption compromises the individual's health and problems, such as rheumatoid arthritis, osteoporosis and periodontal disease, can arise.

Moreover, it has been demonstrated that linalool can induce the cell cycle of U937 cells to arrest at the G0/G1 phase, while HeLa cells arrest at the G2/M phase, and its function facilitates the expression of p53, p21, p27, p16 and p18 (CDKIs) and the non-expression of CDK activity (Chang et al., 2015). Therefore, linalool can inhibit the cell cycle of leukemia cells and cervical cancer cells, and it could thus be used to arrest cell cycle of osteoclast and to develop novel therapeutic agents for periodontal disease.

Linalool-incorporated nanoparticles (LIN-NP) were used as novel anticancer agent against epithelial ovarian cancer (Han et al., 2016). The authors reported that LIN NPs had significant cytotoxicity and apoptotic activity against cancer cells and the treatment increased apoptosis through reactive oxygen species (ROS) generation and a subsequent decrease in mitochondrial membrane potential and increase in caspase-3 levels.

The apoptotic activity of linalool, against *Candida albicans*, was also reported by Khan et al. (2014). Linalool was able to induce chromatin condensation and margination, nuclear envelope separation, nuclear fragmentation, cytoplasmic shrinkage and plasma membrane blebbing in exposed cells. Although, the apoptotic activity of linalool against osteoclast was not found in literature, these findings reinforce the hypothesis that linalool has an apoptotic activity.

Another compound studied in this work was caryophyllene, which was considered toxic to human cells at concentrations above 5 $\mu g.ml^{-1}$. However, according to Molina-Jasso et al. (2009), caryophyllene is considered safe for use in industries and therapeutic purposes. Based on metabolism of sesquiterpenes, hydroperoxides are the product of caryophyllene metabolism, which are readily converted to caryophyllene oxide, a second compound that is little reactive and more stable (Sköld et al., 2006). This metabolic process, associated with the DNA repair system and detoxification in mice, could explain the absence of genotoxic effects *in vivo* studies by Molina-Jasso et al. (2009) and the divergence with the results found in this study, where tests were performed *in vitro*.

Regarding apoptotic activity, caryophyllene was not able to induce apoptosis in OCs. It is noteworthy that β-caryophyllene exhibited strong antibacterial effect and also displayed strong antioxidant effects (Dahham et al., 2015). Because there is a growing body of evidence suggesting oxidative stress playing a pivotal role in periodontal disease initiation and progression (Ramesh et al., 2016), antioxidant properties of caryophyllene may contribute to the development of novel preventive or therapeutic strategies for oral health.

In this study, the E-methyl cinnamate activity on osteoclasts was not considered effective, as it was toxic above 5 $\mu g.ml^{-1}$ and it was not able to induce apoptosis in osteoclasts. Schepetkin et al. (2015) reported that E-methyl cinnamate was not effective in modulating some innate responses like neuthophil migration and ROS production. *O. americanum* essential oil contained about 55% of methyl cinnamate in its composition; however neither the essential oil extracted from *O. americanum* nor methyl cinnamate alone were able to induce apoptosis in OCs. Taken together, these data do not support the candidacy of E-methyl cinnamate for the development of new drugs to control osteoclast activity. However, methyl cinnamate was found to show antifungal and antiaflatoxigenic efficacy at a low concentration (0.6 µl/ml) and the nature of its toxicity was fungicidal (Prakash et al., 2012).

There is little information in the literature on the biological activities of the studied plants in the treatment of periodontal disease, one of the most common pathological conditions of the oral cavity. This study demonstrated that *Ocimum* species studied have a potential biotechnological application in drug formulation for the treatment of periodontal disease, since linalool (50 to 300 $\mu g.ml^{-1}$) induced 75% of apoptotic osteoclasts. So, this study suggests that linalool could be used to control osteoclast activity and paves way for future research on the use of *O. americanum* and *O. basilicum* compounds for the control of osteoclast activity.

Conflicts of interests

The authors declare that they have no conflict of interest.

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REFERENCES

Adams RP (1995). Identification of essential oil components by Gas Chromatography/Mass Spectroscopy. Illinois: Allured Publishing Corporation.

Agra MF, Silva KN, Basílio IJLD, Freitas PFJ, Barbosa-Filho M (2008). Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn. 18:472-508.

Balvers MG, Brouwer-Brolsma EM, Endenburg S, de Groot LC, Kok FJ, Gunnewiek JK (2015). Recommended intakes of vitamin D to optimise health, associated circulating 25-hydroxyvitamin D concentrations, and dosing regimens to treat deficiency: workshop report and overview of current literature. J. Nutr. Sci. 4:23.

Baritaux O, Richard H, Touche J, Derbesy M (1992). Effects of drying and torage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour Fragr. J. 7:267-271.

Blank AF, Alves PB, Fontes SM, Santos MF, Dantas IB, Silva PA, MendoncËa MC, Arrigoni-Blank MF,Costa AG, Silva-Mann R. (2002). Efeito do horário de colheita e secagem no teor e na composição

química de óleo essencial de erva cidreira verdadeira (*Melissa officinalis* L.). Simpósio de plantas medicinais do Brasil, Cuiabá. Resumos. Cuiabá: UFMT.

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006). Characterization of the volatile composition of essential oils of some lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem. 54:1822-1828.

Carović-Stanko K, Liber K, Besendorfer V, Javornik B, Bohanec B, Kolak I, Satovic Z (2010). Genetic relations among basil taxa (*Ocimum* L.) based on molecular markers, nuclear DNA content, and chromosome number. Plant Syst. Evol. 285(1):13-22.

Chang MY, Shieh DE, Chen CC, Yeh CS, Dong HP (2015). Linalool Induces Cell Cycle Arrest and Apoptosis in Leukemia Cells and Cervical Cancer Cells through CDKIs. Int. J. Mol. Sci. 16:28169-28179.

Chowdhury Z, Alamgir ANM, Alauddin M, Islam MS, Chakma K, Hoque MR, Kabir GM (2008). Traditional knowledge related to medicinal and aromatic plants in tribal societies and the quantitative study of alkaloids in medicinal plants of the hill tracts in Bangladesh. Pharmacogn. Mag. 4:15.

Couto MEO (2006). Coleção de plantas medicinais aromáticas e condimentares. Pelotas. Embrapa Clima Temperado. 91p.

Cronquist A (1981). An integrated system of classification of flowering plants. New York: Columbia University Press.

Dahham TYM, Iqbal MA, Ahamed MBK, Ezzat MO, Majid ASA, Majid AMSA (2015). The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of *Aquilaria crassna* Saad S. Molecules 20:11808-11829.

Eguchi T, Koshy G, Umeda M, Iwanami T, Suga J, Nomura Y, Kawanami M, Ishikawa I (2008). Microbial changes in patients with acute periodontal abscess after treatment detected by PadoTest. Oral Dis. 14(2):180-184.

Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR (1998). Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J. Biol. Chem. 273:14363-143637.

Goñi P, López P, Sánchez C, Gómez-Lus R, Becerril R, Nerín C (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem.116:982-989.

Hakkim FL, Arivazhagan G, Boopathy R (2008). Antioxidant property of selected *Ocimum* species and their secondary metabolite content. J. Med. Plants Res. 2(9):250-257.

Han HD, Cho YJ, Cho SK, Byeon Y, Jeon HN, Kim HS, Kim BG, Bae DS, Lopez-Berestein G, Sood AK, Shin BC, Park YM, Lee JW (2016). Linalool-Incorporated Nanoparticles as a Novel Anticancer Agent for Epithelial Ovarian Carcinoma. Mol. Cancer Ther. 15(4):618-27.

Ishikawa I (2007). Host responses in periodontal diseases: A preview. Periodontology 2000(43):9-13.

Khan A, Ahmad A, Khan LA, Manzoor N (2014). *Ocimum sanctum* (L.) essential oil and its lead molecules induce apoptosis in Candida albicans. Res. Microbiol. 165(6):411-419.

Lin CC, Kan WS (1990). Medicinal plants used for the treatment of hepatitis in Taiwan. Am. J. Chin. Med. 18:35-43.

Lorenzi H, Matos FJA (2008). Plantas medicinais no Brasil: nativas e exóticas. São Paulo: Instituto Plantarum, 2008, 2° Edição. 544 p.

Lu SH, Chen TH, Chou TC (2015). Magnolol Inhibits RANKL-induced osteoclast differentiation of raw 264.7 macrophages through heme oxygenase-1-dependent inhibition of NFATc1 expression. J. Nat. Prod. 78(1):61-68.

Manosroi J, Dhumtanom P, Manosroi A (2006). Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett. 235:114-120.

Matsuzaki K, Katayama K, Takahashi Y, Nakamura I, Udagawa N, Tsurukai T, Nishinakamura R, Toyama Y, Yabe Y, Hori M, Takahashi N (1999). Human osteoclast-like cells are formed from peripheral blood mononuclear cells in a coculture with SaOS-2 cells transfected with the parathyroid hormone (PTH)/PTH-related protein receptor gene. Endocrinology 140(2):925-932.

Molina-Jasso D, Alvarez-González I, Madrigal-Bujaidar E (2009). Clastogenicity of beta-caryophyllene in mouse. Biol. Pharm. Bull. 32:520-522.

Mori SA, Silva LAM, Lisboa G, Coradin L (1989). Manual de manejo do herbaceo fanerogamico. CEPLAC-CEPEC.

Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh- Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A,Wagner EF, Penninger JM, Takayanagi H (2011). Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat. Med. 17:1231-1234.

Oliveira JS, Porto LA, Estevam CS, Siqueira RS, Alves PB, Niculau ES, Blank AF, Reinaldo N, Almeida RN, Marchioro M (2009). Phytochemical screening and anticonvulsant property of Ocimum basilicum leaf essential oil. Bol. Latinoam. Caribe Plantas Med. Aromát. 8:195-202.

Pandey AK, Singh P, Tripathi NN (2014). Chemistry and bioactivities of essential oils of some Ocimum species:An Overview. Asian Pac. J. Trop. Biomed. 4:682-694.

Park SN, Lim YK, Feire MO, Cho E, Jin D, Kook JK (2012). Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. Anaerobe 18:369-372.

Paton A (1992). A synopsis of Ocimum L. (Labiatae) in Africa. Kew Bull. 47(3):403-435.

Prakash B, Singh P, Mishra PK, Dubey NK (2012). Safety assessment of Zanthoxylum alatum Roxb. essential oil, its antifungal, antiaflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of *Piper nigrum* L. fruits. Int. J. Food Microbiol. 153(1-2):183-191.

Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz. J. Med. Biol. Res. 36:521-524.

Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S (2016). Herbs as an antioxidant arsenal for periodontal diseases. J. Intercult. Ethnopharmacol. 5(1).

Santos AS, Alves SM, Figueirêdo FJC, Rocha Neto OG (2004). Descrição de sistema e de métodos de extração de óleos essenciais e determinação de umidade de biomassa em laboratório. [Embrapa Amazônia Oriental. Comunicado Técnico, 99], Embrapa Amazônia Oriental, Belém. 6p.

Schepetkin IA, Kushnarenko SV, Özek G, Kirpotina LN, Utegenova GA, Kotukhov YA, Danilova AN, Özek T, K, Başer KHC, Quinn MT (2015). Inhibition of Human Neutrophil Responses by Essential Oil of *Artemisia kotuchovii* and Its Constituents. J. Agric. Food Chem. 63(20):4999-5007.

Seyoum A, Palisson K, Kunga SEW, Lwande WGF, Killeer Hassauali ABGJ (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. Trans. R. Soc. Trop. Med. Hyg. 96:225-231.

Shadia E, El-Aziz ABD, Omer EA, Sabra AS (2007). Chemical composition of *Ocimum americanum* essential oil and the biological effects against, *Agrotis ipsilon*, (Lepidoptera: Noctuidae). Res. J. Agric. Biol. Sci. 3:740-747.

Sköld M, Karlberg AT, Matura M, Börje A (2006). The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. Food Chem. Toxicol. 44:538-545.

Suppakul P, Miltz J, Sonneveld K, Bigger SW (2003). Antimicrobial properties of basil and its possible application in food packaging. J. Agric. Food Chem. 51:3197-3207.

Tohti I, Tursun M, Umar A, Turdi S, Imin H, Moore EN (2006). Aqueous extracts of *Ocimum basilicum* L. (sweet basil) decrease platelet aggregation induced by ADP and thrombin *in vitro* and rats arterio-venous shunt thrombosis *in vivo*. Thromb. Res. 118:733-739.

Ueda-Nakamura T, Mendonca Filho RR, Morgado-Diaz JA, Maza PK, Dias Filho BP, Cortez DAG (2006). Uighur traditional medicine syndrome of Abnormal Savda in men is associated with oxidative stress, which can be improved by Munziq and Mushil of Abnormal Savda. Therapie 59:483-484.

Upur H, Yusup A, Umar A, Moore N (2004). Uighur traditional medicine syndrome of Abnormal Savda in men is associated with oxidative stress, which can be improved by Munziq and Mushil of Abnormal Savda. Therapie. 59:483-484.

Vats V, Grover JK, Rathi SS (2002). Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum*

sanctum Linn. and *Pterocarpus marsupium* Linn. in normal and alloxanized diabetic rats. J. Ethnopharmacol. 79:95-100.
Vieira RF, Grayer RJ, Paton AJ (2003). Chemical profiling of *Ocimum americanum* using flavonoids. Phytochemistry 63:555-567.
Viyoch J, Pisutthanan N, Faikreua A, Nupangta K, Wangtorpol K, Ngokkuen J (2006). Evaluation of *in vitro* antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*. Int. J. Cosmet. Sci. 28:125-133.
Zhang YS, Qiang Shen Q, Jing Li J (2016). Traditional Chinese medicine targeting apoptotic mechanisms for esophageal cancer therapy. Acta Pharmacol. Sin. 37:295-302.