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Full Length Research Paper

Relaxant effect of carvacrol, citronellal and p-cymene, monoterpenes present in *Thymus* and *Cymbopogon* species, in guinea-pig trachea: A comparative study

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Respiratory diseases are responsible for significant morbidity and mortality in worldwide. One of most important respiratory disease is asthma, which the current treatment is based on use of β$_2$-agonists combined to inhaled corticosteroids. However, in recent years, many *in vitro* studies have attempted to validate the use of aromatic medicinal plants and its chemical compounds, such as monoterpenes, as potential therapeutically alternatives. In this work it was demonstrated that three different monoterpenes (carvacrol, citronellal and p-cymene) has relaxant effect in guinea-pig trachea, somehow showing different profile regarding epithelium presence, potency and efficacy.

Key words: Monoterpenes, carvacrol, p-cymene, citronellal, guinea-pig trachea, relaxant effect.

INTRODUCTION

Respiratory diseases are responsible for significant morbidity and mortality in worldwide. Among all respiratory diseases, asthma and chronic obstructive pulmonary disease (COPD) are of the most common in the world (Ambrosino and Paggiaro, 2012). According to World Health Organization (WHO), 300 million people suffer from asthma and this number could reach 400 million by 2025, accounting for 200,000 deaths per year (Meetoo, 2008). Asthma is the main airway chronic disease that causes substantial morbidity among affected individuals, it has no cure, but could be controlled by treatment (Schatz et al., 2006) which currently consists of combating its two main pathophysiology components, the hyper-responsiveness of airways smooth muscle by

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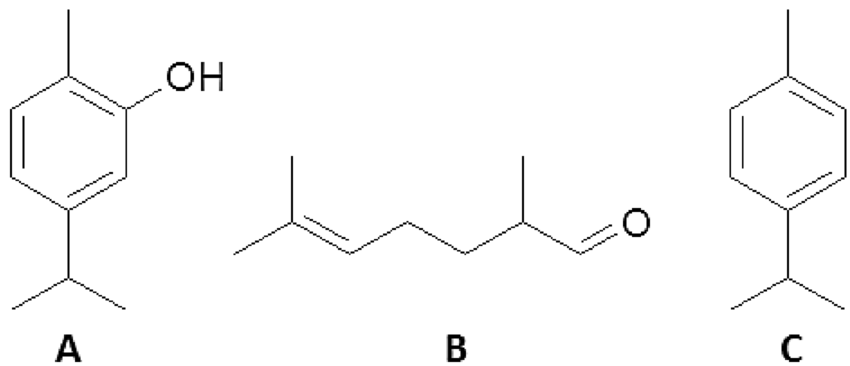

Figure 1. Chemical structures of (A) carvacrol, (B) citronellal and (C) p-cymene.

bronchodilators and lung inflammation by glucocorticoids (Sarr et al., 2012; Camoretti-Mercado, 2009). Asthma treatment guidelines advocate the use of β_2-agonists combined with inhaled corticosteroids (Miller-Larsson and Selroos, 2006). Although in recent years, many *in vitro* studies have attempted to validate the uses in traditional medicine of medicinal plants for treatment of diseases related to the respiratory system by demonstrating pharmacological effects of chemical constituents isolated from these plants (Sarret al., 2010).

Many medicinal plants such as *Eucalyptus citriodora*, *Eucalyptus globulus*, *Menthapiperita*, *Origanumsyriacum*, *Salvia fruticosa* and *Rosmarinus officinalis* have been described to act directly on the respiratory tract, the coughing reflex and the airflow (McKay and Blumberg, 2006, Rakover, et al., 2008). Something common to these species is the fact that they all produce essential oils which are composed of volatile aromatic compounds bio-synthetized by them according to the growth stage, local climate, season, geographical distribution and others (Richter and Schellenberg, 2007). There are several chemical compounds present in the essential oils, however, monoterpenes, isoprene-related compounds, are the major components of these oils and the pharmacological profiles of various medicinal plants have been attributed to them (Quintans-Júnior et al., 2010).

Some monoterpenes have been demonstrated to have effects on the airways, such as thymol, menthol and camphor, including effects on airway smooth muscle (Abanses et al., 2009; Begrow et al., 2010; Burrow et al., 1983; Kawasuji et al., 2005; Millqvist et al., 2012; Turletti, 1951). Some other monoterpenes as eucalyptol, gentiopricrin and carvacrol have direct effect upon smooth muscle contractility (Boskabady and Jandaghi, 2003; Nascimento et al., 2009; Rojas et al., 2000). However, several monoterpenes which could potentially have application in respiratory disorders have not been studied through the pharmacological approach using *in vitro* models. Thus, the present study aimed to investigate and compare the effects of three different monoterpenes, 2-methyl-5-(1-methylethyl) phenol (carvacrol), 7-dimethyl-6-octene-1-al (citronellal) and 4-isopropyl-1-methylbenzene (p-cymene) on airway smooth muscle, using experimental model of the isolated guinea pig trachea (Figure 1).

MATERIALS AND METHODS

Drugs

All the salts used in Krebs solution were purchased from VETEC QuímicaFina LTDA (Rio de Janeiro, BR). The monoterpenes carvacrol, citronellal and p-cymene, carbachol and arachidonic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animals

Guinea pigs of both sexes weighing between 350 to 500 g were used in this study. The animals were kept in the Animal Care Facilities of the Federal University of São Francisco Valley (UNIVASF) at a controlled temperature (22 ± 02°C) and 12/12 h light/dark cycle (lights on 06:00 to 18:00 h) in appropriate cages containing food and water *ad libitum*. The animals were killed by concussion followed by exsanguination by cutting the cervical vessels and their tracheas were removed. Experimental protocols and procedures were approved by the Ethics Committee on Animal Experiments by UNIVASF (CEUA/UNIVASF #27091051).

Protocols

After removing the adhering fat and connective tissue the trachea was cut into rings (lengths of 4 to 5 mm) and were suspended in 10 ml organ baths containing Krebs solution (in mM: NaCl 118, KCl 4.6, $KH_2PO_4.H_2O$ 1.1, $MgSO_4 .7H_2O$ 5.7, $CaCl_2.2H_2O$ 2.5, $NaHCO_3$ 25 and D-glucose 11) at 37 ± 0.1°C and bubbled with 95% O_2 and 5% CO_2 carbogenic mixture. The tracheal rings were preloaded under an isometric tension of 1 g and allowed to incubate for at least 60 min with changes of Krebs solution every 15 min. Isometric

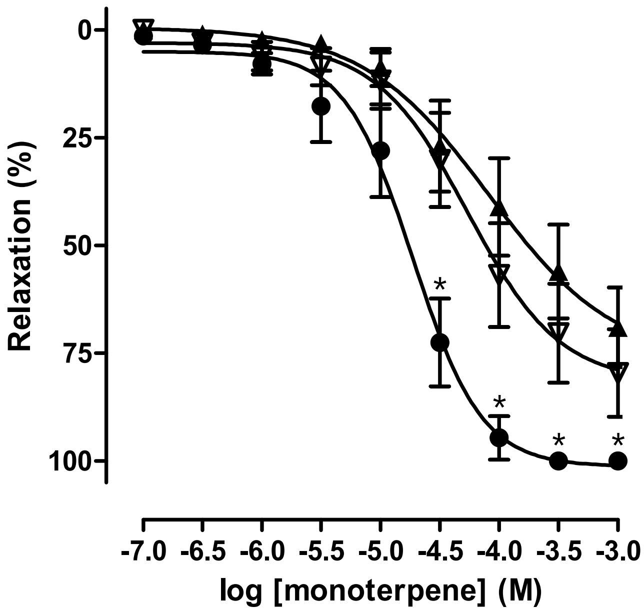

Figure 2. Relaxant effect of carvacrol (●), citronellal (▽) and p-cymene (▲) in tracheal rings with functional epithelium and pre-contracted by CCh (1 µM). The symbols represents means and vertical bars S.E.M, respectively. *p < 0.05 (unpaired Student's t test: carvacrol vs. citronellal/p-cymene).

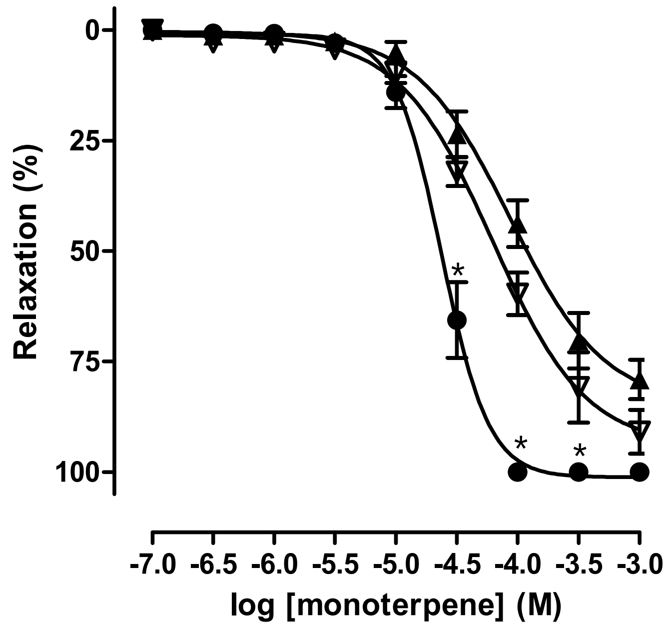

Figure 3. Relaxant effect of carvacrol (●), citronellal (▽) and p-cymene (▲) in tracheal rings without functional epithelium and pre-contracted by CCh (1 µM). The symbols represents means and vertical bars S.E.M, respectively. *p < 0.05 (unpaired Student's t test: carvacrol vs. citronellal/p-cymene).

contractions were monitored by force transducers TRO 015 (Panlab®, Barcelona, Spain) connected to an amplifier (Insight®, RibeirãoPreto, SP,Brazil) and data acquisition was performed through a PC running the software WinDaq (DATAQ Instruments®,Akron, OH, USA). After the resting period a first contraction was induced by carbachol (10^{-6} M). To evaluate the presence of epithelium when the contraction was stable (~10 min after carbachol addition) the arachidonic acid was added to organ bath (10^{-4} M). When the relaxation induced by arachidonic acid reached more than 50% of maximal effect, the tracheal ring was considered with epithelium. Rings without epithelium were obtained by causing friction carefully inside the tracheal tube and the absence of epithelium was confirmed by the absence of relaxation induced by arachidonic acid. Afterward, the organ bath was washed out and after 30 min a second contraction was induced by carbachol and as soon as it became stable (~10 min after carbachol addition), a cumulative concentration-response curve (10^{-7} to 10^{-3} M) was constructed for each monoterpene and its relaxant effect wereregistered.

Statistical analysis

The relaxing effect is represented by the inverse percentage of the contraction force obtained by adding carbachol. The relaxation was considered maximal (100%) when the base line reaches the resting tension level. Data in the graphs were expressed as mean ± standard error of mean (SEM). The half maximal effective concentration (EC_{50}) values were calculated through a non-linear regression equation from the values of relaxation obtained from each single dose-response curve for each monoterpene. Differences between means were considered statistically significant when *p* value calculated by unpaired Student's t test was lower than 0.05. Statistical analyzes were performed using the Graph-Pad Prism© 5.0 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The monoterpenes carvacrol, citronellal and p-cymene induced a relaxing effect in a concentration-dependent manner (range of 10^{-7} to 10^{-3} M) of the tracheal rings pre-contracted with carbachol (10^{-6} M) both in the presence (Figure 2) and in absence (Figure 3) of functional epithelium. The carvacrol had a greater efficacy both in the presence and absence of epithelium than the other monoterpenes, reaching an E_{max} of 100% of relaxing at 10^{-4} M while citronellal attained an E_{max} of 79.6 ± 10.2 and 90.2 ± 5.0% in the presence and absence of epithelium, respectively, and p-cymene an E_{max} of 69.0 ± 9.4 and 79.0 ± 4.4% in the presence and absence of epithelium, respectively. These isoprene compounds showed no statistical difference in their efficacy regarding the presence or absence of epithelium (Figures 2 and 3). The carvacrol was also the monoterpene among the three that has greatest potency to relax the guinea-pig trachea, showing an EC_{50} value of $2.1 \pm 0.6 \times 10^{-5}$ and $2.3 \pm 0.3 \times 10^{-5}$ M in both the presence and absence of epithelium, respectively, with no significant difference between these values. The other two monoterpenes showed no significant differences between their potential for being equipotent in relaxing the trachea. However, citronellal showed EC_{50} values of $1.4 \pm 0.6 \times 10^{-4}$ and $6.9 \pm 1.4 \times 10^{-5}$ M in the presence and absence of epithelium, respectively, with significant difference between these

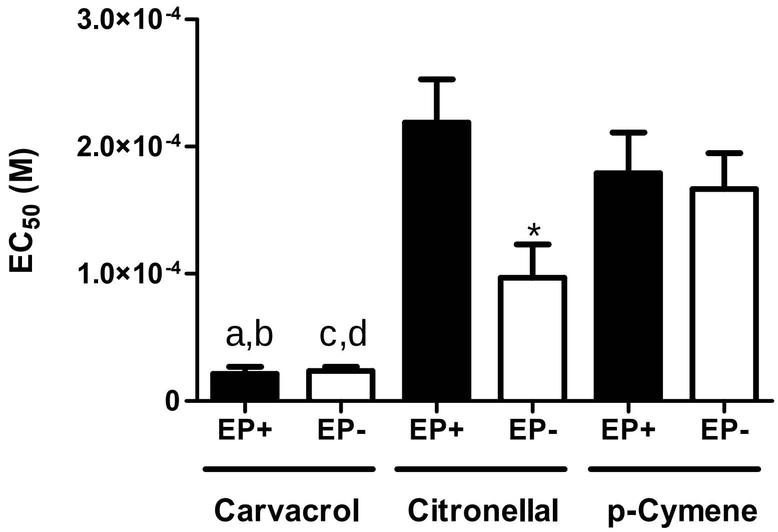

Figure 4. Comparison of EC_{50} values of carvacrol, citronellal and p-cymene in tracheal rings with (EP+) and without (EP-) functional epithelium and pre-contracted by CCh (1 µM). The columns represents means and vertical bars S.E.M, respectively. $^{a}p < 0.05$ (unpaired Student's t test: carvacrol EP+ *vs*. citronellal EP+); $^{b}p < 0.05$ (unpaired Student's t test: carvacrol EP+ *vs*. p-cymene EP+). $^{c}p < 0.05$ (unpaired Student's t test: carvacrol EP- *vs.* citronellal EP-). $^{d}p < 0.05$ (unpaired Student's t test: carvacrol EP- *vs*. p-cymene EP-); $^{*}p < 0.05$ (unpaired Student's t test: citronellal EP+ *vs*. citronellal EP-).

values while p-cymene presented EC_{50} values of $6.3 \pm 0.3 \times 10^{-4}$ and $1.7 \pm 0.3 \times 10^{-4}$ M with no difference between them (Figure 4).

DISCUSSION

Essential oils extracted from aromatic plants of many species have attracted interest from industry and researchers worldwide due to its fragrances as well as their applications in various fields of interest according to their biological activities (Lis-Balchin et al., 1997; Pauli and Knobloch, 1987; Perez-Alfonso et al., 2012). It is speculated that among the various chemical constituents present in these oils monoterpenes stand out as the main aromatic compounds and responsible for various biological activities (Bassole et al., 2003; Cimanga et al., 2002; Hadian et al., 2012; Martins et al., 1999).

Monoterpenes are mostly used in industry to mask odors (Osada et al., 2013), scents for all purposes, food additive (Yu et al., 2012), flavor adjuvant (Fukumoto et al., 2006; Heck, 2010), pharmaceutical adjuvants (Narishetty and Panchagnula, 2004) and as active ingredients in some ointments or topical creams used to relieve symptoms of chest congestion, cough and asthma (Abanses et al., 2009; Ando et al., 1993). Despite these applications, the monoterpenes have several biological activities but the microbial activity is highlighted for many of them, such as thymol (Perez-Alfonso et al., 2012), eugenol (Pei et al., 2009; Sleha et al., 2012; Veras et al., 2012), cinnamaldehyde (Du et al., 2012), alpha-pinene (Sartorelli et al., 2007), limonene (Di Pasqua et al., 2006), trans-cinnamaldehyde, perillaldehyde, citral, eugenol, citronellal (Sato et al., 2006; Sleha et al., 2012), p-cymene (Kisko and Roller, 2005; Rattanachaikunsopon and Phumkhachorn, 2010) and carvacrol (Didry et al., 1993).

Indeed carvacrol is one of the most cited antimicrobial monoterpene in the literature. Moreover carvacrol has activity against many microorganisms, such as *Schistosoma mansoni* (Ait-Ouazzou et al., 2013), *Campylobacter jejuni* (van Alphen et al., 2012), *Mycoplasma hominis* (Sleha et al., 2012), *Penicillium digitatum* and *P. Italicum* (Perez-Alfonso et al., 2012), *Staphylococcus aureus* and *Staphylococcus epidermidis* (Nostro et al., 2012), *Salmonella* ssp. (Inamuco et al., 2012; Luz Ida et al., 2012; Moschonas et al., 2012), *Escherichia coli* (Obaidat and Frank, 2009) and others (Nostro and Papalia, 2012). Furthermore, carvacrol has anticancer (Koparal and Zeytinoglu, 2003; Zeytinoglu et al., 2003), anti-hyperlipidemic (Aristatile et al., 2009), anti-inflammatory (Fachini-Queiroz et al., 2012; Lima et al., 2012), anti-ulcer (Silva et al., 2012), antinociceptive

(Cavalcante Melo et al., 2012; Guimaraes et al., 2010), antidepressant-like (Melo et al., 2011a), hypotensive (Aydin et al., 2007) and vasorelaxant activities (Peixoto-Neves et al., 2010).

Besides the antimicrobial activity some monoterpenes, such as citronellal, has anti-inflammatory (Melo et al., 2011b), antinoceptive (Melo et al., 2010; Quintans-Junior et al., 2010), larvicidal (Barros et al., 2009) and anticancer activities (Osato, 1965; Osato et al., 1961; Osato et al., 1954), while p-cymene modulates cytokine production (Zhong et al., 2012) has anti-inflammatory (Xie et al., 2012) and antinocceptive activity (Bonjardim et al., 2012).

Now, we demonstrate, through this work, that citronellal, p-cymene and carvacrol have relaxing effect on guinea-pig trachea and carvacrol has greater efficacy and potency among the three monoterpenes tested. Although there were already reports that carvacrol has relaxant effect in guinea-pig trachea (Boskabady and Jandaghi, 2003; Van den Broucke and Lemli, 1982) for the first time is reported relaxing effect for citronellal and p-cymene in that organ. In terms of potency, citronellal and p-cymene were equipotent in relaxing the trachea, but citronellal was more effective than p-cymene in doing so, showing a higher value of E_{max}.

Some studies suggest that carvacrol has a putative mechanism of action for some of its biological effects. Some of these studies claim that carvacrol has *in vitro* acetyl cholinesterase inhibitory properties (Jukic et al., 2007), an antagonist-like effect on muscarinic (Boskabady et al., 2011a) and histamine H_1 receptors (Boskabady et al., 2012), and an agonist-like effect on β_2-adrenoceptors (Boskabady et al., 2011b). Additionally, previous studies have described the antioxidant profile of carvacrol (Guimarães et al, 2010) and its action to modulate the release of cytokines, as IL-10 (Lima et al., 2013). Therefore, these activities may be acting synergistically to produce relaxant activity on the guinea pig trachea.

Moreover, we demonstrate that carvacrol induced a relaxing effect on tracheal rings pre-contracted with carbachol, a muscarinic agonist insensitive acetylc holinesterase. Thus, the fact that the supposedly carvacrol possess inhibitory effect on acetyl cholinesterase or an antagonistic effect on H_1 receptors does not explain its relaxing effect in our experimental conditions. However, an anti-muscarinic effect or an agonist effect on beta-adrenergic receptors could explain its mechanism of action regarding our data. However, the lack of information specifically about the relaxing effect of p-cymene and citronellal associated lack of studies about their possible biochemical pathways, which would serve to explain some of its biological effects, not allowing us to speculate much about the mechanism of action of the relaxing effect for these two monoterpenes. It is widely known that the epithelium is described as a layer of cells whose function goes well beyond the lining of the airways and in fact it is responsible for produce and release many mediators of smooth muscle contractile function (Advenier et al., 1988; Filep et al., 1993; Hay et al., 1988; Holroyde, 1986; Schlemper and Calixto, 1995) and its removal modifies the response to drugs that somehow stimulate the production of the so-called epithelium-dependent relaxant factors (Conroy et al., 1995; Daffonchio et al., 1990; Fine et al., 1989; Shikada and Tanaka, 1995).

Our results show that relaxant effect of carvacrol and p-cymene on tracheal rings do not depend of presence of the epithelium. This fact is evidenced by the absence of difference between the EC_{50} and E_{max} values for both monoterpenes when comparing its effects in the presence and absence of epithelium. However, although no significant differences in the E_{max} was evidenced for citronellal in tracheal rings; with epithelium this monoterpene was about two-fold less potent than in rings without epithelium, suggesting that the relaxing effect of citronellal is influenced by factors derived from the tracheal epithelium.

Conclusion

The carvacrol, p-cymene and citronellal have relaxant effect in smooth muscle of trachea. However, carvacrol and p-cymene have its effect irrespective of the tracheal epithelium while citronellal seems to depend of epithelium-derived relaxing factors to reach its effect. Moreover, the carvacrol was more effective and more potent in relaxing the trachea in this study.

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Conflict of interest

The authors report no declarations of interest.

REFERENCES

Abanses JC, Arima S, Rubin BK (2009). Vicks VapoRub induces mucin secretion, decreases ciliary beat frequency, and increases tracheal mucus transport in the ferret trachea. Chest 135:143-148.

Advenier C, Devillier P, Matran R, Naline E (1988). Influence of epithelium on the responsiveness of guinea-pig isolated trachea to adenosine. Brit. J. Pharmacol. 93:295-302.

Ait-Ouazzou A, Espina L, Gelaw TK, de Lamo-Castellvi S, Pagan R, Garcia-Gonzalo D (2013). New insights in mechanisms of bacterial inactivation by carvacrol. J. Appl. Microbiol. 114:173-185.

Ambrosino N, Paggiaro P (2012). The management of asthma and chronic obstructive pulmonary disease: current status and future perspectives. Expert Rev. Respir. Med. 6:117-127.

Ando H, Shimizu H, Takahashi Y, Fukumoto M, Okonogi H, Kadokura M (1993). A basic study for estimating the level of exposure to a nasal inhalant containing a stimulant. Nippon Eiseigaku Zasshi 48:692-697.

Aristatile B, Al-Numair KS, Veeramani C, Pugalendi KV (2009). Antihyperlipidemic effect of carvacrol on D-galactosamine-induced hepatotoxic rats. J. Basic Clin. Physiol. Pharmacol. 20:15-27.

Aydin Y, Kutlay O, Ari S, Duman S, Uzuner K, Aydin S (2007). Hypotensive effects of carvacrol on the blood pressure of normotensive rats. Planta Med. 73:1365-1371.

Barros LA, Yamanaka AR, Silva LE, Vanzeler ML, Braum DT, Bonaldo J (2009). In vitro larvicidal activity of geraniol and citronellal against Contracaecumsp (Nematoda: Anisakidae). Braz. J. Med. Biol. Res. 42:918-920.

Bassole IH, Ouattara AS, Nebie R, Ouattara CA, Kabore ZI, Traore SA (2003). Chemical composition and antibacterial activities of the essential oils of Lippiachevalieri and Lippiamultiflora from Burkina Faso. Phytochemistry 62:209-212.

Begrow F, Engelbertz J, Feistel B, Lehnfeld R, Bauer K, Verspohl EJ (2010). Impact of thymol in thyme extracts on their antispasmodic action and ciliary clearance. Planta Med. 76:311-318.

Bonjardim LR, Cunha ES, Guimaraes AG, Santana MF, Oliveira MG, Serafini MR, Araujo AA, Antoniolli AR, Cavalcanti SC, Santos MR, Quintans-Junior LJ (2012). Evaluation of the anti-inflammatory and antinociceptive properties of p-cymene in mice.Z. Naturforsch C. 67:15-21.

Boskabady MH, Jafari Z, Pouraboli I (2011a). The effect of carvacrol on muscarinic receptors of guinea-pig tracheal chains. Phytother. Res. 25:530-535.

Boskabady MH, Jandaghi P (2003). Relaxant effects of carvacrol on guinea pig tracheal chains and its possible mechanisms. Die Pharm. 58:661-663.

Boskabady MH, Kaveh M, Eftekhar N, Nemati A (2011b). ZatariamultifloraBoiss and Carvacrol Affect beta(2)-Adrenoceptors of Guinea Pig Trachea. Evid-Based Complement. Alt. 2011: 857124.

Boskabady MH, Tabanfar H, Gholamnezhad Z, Sadeghnia HR (2012). Inhibitory effect of ZatariamultifloraBoiss and carvacrol on histamine (H(1)) receptors of guinea-pig tracheal chains. Fundam. Clin. Pharm. 26:609-620.

Burrow A, Eccles R, Jones AS (1983). The effects of camphor, eucalyptus and menthol vapour on nasal resistance to airflow and nasal sensation. Acta Oto-Laryngol. 96:157-161.

Cavalcante MFH, Rios ER, Rocha NF, Cito Mdo C, Fernandes ML, de Sousa DP, de Vasconcelos SM, de Sousa FC (2012). Antinociceptive activity of carvacrol (5-isopropyl-2-methylphenol) in mice. J. Pharm. Pharmacol. 64:1722-1729.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totte J, Pieters L, Vlietinck AJ (2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharmacol. 79:213-220.

Conroy DM, St-Pierre S, Sirois P (1995). Relaxant effects of pituitary adenylatecyclase activating polypeptide (PACAP) on epithelium-intact and -denuded guinea-pig trachea: a comparison with vasoactive intestinal peptide (VIP). Neuropeptides 29:121-127.

Daffonchio L, Hernandez A, Martinotti E, Omini C (1990). Beta-adrenoceptor reactivity after epithelium removal in guinea-pig trachea *in vitro*. Pharmacol. Res. 22:49-58.

Di Pasqua R, Hoskins N, Betts G, Mauriello G (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J. Agric. Food Chem. 54:2745-2749.

Didry N, Dubreuil L, Pinkas M (1993). Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. Die Pharmazie 48:301-304.

Du WX, Avena-Bustillos RJ, Woods R, Breksa AP, McHugh TH, Friedman M, Levin CE, Mandrell R (2012). Sensory evaluation of baked chicken wrapped with antimicrobial apple and tomato edible films formulated with cinnamaldehyde and carvacrol. J. Agric. Food Chem. 60:7799-7804.

Fachini-Queiroz FC, Kummer R, Estevao-Silva CF, Carvalho MD, Cunha JM, Grespan R, Bersani-Amado CA, Cuman RK (2012). Effects of Thymol and Carvacrol, Constituents of Thymus vulgaris L. Essential Oil, on the Inflammatory Response. Evid-Based Complement Alt. 2012:657026.

Filep JG, Battistini B, Sirois P (1993). Induction by endothelin-1 of epithelium-dependent relaxation of guinea-pig trachea in vitro: role for nitric oxide. Brit. J. Pharmacol. 109:637-644.

Fine JM, Gordon T, Sheppard D (1989). Epithelium removal alters responsiveness of guinea pig trachea to substance P. J. Appl. Physiol. 66:232-237.

Fukumoto S, Sawasaki E, Okuyama S, Miyake Y, Yokogoshi H (2006). Flavor components of monoterpenes in citrus essential oils enhance the release of monoamines from rat brain slices. Nutr. Neurosci. 9:73-80.

Guimaraes AG, Oliveira GF, Melo MS, Cavalcanti SC, Antoniolli AR, Bonjardim LR, Silva FA, Santos JP, Rocha RF, Moreira JC, Araujo AA, Gelain DP, Quintans-Junior LJ (2010). Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic Clin. Pharmacol. 107:949-957.

Hadian J, Akramian M, Heydari H, Mumivand H, Asghari B (2012). Composition and in vitro antibacterial activity of essential oils from four Satureja species growing in Iran.Nat. Prod. Res. 98-108.

Hay DW, Muccitelli RM, Horstemeyer DL, Raeburn D (1988). Is the epithelium-derived inhibitory factor in guinea-pig trachea a prostanoid? Prostaglandins 35:625-637.

Heck JD (2010). A review and assessment of menthol employed as a cigarette flavoring ingredient. Food Chem. Toxicol. 48(2):S1-38.

Holroyde MC (1986). The influence of epithelium on the responsiveness of guinea-pig isolated trachea. Brit. J. Pharmacol. 87:501-507.

Inamuco J, Veenendaal AK, Burt SA, Post JA, Tjeerdsma-van Bokhoven JL, Haagsman HP, Veldhuizen EJ (2012). Sub-lethal levels of carvacrol reduce Salmonella Typhimurium motility and invasion of porcine epithelial cells. Vet. Microbiol. 157:200-207.

Jukic M, Politeo O, Maksimovic M, Milos M (2007). *In vitro* acetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone.Phytother. Res. 21: 259-261.

Kawasuji T, Matsubara T, Suzuki H (2005). Effects of terpene alcohols, bisabolol and menthol, on the tracheal smooth muscle. J. Pharmacol. Sci. 97:226P-226P.

Kisko G, Roller S (2005). Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple juice. BMC Microbiol. 5:36.

Koparal AT, Zeytinoglu M (2003). Effects of Carvacrol on a Human Non-Small Cell Lung Cancer (NSCLC) Cell Line, A549. Cytotechnology 43:149-154.

Lima MD, Quintans-Junior LJ, de Santana WA, Martins Kaneto C, Pereira Soares MB, Villarreal CF (2012). Anti-inflammatory effects of carvacrol: Evidence for a key role of interleukin-10. Eur. J. Pharmacol. 699(1-3):112-117

Lis-Balchin M, Deans S, Hart S (1997).A study of the changes in the bioactivity of essential oils used singly and as mixtures in aromatherapy. J. Altern. Complement. Med. 3:249-256.

Luz Ida S, Gomes Neto NJ, Tavares AG, Nunes PC, Magnani M, de Souza EL (2012). Evidence for lack of acquisition of tolerance in Salmonella enteric serovar Typhimurium ATCC 14028 after exposure to subinhibitory amounts of *Origanum vulgare* L. essential oil and carvacrol. Appl. Environ. Microb. 78:5021-5024.

Martins AP, Salgueiro LR, Vila R, Tomi F, Canigueral S, Casanova J, da Cunha AP, Adzet T (1999). Composition of the essential oils of

Ocimum canum, *O. gratissimum* and O. minimum. Planta Med. 65:187-189.
McKay DL, Blumberg JB (2006). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytother. Res. 20:619-633.
Melo FH, Moura BA, de Sousa DP, de Vasconcelos SM, Macedo DS, Fonteles MM, Viana GS, de Sousa FC (2011a). Antidepressant-like effect of carvacrol (5-Isopropyl-2-methylphenol) in mice: involvement of dopaminergic system. Fundam. Clin. Pharm. 25:362-367.
Melo MS, Guimaraes AG, Santana MF, Siqueira RS, De Lima Ado C, Dias AS, Santos MR, Onofre AS, Quintans JS, De Sousa DP, Almeida JR, Estevam CS, Araujo BS, Quintans-Junior LJ (2011b). Anti-inflammatory and redox-protective activities of citronellal. Biol. Res. 44: 363-368.
Melo MS, Sena LC, Barreto FJ, Bonjardim LR, Almeida JR, Lima JT, De Sousa DP, Quintans-Junior LJ (2010). Antinociceptive effect of citronellal in mice. Pharm. Biol. 48: 411-416.
Miller-Larsson A, Selroos O (2006). Advances in asthma and COPD treatment: combination therapy with inhaled corticosteroids and long-acting beta 2-agonists. Curr. Pharm. Des. 12:3261-3279.
Millqvist E, Ternesten-Hasseus E, Bende M (2012). Inhalation of menthol reduces capsaicin cough sensitivity and influences inspiratory flows in chronic cough. Resp. Med. 107(3): 433-438.
Moschonas G, Geornaras I, Stopforth JD, Wach D, Woerner DR, Belk KE, Smith GC, Sofos JN (2012). Activity of caprylic acid, carvacrol, epsilon-polylysine and their combinations against Salmonella in not-ready-to-eat surface-browned, frozen, breaded chicken products. J. Food. Sci. 77: M405-411.
Narishetty ST, Panchagnula R (2004). Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J. Control. Release 95: 367-379.
Nascimento NR, Refosco RM, Vasconcelos EC, Kerntopf MR, Santos CF, Batista FJ, De Sousa CM, Fonteles MC (2009). 1,8-Cineole induces relaxation in rat and guinea-pig airway smooth muscle. J Pharm Pharmacol. 61: 361-366.
Nostro A, Cellini L, Zimbalatti V, Blanco AR, Marino A, Pizzimenti F, Giulio MD, Bisignano G (2012). Enhanced activity of carvacrol against biofilm of *Staphylococcus aureus* and *Staphylococcus epidermidis* in an acidic environment. Acta Pathol. Microbiol. Immunol. Scand. 120:967-973.
Nostro A, Papalia T (2012). Antimicrobial activity of carvacrol: current progress and future prospectives. Recent Patents on Anti-Infective Drug Discov. 7:28-35.
Obaidat MM, Frank JF (2009). Inactivation of Salmonella and *Escherichia coli* O157:H7 on sliced and whole tomatoes by allylisothiocyanate, carvacrol, and cinnamaldehyde in vapor phase. J. Food Protect. 72:315-324.
Osada K, Hanawa M, Tsunoda K, Izumi H (2013). Evaluation of the Masking of Dimethyl Sulfide Odors by Citronella, Limonene and Citral through the Use of Trained Odor Sensor Mice. Chem. Senses 38:57-65.
Osato S (1965). Chemotherapy of human carcinoma with citronellal and citral and their action on carcinoma tissue in its histological aspects up to healing. Tohoku J. Exp. Med. 86:102-147.
Osato S, Mori H, Morita M (1961). Experiments on the chemotherapeutic treatment of carcinoma with citral and citronellal and those combined with PCMB. Tohoku J. Exp. Med. 75:223-233.
Osato S, Oda K, Sato F (1954). Direct action of citral and citronellal on cancer cells. Cr. Soc. Biol. 148:768-769.
Pauli A, Knobloch K (1987). Inhibitory effects of essential oil components on growth of food-contaminating fungi. Z. Lebensm. Unters. For. 185:10-13.
Pei RS, Zhou F, Ji BP, Xu J (2009). Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against E. coli with an improved method. J. Food Sci. 74:M379-383.
Peixoto-Neves D, Silva-Alves KS, Gomes MD, Lima FC, Lahlou S, Magalhaes PJ, Ceccatto VM, Coelho-de-Souza AN, Leal-Cardoso JH (2010). Vasorelaxant effects of the monoterpenic phenol isomers, carvacrol and thymol, on rat isolated aorta. Fundam. Clin. Pharm. 24:341-350.
Perez-Alfonso CO, Martinez-Romero D, Zapata PJ, Serrano M, Valero D, Castillo S (2012). The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay. Int. J. Food Microbiol. 158:101-106.
Quintans-Junior LJ, Melo MS, De Sousa DP, Araujo AA, Onofre AC, Gelain DP, Goncalves JC, Araujo DA, Almeida JR, Bonjardim LR (2010). Antinociceptive effects of citronellal in formalin-, capsaicin-, and glutamate-induced orofacial nociception in rodents and its action on nerve excitability. J. Orofac. Pain 24:305-312.
Rakover Y, Ben-Arye E, Goldstein LH (2008). The treatment of respiratory ailments with essential oils of some aromatic medicinal plants. Harefuah. 147:783-788, 838.
Rattanachaikunsopon P, Phumkhachorn P (2010). Synergistic antimicrobial effect of nisin and p-cymene on Salmonella Enterica serovar Typhi in vitro and on ready-to-eat food. Biosci. Biotech. Biochem. 74:520-524.
Richter J, Schellenberg I (2007). Comparison of different extraction methods for the determination of essential oils and related compounds from aromatic plants and optimization of solid-phase microextraction/gas chromatography. Anal. Bioanal. Chem. 387:2207-2217.
Rojas A, Bah M, Rojas JI, Gutierrez DM (2000). Smooth muscle relaxing activity of gentiopicroside isolated from *Gentiana spathacea*. Planta Med. 66:765-767.
Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima ME, Moreno PR (2007). Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. Phytother. Res. 21:231-233.
Sato K, Krist S, Buchbauer G (2006). Antimicrobial effect of trans-cinnamaldehyde, (-)-perillaldehyde, (-)-citronellal, citral, eugenol and carvacrol on airborne microbes using an airwasher. Biol. Pharm. Bull. 29:2292-2294.
Schlemper V, Calixto JB (1995). Mechanisms involved in the relaxant response of bradykinin in epithelium intact strips of the guinea-pig trachea. Eur. J. Pharmacol. 282:177-184.
Shikada K, Tanaka S (1995). K+ channel openers produce epithelium-dependent relaxation of the guinea-pig trachea. Eur. J. Pharmacol. 282:193-197.
Silva FV, Guimaraes AG, Silva ER, Sousa-Neto BP, Machado FD, Quintans-Junior LJ, Arcanjo DD, Oliveira FA, Oliveira RC (2012). Anti-inflammatory and anti-ulcer activities of carvacrol, a monoterpene present in the essential oil of oregano. J. Med. Food 15:984-991.
Sleha R, Mosio P, Vydrzalova M, Jantovska A, Bostikova V, Mazurova J (2012). In vitro antimicrobial activities of cinnamon bark oil, anethole, carvacrol, eugenol and guaiazulene against Mycoplasma hominis clinical isolates. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 156:1-4.
Turletti TA (1951). Thymol in diseases of the respiratory tract. Rass Int. Clin. Ter. 31:451-455.
van Alphen LB, Burt SA, Veenendaal AK, Bleumink-Pluym NM, van Putten JP (2012). The natural antimicrobial carvacrol inhibits Campylobacter jejuni motility and infection of epithelial cells. PloS one 7(9):1-7.
Van den Broucke CO, Lemli JA (1982). Antispasmodic activity of Origanumcompactum. Part 2: Antagonistic effect of thymol and carvacrol. Planta Med. 45:188-190.
Veras HN, Rodrigues FF, Colares AV, Menezes IR, Coutinho HD, Botelho MA, Costa JG (2012). Synergistic antibiotic activity of volatile compounds from the essential oil of Lippiasidoides and thymol. Phytotherapy 83:508-512.
Xie G, Chen N, Soromou LW, Liu F, Xiong Y, Wu Q, Li H, Feng H, Liu G (2012). p-Cymene protects mice against lipopolysaccharide-induced acute lung injury by inhibiting inflammatory cell activation. Molecules 17:8159-8173.

Yu H, Zhang ZL, Chen J, Pei A, Hua F, Qian X, He J, Liu CF, Xu X (2012). Carvacrol, a food-additive, provides neuroprotection on focal cerebral ischemia/reperfusion injury in mice. PloS one 7(3):1-8.

Zeytinoglu H, Incesu Z, Baser KH (2003). Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-RAS oncogene. Phytomedicine 10:292-299.

Zhong W, Chi G, Jiang L, Soromou LW, Chen N, Huo M, Guo W, Deng X, Feng H (2012). P-Cymene Modulates *In Vitro* and *In Vivo* Cytokine Production by Inhibiting MAPK and NF-kappaB Activation. Inflammation 36(3):529-537.