Accepted Manuscript

Essential oils and its bioactive compounds modulating cytokines: A systematic review on anti-asthmatic and immunomodulatory properties

Gopalsamy Rajiv Gandhi , Alan Bruno Silva Vasconcelos , Govindasamy Hari Haran , Valdete Kaliane da Silva Calisto , Gnanasekaran Jothi , Jullyana de Souza Siqueira Quintans , Luis Eduardo Cuevas , Narendra Narain , Lucindo José Quintans Júnior , Rosana Cipolotti , Ricardo Queiroz Gurgel

PII: S0944-7113(19)30025-X
DOI: https://doi.org/10.1016/j.phymed.2019.152854
Article Number: 152854
Reference: PHYMED 152854

To appear in: *Phytomedicine*

Received date: 8 December 2018
Revised date: 28 January 2019
Accepted date: 29 January 2019

Please cite this article as: Gopalsamy Rajiv Gandhi , Alan Bruno Silva Vasconcelos , Govindasamy Hari Haran , Valdete Kaliane da Silva Calisto , Gnanasekaran Jothi , Jullyana de Souza Siqueira Quintans , Luis Eduardo Cuevas , Narendra Narain , Lucindo José Quintans Júnior , Rosana Cipolotti , Ricardo Queiroz Gurgel , Essential oils and its bioactive compounds modulating cytokines: A systematic review on anti-asthmatic and immunomodulatory properties, *Phytomedicine* (2019), doi: https://doi.org/10.1016/j.phymed.2019.152854

Essential oils and its bioactive compounds modulating cytokines: A systematic review on anti-asthmatic and immunomodulatory properties

Gopalsamy Rajiv Gandhi [a, b], Alan Bruno Silva Vasconcelos [c], Govindasamy Hari Haran [d], Valdete Kaliane da Silva Calisto [a], Gnanasekaran Jothi [d], Jullyana de Souza Siqueira Quintans [b], Luis Eduardo Cuevas [e], Narendra Narain [f], Lucindo José Quintans Júnior [b], Rosana Cipolotti [a], Ricardo Queiroz Gurgel [a, *]

[a] *Division of Paediatrics, Department of Medicine, Federal University of Sergipe, Rua Cláudio Batista, s/n, Cidade Nova, Aracaju, 49.100-000 Sergipe, Brazil*

[b] *Laboratory of Neuroscience and Pharmacological Assays (LANEF), Department of Physiology, Federal University of Sergipe, São Cristóvão, 49.100-000 Sergipe, Brazil*

[c] *Department of Physiology, Federal University of Sergipe, São Cristóvão, 49.100-000 Sergipe, Brazil*

[d] *Department of Biochemistry, Srimad Andavan Arts and Science College (Autonomous), Tiruchirappalli, 620005 Tamil Nadu, India*

[e] *Liverpool School of Tropical Medicine, Pembroke Place Liverpool, Liverpool, UK*

[f] *Laboratory of Flavor and Chromatographic Analysis, Federal University of Sergipe, São Cristóvão, Aracaju, Sergipe 49.100-000, Brazil*

*Corresponding author: Prof. Dr. Ricardo Queiroz Gurgel, Associate Professor, Division of Paediatrics, Department of Medicine, Federal University of Sergipe, Aracaju, 49.100-000 Sergipe, Brazil. Phone: +55-79-99770480; Fax: +55-79-3212-6640; *E-mail*: ricardoqgurgel@gmail.com.

Table of contents

Abstract

Background: Asthma, the main inflammatory chronic condition affecting the respiratory system, is characterized by hyperresponsiveness and reversible airway obstruction, recruitment of inflammatory cells and excessive production of mucus. Cytokines as biochemical messengers of immune cells, play an important role in the regulation of allergic inflammatory and infectious airway processes. Essential oils of plant origin are complex mixtures of volatile and semi volatile organic compounds that determine the specific aroma of plants and are categorized by their biological activities.

Purpose: We reviewed whether essential oils and their bioactive compounds of plant origin could modulate cytokines' immune responses and improve asthma therapy in experimental systems *in vitro* and *in vivo*.

Methods: Electronic and manual search of articles in English available from inception up to November 2018 reporting the immunomodulatory activity of essential oils and their bioactive compounds for the management of asthma. We used PubMed, EMBASE, Scopus and Web of Science. Publications reporting preclinical experiments where cytokines were examined to evaluate the consequence of anti-asthmatic therapy were included.

Results: 914 publications were identified and 13 were included in the systematic review. Four articles described the role of essential oils and their bioactive compounds on bronchial asthma using cell lines; nine *in vivo* studies evaluated the anti-inflammatory efficacy and immunomodulating effects of essential oil and their secondary metabolites on cytokines production and inflammatory responses. The most important immunopharmacological mechanisms reported were the regulation of cytokine production, inhibition of reactive oxygen species accumulation, inactivation of eosinophil migration and remodeling of the airways and lung tissue, modulation of FOXP3 gene expression, regulation of inflammatory cells in the airways and decreasing inflammatory mediator expression levels.

Conclusion: Plant derived essential oils and related active compounds have potential therapeutic activity for the treatment of asthma by modulating the release of pro-inflammatory (TNF-α, IL-1β, IL-8), Th17 (IL-17), anti-inflammatory (IL-10), Th1 (IFN-γ, IL-2, IL-12) and Th2 (IL-4, IL-5, IL-6, IL-13) cytokines and the suppression of inflammatory cell accumulation.

Keywords: Asthma, Allergy, Cytokines, Essential oil, Inflammation, Immunomodulatory

Abbreviations: BALF, Bronchoalveolar Lavage Fluid; COPD, Chronic Obstructive Pulmonary Disease; DEP, Diesel Exhaust Particles; ELISA, Enzyme-Linked Immunosorbent Assay; OVA, Ovalbumin; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; qPCR, Quantitative Real- time Polymerase Chain Reaction; ROS, Reactive Oxygen Species;

Introduction

Asthma is a chronic inflammation of the airways characterized by the infiltration of inflammatory cells, inflammation, hyperresponsiveness and remodeling **(**Murdoch and Lloyd, 2010) that can be induced or exacerbated by exposure to environmental triggers (Hardy et al., 2015). Its symptoms include excessive production of mucus leading to airway obstruction (Doeing and Solway, 2013) and is often accompanied by infiltration of the airways by eosinophils, mast cells and lymphocytes, thickening of the bronchial walls, and hypertrophy/hyperplasia of airway smooth muscle (Cheng et al., 2018).

The prevalence of asthma has increased in recent decades and is currently one of the most common causes of respiratory morbidity in the world (Nunes et al., 2017), affecting individuals across the age spectrum (Tang et al., 2018). It is estimated that more than 300 million people worldwide have asthma (Nunes et al., 2017). Inflammation of the airways in asthma is associated with stimulation of T helper (Th) 2 cell-derived immune responses and production of Interleukins (IL)-4, 5, and 13 (Ku et al., 2016) and other cytokines, such as IL-1 and 33, tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β), play a pivotal role in the pathophysiology of allergic reactions (Verheijden et al., 2016; Tettamanti et al., 2018). Recent experimental studies have shown that maintaining the Th1/Th2 immune balance could protect against asthma exacerbations (Rao et al., 2017) and several trials have aimed to enhance the inhibition of Th2 cell-derived cytokines such as IL-4 and 5 (Rivera et al., 2011).

Herbal remedies are a popular form of complementary or alternative medicine for asthma and nearly 40% of asthmatics have used herbal remedies (Ernst, 1999). Essential oils extracted from plants are mixtures of volatile compounds, mainly mono- and sesquiterpenoids, phenylpropanoids containing hundreds of bioactive chemical constituents. These oils have antifungal, acaricidal, antiviral and bactericidal properties and could have potential roles for the management asthma (Pina et al., 2018). Thanks to their volatility these oils can easily reach the upper and lower respiratory tract (Levy et al., 2018), where they could reduce IgE, IL-4, 5 and 13 levels and inflammatory cells **(**Horváth and Kamilla, 2015)**.** This review examines *in vitro* and *in vivo* studies reporting the effect of essential oils and their bioactive compounds in anti-asthmatic activity, highlighting the specific cytokines immunomodulated.

Materials and methods

The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

Search strategy

Peer reviewed publications in English were extracted from the PubMed, EMBASE, Scopus and Web of Science databases, restricted to Medical Subjects Headings Index (MeSH/DeCS) up to November 2018. The search used the keywords: "Essential oil", "Medicinal Plants", "Bio-active Compounds", “Cytokines", "Asthma", "Anti-asthmatic Effect", "Natural Products", "Inflammation", "Immunomodulatory" and "Immune Response". In addition, we reviewed the references listed in the articles selected to identify additional reports not included in the databases.

Study selection

Two authors (GRG and ABSV) extracted and checked the titles and abstracts of the articles independently. Studies investigating the anti-asthmatic action of essential oils and bioactive compounds in preclinical models and discussing possible mechanisms of action through specific cytokine-mediated signaling pathways were included. We excluded human studies, review articles, meta-analyses, book chapters, conference abstracts, editorials/letters, patents and case reports. Disagreements among the two authors were resolved by a third author (RQG) through discussion.

Data extraction

Data were extracted and summarized in tables. Table 1 summarizes data of *in vitro* studies, including a) author's name and publication year b) substances and their concentrations c) cytokines assessed d) components identified e) cell lines or strains used f) proposed mechanisms of biochemical results. Table 2 provides data on experimental studies, including a) author's name and year b) experiment design c) substances and concentrations d) dose/route of administration/animal model e) outcomes f) biochemical mechanisms and results.

Methodological quality and risk of bias

The methodological quality of the studies was assessed using standard checklists and mandatory statements of random allocation and concealment of treatment, compliance with welfare regulations, blinding of drug administration, evaluation of outcomes, depiction of animal losses and comprehensiveness of outcome data (Hooijmans et al., 2014). The quality analysis was depicted using colors as suggested by Roskosk-Jr (2017).

Data analysis

The data is presented as a narrative. Pooling statistics and meta-analysis were not used due to the heterogeneity of the studies.

Results and discussion

Search results

Figure 1 presents a flowchart of the search. Nine hundred and fourteen articles were identified (PubMed: 381, EMBASE: 99, Scopus: 228 and Web of Science: 206), including 251 duplicates. Of these, 640 were excluded after screening the abstracts because they did not report cytokines, were case reports or review articles. Twenty-three publications were selected for full-text review and of these, thirteen, four *in vitro* and nine *in vivo,* met the eligibility criteria.

Study characteristics and description

Four *in vitro* studies investigated the effect of plant-derived essential oils and their components against inflammatory airway disease in cell lines of bronchial asthma. A further nine *in vivo* studies assessed the essential oils and their components effect in mouse models of asthma characterized by eosinophil-dominant inflammation in actively sensitized mice. The chemical structures of the essential oil components are shown in Figures 2 and 3. Most of the studies used monoterpenes, sesquiterpene and triterpene alcohols (n = 6) and quinones (n = 2). Two studies used cyclic monoterpene and three used crude essential oils. Four studies originated from Japan, three from Taiwan, two each from USA and Iran and one each from Brazil and Germany.

In vitro experiments were performed in human bronchial epithelial (BEAS-2B), lung mucoepidermoid carcinoma (H292), lung carcinoma (A549), eosinophilic leukemia (HL-60 clone 15) and mouse splenocytes cell lines. Numerous experimental approaches were used in the *in vitro* studies, including the inhibition of eosinophil migration, cytokine assays, level of reactive oxygen species and determination of levels and expression of molecules in innate immune responses. The main finding of the *in vivo* studies is that mice strains, such as BALB/c, are suited for experimental protocols of asthmatic inflammatory reactions. BALB/c mice models have been used extensively in asthma-related inflammatory disease with identical airway responsiveness and bronchial inflammation with hyperproduction of Th2 cytokines to humans (Gueders et al., 2009). This model was recurrently reported in the *in vivo* studies included.

Two experimental mouse models of allergic inflammation and asthmatic disorders have been used to describe the immunomodulation effect of essential oils and their components. These include the ovalbumin-sensitized airway inflammation and the *Dermatophagoides farinae*-induced airway hyperresponsiveness and eosinophilic infiltration. Several methods have been used to study changes in mouse models of asthma, including the assessment of airway responsiveness, leukocyte counts, proinflammatory and inflammatory mediators and markers, cytokine levels, antibody titres, lung resistance, histopathology and histomorphometric analyses, levels of immunostimulatory dependent signalling molecules and transcription factors.

In vitro studies of cytokine responses to essential oil and constituents in asthmatic condition

In vitro studies investigating the systemic treatment of asthma with essential oils of ginger and bioactive compounds, such as limonene from *Citrus junos*, terpenoids from *Zingiber officinale*, carvacrol and thymol have used established cell lines models of asthmatic syndromes via regulating proinflammatory, Th1 and Th2 cytokines to enhance immune responses in experimental bronchial asthma (Table 1). Limonene from *Citrus junos* inhibits the diesel exhaust particles (DEP)-stimulated p38 mitogen activated protein kinase (MAPK) signaling pathway and modulates eotaxin-induced chemotaxis by inhibiting TNF-α, IL-1β, IL-8 and IL-10 (Hirota et al., 2010). In addition, monocyte chemoattractant protein (MCP)-1 production decreases, suggesting that limonene might decrease monocyte and eosinophil infiltration in the lungs. The bioactive constituents of essential oils of various plants may have potential therapeutic roles for the management of bronchial asthma through their role in controlling reactive oxygen species (ROS) production, inactivating eosinophil migration and ameliorating oxidative damage to the lung (Beck-Speier et al., 2005).

Regarding the attenuation of airway inflammation, the increased number of inflammatory cells in the bronchioalveolar lavage fluid (BALF) of rats after lipopolysaccharide treatment is significantly reduced by a ginger extract (Aimbire et al., 2007). Ginger volatile oils and their terpenoid compounds have anti-inflammatory and regulatory effects on lipopolysaccharide-stimulated BEAS-2B-induced IL-8 and TNF-α secretion and might be promising against inflammatory airway diseases (Podlogar and Verspohl, 2011).

Carvacrol or cymophenol is considered the foremost constituent of several plants including *Zataria multiflora,* that demonstrate beneficial properties on respiratory diseases, including asthma (Alavinezhad et al., 2017). It has been hypothesized that carvacrol from *Carum copticum* essential oil has relaxant effects on smooth muscles of guinea pig tracheal chains and bronchodilator effects (Boskabady et al., 2003). The immunologic feature of asthma is a balance shift from Th1 to Th2 (Jalali et al., 2013). The increased cytokine production of IL-4, -5 and -13 has been shown in BALF and airway biopsies of patients with mild or asymptomatic asthma (Walker et al., 1992). Th2 cytokines are required for the development of airway eosinophilia and to stimulate an inflammatory response that results in asthma (Ray and Cohn, 1999). Th1 inhibits Th2 responses through the secretion of INF-γ, IL-2 and TGF-β and thus the goal of asthma therapy should be the balance of Th1/Th2 (Randolph et al., 1999). Carvacrol potentiates anti-inflammatory reactions through the improved balance of Th1 (IFN- γ) and Th2 (IL-4) cytokine gene expression in splenocytes of sensitized mice. The immune modulatory effect of carvacrol is more effective than dexamethasone in sensitized mice splenocytes, indicating it has potential therapeutic value in allergy, autoimmunity and infection (Kianmehr et al., 2016).

The major phenolic monoterpenoids found in genera *Origanum* and *Thymus* are carvacrol and thymol, the most abundant essential oil constituents of various plants (Alavinezhad et al., 2018). In *in vitro* T-cell models, carvacrol and thymol can affect transcriptional factors that regulate inflammation by reducing IL-2 and IFN-γ production (Gholijani et al., 2015, 2016). Carvacrol and thymol inhibit the effects of chitin on type-2 promoting cytokines IL-25 and IL-33 released in asthmatic conditions. Chitin is a component of the cell walls of fungi, crab, shrimp, insects and house dust mites which correlates with asthma (Koch et al., 2015). Previous reports have found increases in IL-25 and IL-33 or the receptors in mice lungs inflamed after chitin administration (Yasuda et al., 2012; Van Dyken et al., 2014). Moreover, IL-33 is involved in other pathological conditions, such as cardiovascular diseases, arthritis, infection, sepsis, atherosclerosis, neurological disorders, cancer and allergy (Pan et al., 2018). The direct administration of chitin to airway epithelial cells is likely to contribute to allergic airway reactions and carvacrol and thymol may inhibit epithelial pro-inflammatory cell responses to chitin by inhibiting type-2 promoting cytokine release and immune responses (Khosravi and Erle, 2016).

In vivo studies of cytokine responses to essential oil and constituents in asthmatic condition

Thymoquinone reduces elevated levels of IL-4, 5 and 13 secretion and increases IL-10 in ovalbumin (OVA)-sensitized and challenged mice (EI Gazzar et al., 2006). IL-4 is required for Th2 cell differentiation and isotype switching in B cells from IgM to IgE production **(**Kopf et al., 1993; Holgate, 2004), whereas, IL-5 and IL-13 regulate growth, differentiation, and survival of eosinophils (Domae et al., 2003). IL-13 promotes IgE isotype switching in B cells and mucus production by goblet cells in the airway mucosa (Zhu et al., 1999). Thymoquinone causes obstruction of lung tissue eosinophilia and goblet cells hyperplasia which could be due to its intense action on Th2 cytokines and inflammatory cells in the airways.

EI Mezayen et al., (2006) reported that thymoquinone attenuates OVA-induced airway inflammation by inhibiting cyclooxygenase-2 (COX-2) expression and prostaglandin D2 production and IL-4, 5 and 13 levels. The inhibition of prostaglandin D2 synthesis plays an important role in modulating Th2 cytokine levels, lung eosinophilia, goblet cell hyperplasia and mucus hyperproduction in allergic airway inflammation (Larche et al., 2002; Herrick and Bottomly, 2003). Thymoquinone suppressed the immunologic and inflammatory responses induced by Th2 cytokines in a mouse model of airway inflammation and had anti-inflammatory effects in allergic lung responses.

Perilla oil diminished bronchoalveolar inflammation by decreasing the secretion of pro-inflammatory and Th1 cytokines into the local lung and airway tissues but failed to regulate the Th1/Th2 balance toward the Th1 pole in Th2-skewed allergic airway inflammation (Chang et al., 2008). Limonene treated mice had reduced IFN-γ, IL-5, IL-13 and TGF-β levels and lower numbers of eosinophils in the lungs of *Dermatophagoides farina*-induced airway hyperresponsiveness asthma model (Hirota et al., 2012). IL-13 is linked to mucus hypersecretion by hyperplastic goblet cells that create airway mucus plugs, especially in peripheral airways of asthmatics (Li et al., 2003). TGF-β is associated with the development of airway remodeling in asthma and correlates with thickening of the basement membrane (Matsukuara et al., 2010; Tian et al., 2011).

Lavender essential oil inhalation extinguishes eosinophils in BALF and lung tissue concomitantly with a decrease in IL-5 and IL-13 levels in bronchial tissue of experimental asthmatic mice (Ueno-Iio et al., 2014). Eosinophils play a key role in the pathogenesis of bronchial asthma, while IL-5 plays a key role in the development and migration of eosinophils and correlates with the severity of eosinophilic inflammation (Sanderson, 1992). IL-13 remodels the infiltration of inflammatory cells into BALF and peri-bronchial and perivascular tissues (Zhou et al., 2012).

Farnesol, a sesquiterpene alcohol widely present in fruits, vegetables and essential oils (Duncan and Archer, 2008) restores IL-2. IL-4, IL-5, IL-10, IL-1β and TNF-α levels in distorted BALF due to OVA sensitization and challenge in asthmatic mice, suggesting it may have potential to modulate the Th1/Th2 balance in lungs (Ku and Lin, 2015). Th2 cells are the major source of IL-10 production, although excessive IL-10 secretion may inhibit the production of IL-1β and TNF-α (Iyer and Cheng, 2012). Farnesol supplementation ameliorates serum lipid profiles in OVA-sensitised and challenged mice and reduces increased non-specific IgE, IgA, IgM, and IgG levels after OVA sensitisation and challenge, suggesting that its supplementation regulates Th2 cytokine ratios in BALF and reduces inflammation.

Zataria multiflora has a relaxant effect on tracheal responsiveness, bronchodilator and regulation of inflammatory mediators in OVA-sensitized guinea pigs (Boskabady et al., 2014) and a preventive effect on emphysema and pathological changes of the lung and systematic inflammation in guineas pigs' models of chronic obstructive pulmonary disease (COPD) (Gholami Mahtaj et al., 2015). The extract of *Zataria multiflora* thymol as main constituent decreases pro-inflammatory cytokines (IL-4 and IL-17 and TGF-β) but increases anti-inflammatory (IFN-γ) cytokine and Forkhead box P3 (FOXP3) gene expression in splenocytes of asthmatic mice, suggesting a specific therapeutic effect in allergy, autoimmunity and infection potentiating Th1 and suppressing Th2 and Th17 cells. (Kianmehr et al., 2017)

The alcoholic monoterpenes citronellol, α-terpineol and carvacrol largely present in plants of the genus *Cymbopogon*, *Eucalyptus* and *Origanum* are used for the treatment of inflammatory diseases (Guimaraes et al., 2013). Citronellol, α-terpineol and carvacrol modulates eosinophil migration and decreases TNF-α levels in the mice pleural cavity after induction by OVA. These effects can be associated with the monoterpenes' ability to inhibit important targets of inflammatory mediators and could be prospective candidates as drugs in the therapy of allergic inflammation and asthma.

Methodological quality/risk of bias

The methodological features of the *in vivo* studies are detailed in Figure 4. All papers selected reported the allocation sequence generation with random procedures. However; none of them reported blinding procedures. The animal experiments had a lack of blinding of the interventions allocated (performance bias) and outcome assessment (detection bias) (Fig. 5).

Conclusions

This review assessed the anti-inflammatory and immune-modulating activities of essential oils and their bioactive compounds linked to cytokine expression and secretion in airway pathologic reactions, highlighting the immunoregulatory mechanisms enhancing cytokine responses, triggering of immune cells, orientate immune regulation and decreased inflammation associated with asthma. The pharmacology and pharmacokinetics of several plant-derived essential oils remain to be clearly established using cellular and animal models of asthma.

Conflicts of interest

The authors declare they have no conflicts of interest regarding the publication of this paper.

Acknowledgment

GRG gratefully acknowledges the Brazilian Programa Nacional de Pós Doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PNPD/CAPES) for the provision of a Postdoctoral Internship (Number: 1814096).

References

Abdureyim, S., Amat, N., Umar, A., Upur, H., Berke, B., Moore, N., 2011. Anti-inflammatory, immunomodulatory, and heme oxygenase-1 inhibitory activities of ravan napas, a formulation of Uighur traditional medicine, in a rat model of allergic asthma. Evid. Based Complement. Altern. Med. 2011, 725926.

Aimbire, F., Penna, S.C., Rodrigues, M., Rodrigues, K.C., Lopes-Martins, R.A., Sertie, J.A., 2007. Effect of hydroalcoholic extract of *Zingiber officinalis* rhizome on LPS-induced rat airway hyperactivity and lung inflammation. Prostoglandins Leukot Essent. Fattty Acids 77, 129-138.

Alavinezhad, A., Khazdair, M.R., Boskabady, M.H., 2018. Possible therapeutic effect of carvacrol on asthmatic patients: A randomized, double blind, placebo-controlled, Phase II clinical trial. Phytother. Res. 32, 151-159.

Alavinezhad, A., Hedayati, M., Boskabady, M.H., 2017. The effect of *Zataria multiflora* and carvacrol on wheezing, FEV1 and plasma levels of nitrite in asthmatic patients. Avicenna J. Phytomed. 7, 531-541.

Beck-Speier, I., Dayal, N., Karg, E., Maier, K.L., Schumann, G., Schulz, H., Semmler, M., Takenaka, S., Stettmaier, K., Bors, W., Ghio, A., Samet, J.M., Heyder, J., 2005. Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particles. Free Radic. Biol. Med. 38, 1080-1092.

Boskabady, M.H., Jalali, S., Farkhondeh, T., Byrami, G., 2014. The extract of *Zataria multiflora* affect tracheal responsiveness, serum levels of NO, nitrite, PLA2, TP and histamine in sensitized Guinea pigs. J. Ethanopharmacol. 156, 301-308.

Boskabady, M.H., Ramazani, M., Tabei, T., 2003. Relaxant effects of different fractions of essential oil from *Carum copticum* on guinea pig tracheal chains. Phytother. Res. 17, 1145-1149.

Chang, H.H., Chen, C.S., Lin, J.Y., 2008. Dietary perilla oil inhibits proinflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice. Lipids 43, 499-506.

Cheng, Z., Wang, X., Dai, L.L., Jia, L.Q., Jing, X.G., Liu, Y., Wang, H., Li, P.F., An, L., Liu, M., 2018. Thymic stromal lymphopoietin signaling pathway inhibition attenuates airway inflammation and remodeling in rats with asthma. Cell Physiol. Biochem. 47, 1482-1496.

Doeing, D.C., Solway, J., 2013. Airway smooth muscle in the pathophysiology and treatment of asthma. J. Appl. Physiol. 114, 834-843.

Domae, M., Sagara, H., Sakaue, M., Fukuda, T., Kamikawa, Y., 2003. The antiallergic drug oxatomide promotes human eosnophil apoptosis and suppresses IL-5-induced eosinophil survival. J. Allergy Clin. Immunol. 111, 567-572.

Duncan, R.E., Archer, M.C., 2008. Farnesol decreases serum triglycerides in rats: identification of mechanisms including up-regulation of PPARα and down-regulation of fatty acid synthase in hepatocytes. Lipids 43, 619-627.

El Gazzar, M., El Mezayen, R., Nicolls, M.R., Marecki, J.C., Dreskin, S.C., 2006. Downregulation of leukotriene biosynthesis by thymoquinone attenuates airway inflammation in a mouse model of allergic asthma. Biochim. Biophys. Acta 1760, 1088-1095.

El Mezayen, R., El Gazzar, M., Nicolls, M.R., Marecki, J.C., Dreskin, S.C., Nomiyama, H., 2006. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol. Lett. 106, 72-81.

Ernst, E., 1998. Complementary therapies for asthma: What patients use, J. Asthma Allergy Educ. 35, 667-671.

Gholami Mahtaj, L., Boskabady, M., Mohamadian Roshan, N., 2015. The effect of *Zataria multiflora* and its constituent, carvacrol, on tracheal responsiveness and lung pathology in guinea pig model of COPD. Phytother. Res. 29, 730-736.

Gholijani, N., Gharagozloo, M., Farjadian, S., Amirghofran, Z., 2016. Modulatory effects of thymol and carvacrol on inflammatory transcription factors in lipopolysaccharide-treated macrophages. J. Immunotoxicol. 13, 157-164.

Gholijani, N., Gharagozloo, M., Kalantar, F., Ramezani, A., Amirghofran, Z., 2015. Modulation of cytokine production and transcription factors activities in human jurkat T cells by thymol and carvacrol. Adv. Pharm. Bull. 5, 653-660.

Gueders, M.M., Paulissen, G., Crahay, C., Quesada-Calvo, F., Hacha, J., Hove, C.V., Tournoy, K., Louis, R., Foidart, J., Noël, A., Cataldo, D.D., 2009. Mouse models of asthma: a comparison between C57BL/6 and BALB/c strains regarding bronchial responsiveness, inflammation, and cytokine production. Inflamm. Res. 58, 845-854.

Guimaraes, A.G., Quintans, J.S., Quintans-Junior, L.J., 2013. Monoterpenes with analgesic activity - A systematic review. Phytother. Res. 27, 1-15.

Hardy, C.L., Rolland, J.M., O'Hehir, R.E., 2015. The immunoregulatory and fibrotic roles of activin A in allergic asthma. Clin. Exp. Allergy 45, 1510-1522.

Herrick, C.A., Bottomly, K., 2003. To respond or not to respond: T cells in allergic asthma. Nat. Rev. Immunol. 3, 405-412.

Hirota, R., Nakamura, H., Bhatti, S.A., Ngatu, N.R., Muzembo, B.A., Dumavibhat, N., Eitoku, M., Sawamura, M., Suganuma, N., 2012. Limonene inhalation reduces allergic airway inflammation in *Dermatophagoides farina* treated mice. Inhal. Toxicol. 24, 373-381.

Hirota, R., Roger, N.N., Nakamura, H., Song, H.S., Sawamura, M., Suganuma, N., 2010. Anti-inflammatory effects of limonene from yuzu (*Citrus junos* Tanaka) essential oil on eosinophils. J. Food Sci. 75, 87-92.

Holgate, S.T., 2004. Cytokine and anti-cytokine therapy for the treatment of asthma and allergic disease. Cytokine 28, 152-157.

Hooijmans, C.R., Rovers, M.M., De-Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 43.

Horváth, G., Kamilla Á.C.S., 2015. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review. Flavour Fragr. J. 30, 331-341.

Iyer, S.S., Cheng, G., 2012. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit. Rev. Immunol. 32, 23-63.

Jalali, S., Boskabady, M.H., Haeri-Rohani, A., Eidi, A., 2013. The effect of carvacrol on serum cytokines and endothelium levels of ovalbumin sensitized guinea-pigs. Iran J. Basic Med. Sci. 16, 615-619.

Khosravi, A.R., Erle, D.J., 2016. Chitin-induced airway epithelial cell innate immune responses are inhibited by Carvacrol/Thymol. Plos One 11, e0159459.

Kianmehr, M., Haghmorad, D., Nosratabadi, R., Rezaei, A., Alavinezhad, A., Boskabady, M.H., 2017. The effect of *Zataria multiflora* on Th1/Th2 and Th17/T regulatory in a mouse model of allergic asthma. Front. Pharmacol. 8, 458.

Kianmehr, M., Rezaei, A., Boskabady, M.H., 2016. Effect of carvacrol on various cytokines genes expression in splenocytes of asthmatic mice. Iran J. Basic Med. Sci. 19, 402-410.

Koch, B.E., Stougaard, J., Spaink, H.P., 2015. Keeping track of the growing number of biological functions of chitin and its interaction partners in biomedical research. Glycobiology. 25, 469-482.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, G., Kohler, G., 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine response. Nature 362, 245-248.

Ku, C.M., Lin, J.Y., 2015. Farnesol, a sesquiterpene alcohol in herbal plants, exerts anti-inflammatory and antiallergic effects on ovalbumin-sensitized and challenged asthmatic mice. Evid. Based Complement. Alternat. Med. 2015, 387357.

Ku, C.M., Lin, J.Y., 2016. Farnesol, a sesquiterpene alcohol in essential oils, ameliorates serum allergic antibody titres and lipid profiles in ovalbumin-challenged mice. Allergol. Immunopathol. 44, 149-159.

Larche, M., Robinson, D.S., Kay, A.B., 2002. The role of T lymphocytes in the pathogenesis of asthma. J. Allergy Clin. Immunol. 111, 450-463.

Levy, J., Neukirch, C., Larfi, I., Demoly, P., Thabut, G., 2018. Tolerance to exposure to essential oils in patients with allergic asthma. J Asthma 9, 1-20.

Li, N., Hao, M., Phalen, R.F., Hinds, W.C., Nel, A.E., 2003. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin. Immunol. 109, 250-265.

Matsukura, S., Odaka, M., Kurokawa, M., Kuga, H., Homma, T., Takeuchi, H., Notomi, K., Kokubu, F., Kawaguchi, M., Schleimer, R.P., Johnson, M.W., Adachi, M., 2010. Transforming growth factor-β stimulates the expression of eotaxin/CC chemokine ligand 11 and its promoter activity through binding site for nuclear factor-κβ in airway smooth muscle cells. Clin. Exp. Allergy 40, 763-771.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann. Intern. Med. 151, 264-269.

Murdoch, J.R., Lloyd, C.M., 2010. Chronic inflammation and asthma. Mutat. Res. 690, 24-39.

Nunes, C., Pereira, A.M., Morais-Almeida, M., 2017. Asthma costs and social impact. Asthma Res. Pract. 3, 1-11.

Pan, D., Buchheit, K.M., Samuchiwal, S.K., Liu, T., Cirka, H., Raff, H., Boyce, J.A., 2018. COX-1 mediates IL-33-induced extracellular signal-regulated kinase activation in mast cells: Implications for aspirin sensitivity. J. Allergy Clin. Immunol. 18, 30996-5.

Pina, L.T.S., Ferro, J.N.S., Rabelo, T.K., Oliveira, M.A., Scotti, L., Scotti, M.T., Walker, C.I.B., Barreto, E.O., Quintans Júnior, L.J., Guimarães, A.G., 2018. Alcoholic monoterpenes found in essential oil of aromatic spices reduce allergic inflammation by the modulation of inflammatory cytokines. Nat. Prod. Res. 2, 1-5.

Podlogar, J.A., Versphol, E.J., 2011. Antiinflammatory effects of ginger and some of its components in human bronchial epithelial (BEAS-2B) cells. Phytother. Res. 26, 333-336.

Randolph, D.A., Stephens, R., Carruthers, C.J., Chaplin, D.D., 1999. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. J. Clin. Invest. 104,1021-1029.

Rao, S.S., Mu, Q., Zeng, Y., Cai, P.C., Liu, F., Yang, J., Xia, Y., Zhang, Q., Song, L.J., Zhou, L.L., Li, F.Z., Lin, Y.X., Fang, J., Greer, P.A., Shi, H.Z., Ma, W.L., Su Y., Ye, H., 2017. Calpain-activated mTORC2/Akt pathway mediates airway smooth muscle remodelling in asthma. Clin. Exp. Allergy, 47, 176-189.

Ray, A., Cohn, L., 1999. Th2 cells and GATA-3 in asthma; new insights into the regulation of airway inflammation. J. Clin. Invest. 104, 985-993.

Rivera, D.G., Hernandez, I., Merino, N., Luque, Y., Alvarez, A., Martin, Y., Amador, A., Nuevas, L., Delgado, R., 2011. *Mangifera indica* L. extract (Vimang) and mangiferin reduce the airway inflammation and Th2 cytokines in murine model of allergic asthma. J. Pharm. Pharmacol. 63, 1336-1345.

Roskosk Jr, R., 2017. Guidelines for preparing color figures for everyone including the colorblind. Pharmacol. Res. 119, 240-241.

Sanderson, C.J., 1992. Interleukin-5, eosinophils and disease. Blood 79, 3101-3109.

Tang, W., Shang, Y., Xiao, B., Wen, P., Lyu, R., Ning, K., 2018. The cell research trends of asthma: A stem frequency analysis of the literature. J. Healthc. Eng. 2018, 9363820.

Tettamanti, L., Kritas, S.K., Gallenga, C.E., D'Ovidio, C., Mastrangelo, F., Ronconi, G., Caraffa, A., Toniato, E., Conti, P., 2018. IL-33 mediates allergy through mast cell activation: Potential inhibitory effect of certain cytokines. J. Biol. Regul. Homeost. Agents. 5, 1061-1065.

Tian, X.R., Tian, X.L., Bo, J.P., Li, S.G., Liu, Z.L., Niu, B., 2011. Inhibition of allergic airway inflammation by antisense-induced blockade of STAT6 expression. Clin. Med. J. 124, 26-31.

Ueno-Iio, T., Shibakura, M., Yokota, K., Aoe, M., Hyoda, T., Shinohata, R., Kanehiro, A., Tanimoto, M., Kataoka, M., 2014. Lavender essential oil inhalation suppresses allergic airway inflammation and mucous cell hyperplasia in a murine model of asthma. Life Sci. 108, 109-115.

Van Dyken, S.J., Mohapatra, A., Nussbaum, J.C., Molofsky, A.B., Thornton, E.E., Ziegler, S.F., McKenzie, A.N., Krummel, M.F., Liang, H.E., Locksley, R.M., 2014. Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and gamma delta T cells. Immunity. 40, 414-424.

Verheijden, K.A., Braber, S., Leusink-Muis, T., Thijssen, S., Boon, L., Kraneveld, A.D., Garssen, J., Folkerts, G., Willemsen, L.E., 2016. Regulatory T cell depletion abolishes the protective effect of dietary galacto-oligosaccharides on eosinophilic airway inflammation in house dust mite-induced asthma in mice. J. Nutr. DOI: 10.3945/jn.115.224402.

Walker, C., Bode, E., Boer, L., Hansel, T.T., Blaser, K., Virchow, J.C., 1992. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am. Rev Respir. Dis. 146,109-115.

Yasuda, K., Muto, T., Kawagoe, T., Matsumoto, M., Sasaki, Y., Matsushita, K., Taki, Y., Futatsugi-Yumikura, S., Tsutsui, H., Ishii, K.J., Yoshimoto, T., Akira, S., Nakanishi, K., 2012. Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. Proc. Natl. Acad. Sci. U S A. 109, 3451-3456.

Zhou, X., Hu, H., Balzar, S., Trudeau, J.B., Wenzel, S.E., 2012. MAPK regulation of IL-4/IL-13 receptors contributes to the synergistic increase in CCL11/eotaxin-1 in response to TGF-β1 and IL-13 in human airway fibroblasts. J. Immunol. 188, 6046-54.

Zhu, Z., Homer, R.J., Wang, Z., Chen, Q., Geba, G.P., Wang, J., Zhang, Y., Elias, J.A., 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. J. Clin. Invest. 103, 779-788.

Figures legends

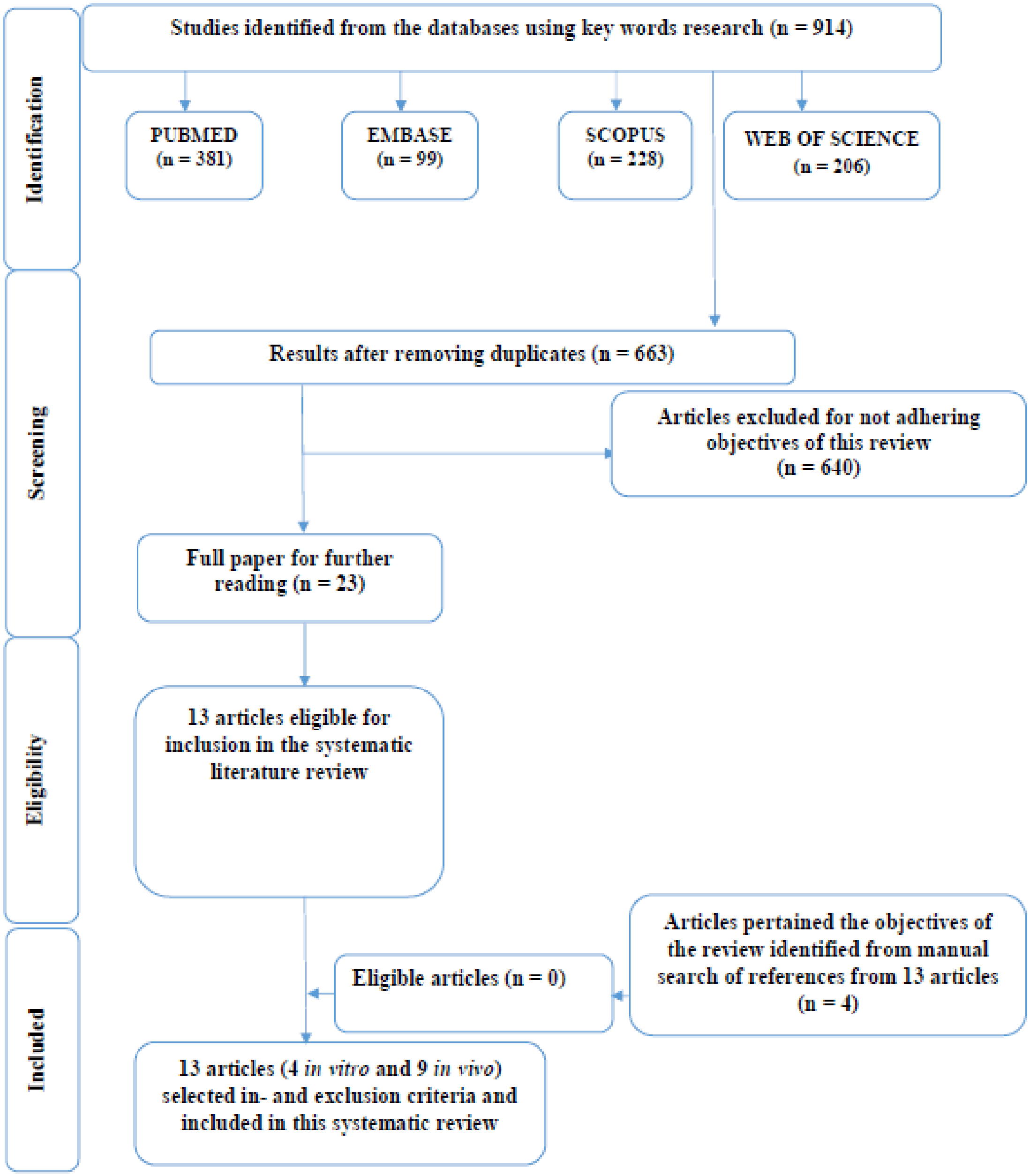

Fig. 1.

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of article search and selection included in this review.

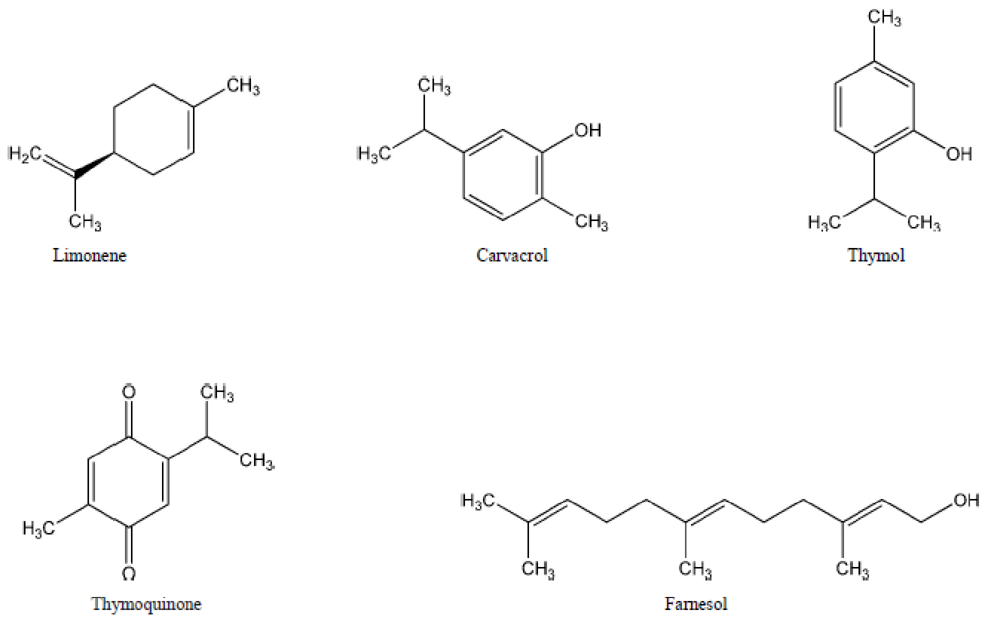

Fig. 2.

Fig. 2. Chemical structures of essential oil constituents (I)

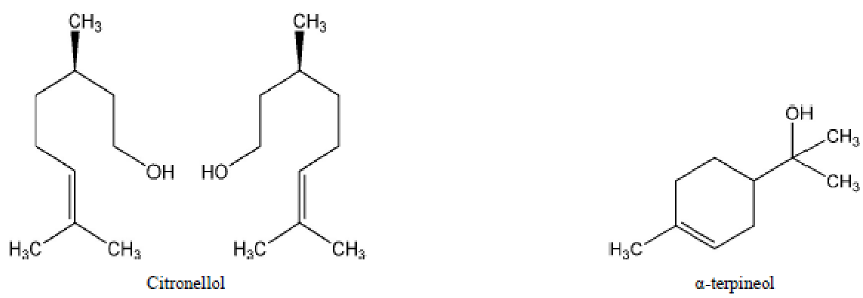

Fig. 3.

Fig. 3. Chemical structures of essential oil constituents (II)

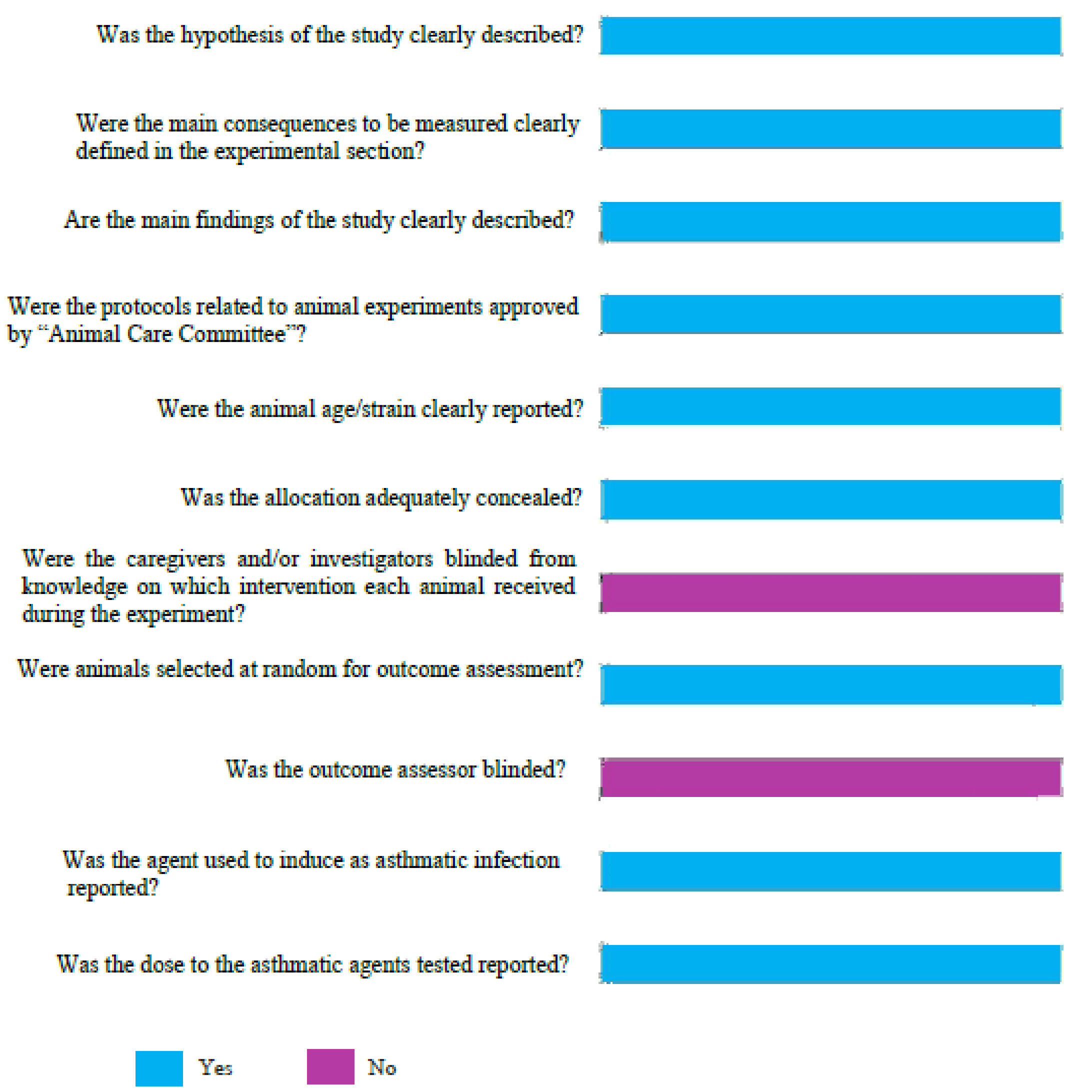

Fig. 4.

Fig. 4. Analysis on methodological quality and results of studies. Blue and magenta bars represent the proportion of studies for which the item was or was not applicable.

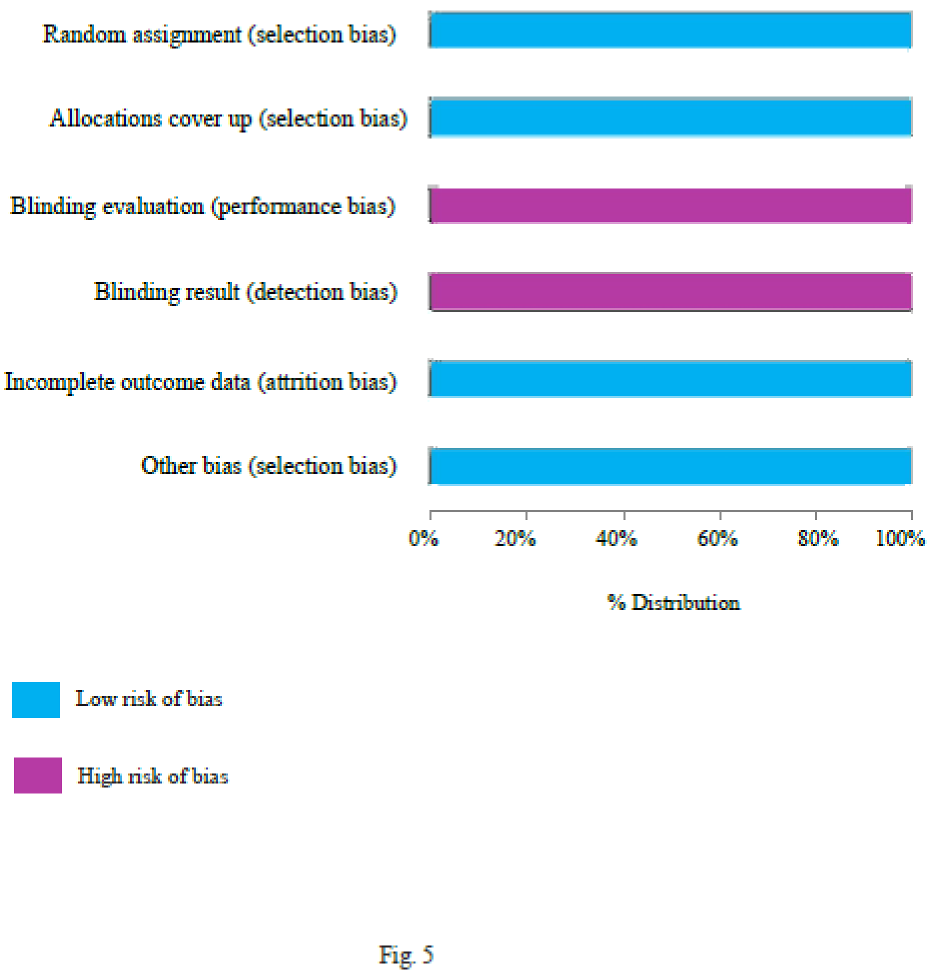

Fig. 5. Risk of bias level. Blue: Low; Magenta: High.

Table 1 *In vitro* **s**tudies of cytokine responses to essential oil and bioactive constituents in asthmatic condition

Authors, Year, Country	**Substance**	**Concentration**	**Cell lines**	**Objectives**	**Cytokines studied**	**Assays**	**Improved Characteristics**
Hirota et al., 2010; Japan	Limonene from *Citrus junos*	7.34 mMol/L	HL-60 clone 15 cells (Human leukemia cell line)	To investigate anti-inflammatory effect of limonene on human eosinophilic leukemia	Tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), IL (interleukin)-8, IL-10	ELISA	Limonene may have potential anti-inflammatory efficacy against bronchial asthma by inhibiting cytokines production
Podlogar and Verspohl 2011; Germany	Ginger volatile oil (*Zingiber officinale*)	0.1-1 µg/mL	BEAS-2B (Human bronchial epithelial cell line)	Ginger volatile oil and its compounds were tested against human bronchial epithelial cells	IL-8 and TNF-α	ELISA	Ginger oil and its terpenoid compounds could be used as anti-inflammatory drugs in respiratory infections

Kianmehr et al., 2016; Iran	Carvacrol	75, 150 and 300 µg/mL	Splenocytes	Effects of carvacrol on cytokines genes expression in splenocytes of asthmatic	IL-4, IFN-γ, transforming growth factor beta (TGF-β) and IL-17	qPCR	Immunomodulatory effect of carvacrol indicating increased IFN-γ and FOXP3 but decreased IL-4, TGF-β, and IL-17 expression

Table 1 Continued

Authors, Year, Country	**Substance**	**Concentration**	**Cell lines**	**Objectives**	**Cytokines studied**	**Assays**	**Improved Characteristics**
Khosravi and Erle 2016; USA	Carvacrol and Thymol	200 µM (equal to 30 µg/mL)/ 80 µg/mL	Beas-2B, H292 (Mucoepidermoid carcinoma cells) and A549 (Adenocarcinomic human alveolar basal epithelial cells)	To hypothesize that chitin directly stimulates airway epithelial cells to release cytokines that promotes type 2 immune responses	IL-25 and IL-33	ELISA	Direct effects of chitin on airway epithelial cells are likely to contribute to allergic airway diseases like asthma, and that carvacrol/thymol directly inhibits epithelial cell pro-inflammatory

	responses to chitin

Table 2 *In vivo* **s**tudies of cytokine responses to essential oil and bioactive constituents in asthmatic condition

Authors, Year, Country	**Substance**	**Strains/ Animal**	**Dose/ Route**	**Objectives**	**Cytokines studied**	**Assays**		**Improved Characteristics**
						Biochemical	**Molecular**	
EI Mezayen et al; 2006; Japan	Thymoquinone	BALB/c Mice	3 mg/kg (*i.p.*)	To investigate the potential anti-inflammatory role of thymoquinone by examining its effect on mouse challenged through the airways with ovalbumin (OVA)	IL-4, IL-5 and IL-13	ELISA	Western blot	Thymoquinone has an anti-inflammatory effect during the allergic response in the lung through the inhibition of Th2 cytokine mediated immune response
EI Gazzar et al; 2006; USA	Thymoquinone	BALB/c Mice	3mg/kg (*i.p.*)	To examine the anti-inflammatory effect of thymoquinone in a mice sensitized and challenged with OVA	IL-4, IL-5, IL-13 and IL-10	ELISA		Anti-inflammatory effect of thymoquinone on allergic lung is mediated by a decrease in Th2 cytokine production

Table 2 Continued

Authors, Year, Country	**Substance**	**Strains/ Animal**	**Dose/ Route**	**Objectives**	**Cytokines studied**	**Assays Biochemical Molecular**	**Improved Characteristics**
Chang et al., 2008; Taiwan	5 % Perilla oil	BALB/c Mice	Experimental food-oral	To evaluate the anti-inflammatory effect of perilla oil on OVA-sensitized and challenged asthmatic mice	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, Interferon gamma (IFN-γ) and TNF-α	ELISA	Dietary perilla oil alleviates inflammation by decreasing the secretion of pro-inflammatory cytokines in BALF, but failed to regulate the Th1/Th2 balance during allergic airway inflammation.
Hirota et al., 2012; Japan	Limonene isolated from *Citrus junos*	BALB/c Mice	4 µg (*i.t.*)	To evaluate the inhibitory effect of limonene on *Dermatophagoides farinae*-induced airway hyper	IFN-γ, IL-5, IL-13, and TGF-β	ELISA	The goblet cell metaplasia, thickness of airway smooth muscle, and fibrosis were markedly decreased in limonene

				responsiveness and airway modeling in a mice model of asthma			treated mice. It has a potential to reduce airway remodeling and hyperresponsiveness

Table 2 Continued

Authors, Year, Country	**Substance**	**Strains/ Animal**	**Dose/ Route**	**Objectives**	**Cytokines studied**	**Assays Biochemical Molecular**	**Improved Characteristics**
Ueno-Iio et al., 2014; Japan	Lavender essential oil isolated from *Lavandula angustifolia*	BAL B/c Mice	Aerosolized 5-20 µL of Lvn	To evaluate the anti-inflammatory effect of lavender essential oil on OVA-induced bronchial asthma	IL-4, IL-5 and IL-13	ELISA	Essential oil inhibits allergic inflammation and mucous cell hyperplasia with suppression of Th2 cytokines
Ku and Lin, 2015; Taiwan	Farnesol	BAL B/c Mice	5, 25 and 100 mg/kg (mixed in feed)	To investigate the effect of farnesol on OVA-sensitized and challenged asthmatic mice	IL-4, IL-5, IL-2, IFN-γ, IL-1β, TNF-α and IL-10	ELISA	Farnesol supplementation may be beneficial to improve the Th2-cytokine mediated allergic asthmatic inflammation

Ku and Lin, 2016; Taiwan	Farnesol	BALB/c Mice	5, 25 and 100 mg/kg (mixed in feed)	To investigate the effect of farnesol on allergic asthma by OVA-sensitized and mice	IL-2, IL-4, IL-5 and IFN-γ	ELISA		Farnesol improved allergic antibody levels and regulated Th2 responses towards Th1 immune balance and ameliorate inflammation

Table 2 Continued

Authors, Year, Country	Substance	Strains/ Animal	Dose/ Route	Objectives	Cytokines studied	Assays Biochemical	 Molecular	Improved Characteristics
Kianmehr et al., 2017; Iran	Thymol from *Zataria multiflora*	BALB/c Mice	200, 400 and 800 µg/mL (*p.o.*)	To investigate the effect of *Z. multiflora* containing thymol on cytokine genes expression on OVA-injected experimental mouse model of asthma	IFN-γ, IL-4, TGF-β and IL-17		Real time-PCR	*Z. multiflora* containing thymol decreased pro inflammatory cytokines but increased anti-inflammatory cytokines gene expression and the number of Treg in splenocytes of asthmatic mice

Pina et al., 2018; Brazil	Citronellol, α-terpineol and carvacrol	Swiss Mice	25, 50 or 100 mg/kg (*i.p.*)	Effects of citronellol, α-terpineol and carvacrol on allergic airway inflammation established by OVA	TNF-α	ELISA	Monoterpenes decreased leucocyte migration and TNF-α levels, it can be an alternative for treatment of allergic airway inflammation

Graphical Abstract