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RESEARCH ARTICLE

IN VITRO ANTICHOLINERGIC ACTIVITY OF SELECTED CULINARY SPICES ON SHEEP AIRWAY SMOOTH MUSCLE

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ABSTRACT

In vitro anticholinergic activity of selected culinary spices on sheep tracheal smooth muscle by organ bath studies was carried out. The anticholinergic activity of plant extracts was determined in vitro by using organ bath in which sheep tracheal smooth muscle was mounted in Krebs-Henseleit solution and its contractile activity for acetylcholine, a known cholinergic spasmogen was recorded in the presence and absence of extracts to determine the anticholinergic potential of the extracts of culinary spices. The percentile contractile response of acetylcholine in the presence and absence of tested plant extracts were recorded. Among the eight spices tested, acetone extracts *of Illicium verum* (fruit), *Cinnamomum zeylanicum* (bark*), Myristica fragrans* (seed), *Pimpinella anisum* (seed) and *Terminalia chebula* (seed) revealed considerable anticholinergic property. The anticholinergic property of these extracts may be helpful in treatment of upper respiratory tract problems including bronchial asthma.

INTRODUCTION

In mammals, cholinergic parasympathetic nerves provide the dominant innervation to the lungs and airway smooth muscles. Release of acetylcholine from these nerves regulates airway tone. Airway smooth muscle contraction, mucus secretion and vasodilation are regulated through interaction of muscarinic acetylcholine receptors found on airway smooth muscle, mucous glands and pulmonary vasculature (Colebatch *et al.,* 1963; Baker *et al.,* 1985; Laitinen *et al.,* 1987).

It was recently demonstrated that activation of muscarinic receptors also interacts with several cytokines and growth factors that play important role in pathogenesis of asthma. Epithelial damage during allergic airway inflammation plays a key role in asthma and exposes sensory nerve endings in the sub-mucosa to airway lumen, which promotes reflex mechanisms leading to enhanced vagal release of acetylcholine. Due to this, there will be an exaggerated airway smooth muscle hyper response that obstructs the air ways to a variety of pharmacological, chemical and physical stimuli and is responsible for recurrent episodes of wheezing and breathlessness (Gosens *et al.,* 2012). Further, the loss of epithelial barrier integrity associated with airway inflammation may increase exposure of airway smooth muscle to inhaled contractile agonist (Holgate *et al.,* 2009).

Recently increasing attention has been paid to the use of polyphenolic substances (bioactive molecules in plant foods) in the field of respiratory tract infections. They are able to inhibit the release and synthesis of broncho-constricting mediators and to impair the increased amount of inflammatory cytokines, chemokines, eosinophils during immune-allergic airway inflammation resulting in bronchodilator and anti-inflammatory effects. It is well documented that phenolic compounds like rosmarinic acid, luteolin derivatives and hesperidin present in the volatile oil of *Rosmarinus officinalis* Linn., thymol present in oil of thyme (*Thymus vulgaris* Linn.), chrysoeriol present in rooibos tea (*Aspalathus linearis* Linn.), silymarin, isoquercitrin, isoliquiritigenin and isokaempferide obtained from alcoholic extracts of *Silybum marianum* Linn., *Argemone platycera* Linn., *Glycyrrhiza glabra* Linn. (Licorice), *Amburana cearensis* Linn. showed bronchodilatory effects in various *in-vitro* and *in-vivo* laboratory models (Aqel *et al.,* 1991; Okamura *et al.,* 1994; Al-Sereiti *et al.,* 1999; Breschi *et al.,* 2002; Fernandez *et al.,* 2005; Khan *et al.,* 2006; Leal *et al.,* 2006; Liu *et al.,* 2008a;). It is also reported that the risk of asthma is lower with higher dietary polyphenolic compounds like flavonoids (Knekt *et al.,* 2002). Recently it has also been documented that epigenin, flavin-7, gnaphaliin A and B, luteolin, naringenin, provinol, quercetin, resveratrol and rutin like polyphenolic compounds showed bronchorelaxant effects both *in-vitro* and animal models (Das *et al.,* 2003; Jung *et al.,* 2007; Lin *et al.,* 2008; Moon *et al.,* 2008; Choi *et al.,* 2009; Franova *et al.,* 2009; Joskova *et al.,*

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2009; Joskova *et al.,* 2011; Lee *et al.,* 2009; Park *et al.,* 2009; Rodriguez-Ramos *et al.,* 2011). It has been established that ovine airway smooth muscle is a model for pharmacological evaluation of bronchorelaxant activity of capsaicin, an active component of chilli peppers (capsicum) on cholinergic transmission (Mustafa *et al.,* 1999).

MATERIALS AND METHODS

Plant Material Collection and Authentication

Culinary spices *Cinnamomum Zeylanicum Bl.* (bark), *Cuminum cyminum L.*(seed), *Illicium verum* (hook fruit), *Menthe piperata* (leaf), *Myristica Fragarn, Houtt.*(seed), *Nigella sativa Linn.* (seed), *Pimpinella anisum L.* (seed) and *Terminalia chebula Retz.* (seed), were brought from Kaleswara Rao Market Vijayawada, Krishna District, Andhra Pradesh, India. The plant materials were identified and authenticated by A.Rama Krishna MS.c., MPhil. Sr. Lecture, Head of the Department of Botany, Vemulapalli Kodanda Ramaiah College, Buddhavaram, Gannavaram Mandal, Krishna District, Andhra Pradesh, India.

Extraction procedure

Shade dried plant parts were reduced to fine powder and 10g of powder was taken into a 250 ml conical flask and 100 ml of acetone was added. After thorough mixing the flask was kept on a rotary shaker at 190 – 220 r/min for 24 hours and it was filtered with whatman filter paper No1. The filtrate was evaporated until dry in a water bath at 80^{o}C.

Chemicals

Dimethyl sulfoxide (DMSO) and magnesium heptahydrate (Merck Specialties Private Limited, Mumbai, India); sodium chloride (Qualigens Fine Chemicals, Mumbai, India); sodium bicarbonate (Fischer Inorganics and Aromatics Limited, Madras, India); calcium chloride, potassium chloride and potassium dihydrogen orthophosphate (s.d-fine Chem. Limited, Mumbai, India); Acetylcholine, indomethacin and Propranalol (Calbiochem^{R}, an affiliate of Merck, Darmstadt, Germany).

Composition of Modified Krebs-Henseleit Solution

Modified Krebs-Henseleit solution consisted of sodium chloride (NaCl) 118mM, sodium bicarbonate ($NaHCO_3$) 25mM, anhydrous dextrose 11.1mM, potassium dihydrogen orthophosphate (KH_2PO_4) 1.2mM, potassium chloride (KCl) 4.8mM, magnesium sulphate heptahydrate ($Mg_2SO_4.7H_2O$) 1.2mM and calcium chloride ($CaCl_2$) 1.2mM with pH maintained around 7.4. To this, 10^{-4}M indomethacin was added to prevent epithelium related non-specific relaxant molecules.

Instrument

Muscle tension (contractile response) experiments were performed by using polygraph INCO-Niviqure digital data acquisition system, version 60.1.1 (Niviqure Meditech Pvt. Ltd., Bangalore, India) and an organ bath (INCO, Ambala, India) containing an isometric force transducer to measure tension (contractile response) in muscle, a 30ml tissue (muscle) chamber, a tissue holder connected to an aerator, a water bath, a thermostat connected to a heater and a stirrer.

Preparation of sheep isolated tracheal strips

The fresh sheep tracheas were obtained from the local slaughter house in chilled modified Krebs-Henseleit solution to the laboratory within 30 minutes. A piece of trachea was selected and cleaned of adhering adipose and connective tissue and then opened transversely along the cartilaginous rings, thereafter, the rings were pinned flat on a cork board and the strips of smooth muscle were dissected free from the underlying cartilage (Mustafa *et al.,* 2008). The smooth muscle preparations were suspended in 20 ml organ bath containing modified Krebs-Henseleit solution and tied to an isometric force transducer. The bath temperature was maintained at 37^{0}C and aerated continuously. The tension was recorded using a polygraph digital data acquisition system linked to isometric transducer connected to a recorder. Tracheal strips were suspended at a preset-tension of 0.5 g and allowed to equilibrate for about 60 minutes, during which time they were washed thrice with Krebs-Henseleit solution at 15 minutes interval before adding the spasmogen.

Muscle-tension experiments with receptor-operated agonist Acetylcholine and acetone extract of culinary spices on sheep tracheal smooth muscle

Acetylcholine cumulative dose response curves were obtained with graded doses of acetylcholine [5×10^{-9}M to 3×10^{-4}M] applied on isolated sheep tracheal smooth muscles in the presence and absence of extract at a dose level of 0.25 mg. mL^{-1}. A known β-receptor antagonist, propranolol was added at a dose of 10^{-6}M to the bath along with extract to block the non specific relaxation, if any produced by β-receptor stimulation. The strips were incubated with extracts along with propranolol for about 10 minutes.

RESULTS

The representative physiographic recording of acetylcholine cumulative dose response is presented in Fig 1. Maximum cumulative acetylcholine dose-dependent contractile response in the absence of extracts was taken as 100% (Fig 2) and percent contractile response in the presence of extracts (0.25 mg/ml) was calculated and represented in Table 1 and Fig 1. Among the eight culinary spices taken, *Illicium verum* (hook fruit) revealed strongest anticholinergic activity by complete inhibition of acetylcholine contractile response (Fig 3), contractile response of acetylcholine in presence of *Cinnamomum zeylanicum* (bark) (Fig 4), *Terminalia chebula* (seed) (Fig 5), *Myristica fragarns* (seed) (Fig 6) and *Pimpinella anisum* (seed) (Fig 7), were 12.5, 28.57, 28.57 *and* 47.6%, respectively, whereas extracts of *Cuminum cyminum* (seed), *Menthe piperata* (leaf) and *Nigella sativa* (seed) enhanced the contractile response of acetylcholine.

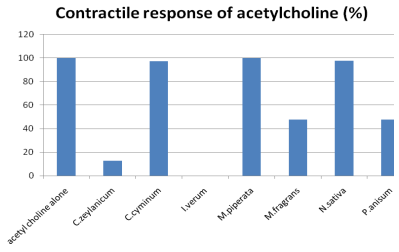

Fig 1 Percentage contractile response of acetylcholine on sheep tracheal smooth muscle after incubation in the presence of extracts of selected culinary spices at a bath concentration of 0.25mg/ml

METHODS

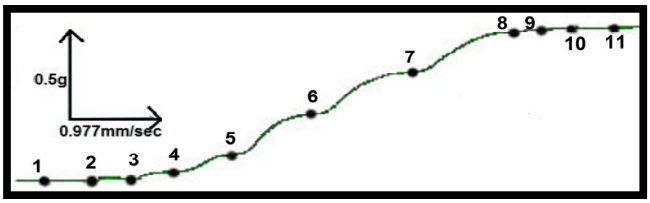

Figure 2 Representative physiographic recording of acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in sheep tracheal smooth muscle

(Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

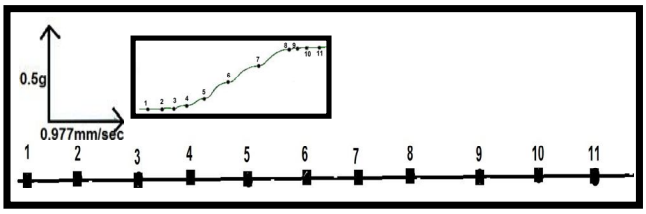

Figure 3 Representative physiographic recording of acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in the presence of *Illicium verum* (fruit) in sheep tracheal smooth muscle

(Graph in the insert represents normal cumulative dose response of acetylcholine in sheep skeletal smooth muscle; Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

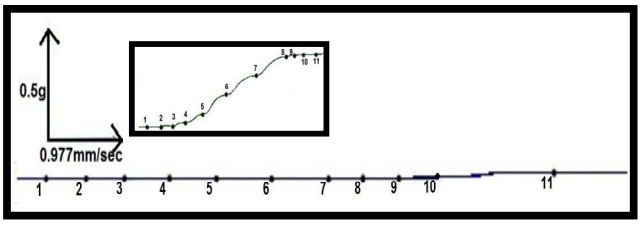

Figure 4: Representative physiographic recording of acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in the presence of *Cinnamomum zeylanicum* in sheep tracheal smooth muscle.

(Graph in the insert represents normal cumulative dose response of acetylcholine in sheep tracheal smooth muscle; Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

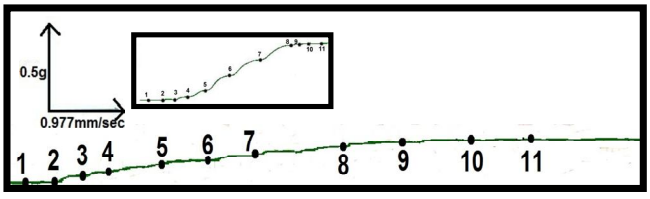

Figure 5 Representative physiographic recording of acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in the presence of *Terminalia chebula* in sheep tracheal smooth muscle

(Graph in the insert represents normal cumulative dose response of acetylcholine in sheep tracheal smooth muscle; Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

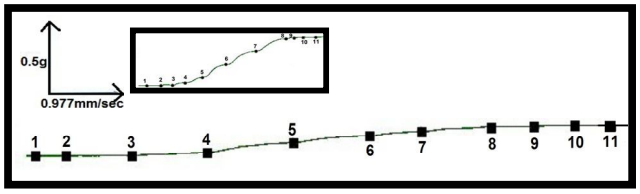

Figure 6 Representative physiographic recording of Acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in the presence of *Myristica fragrans (seed)* in sheep tracheal smooth muscle.

(Graph in the insert represents normal cumulative dose response of acetylcholine in sheep tracheal smooth muscle; Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

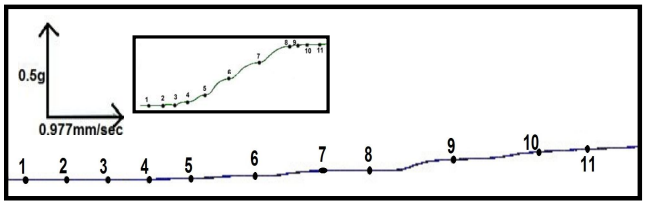

Figure 7 Representative physiographic recording of acetylcholine (5.0 x 10^{-9} to 3.0 x 10^{-4} M) cumulative dose response in the presence of *Pimpinella anisum* in sheep tracheal smooth muscle

(Graph in the insert represents normal cumulative dose response of acetylcholine in sheep tracheal smooth muscle; Numbers represent different doses (M) of acetylcholine; Dose 1 = 5.0 x 10^{-9}, Dose 2 = 1.5 x 10^{-8}, Dose 3 = 5.0 x 10^{-8}, Dose 4 = 1.5 x 10^{-7}, Dose 5 = 5.0 x 10^{-7}, Dose 6 = 1.5 x 10^{-6}, Dose 7 = 5.0 x 10^{-6}, Dose 8 = 1.5 x 10^{-5}, Dose 9 = 5.0 x 10^{-5}, Dose 10= 1.5 x 10^{-4}, Dose 11= 3.0 x 10^{-4})

Table 1 Percentage contractile response of acetylcholine on sheep tracheal smooth muscle in the presence of selected indigenous plant extracts at a concentration of 0.25mg/ml in the tissue chamber

Plant	Contractile response of acetylcholine (%)
C.zeylanicum	12.5
C.cyminum	97.2
I.verum	**0**
M.piperata	100
M.fragrans	47.6
N.sativa	97.6
P.anisum	47.6
T.chebula	28.57

C.zeylanicum = Cinnamomum zeylanicum, Bl (Bark), C.cyminum = Cuminum cyminum, L. (Seed), I.verum = Illicium verum (Fruit), *M.piperata = Mentha piperata* (hole plant) *M.fragrans = Myristica fragrans Houtt(seed), N.sativa = Nigella sativa, Linn(seed), P.anisum = Pimpinella anisum (seed), T.chebula = Terminalia chebula, Retz.* (seed)

DISCUSSION

Hyper responsiveness of the airway, inflammation, bronchial smooth muscle contraction and remodeling are the characteristics of Asthma. Corticosteroids, phosphodiesterase inhibitors like theophylline and β_2 agonists are employed in the treatment of asthma; they are limited for temporary relief and at higher doses they may lead to side effects. Certain herbal alternatives provide symptomatic relief in asthma and assist in inhibition of disease progression by bronchodilation, mast cell stabilization and immunomodulation.

The muscarinic, acetylcholine is involved in the regulation of broncho-constriction and muscarinic (M_3) receptors represent a primary target of acetylcholine in the airways (Fisher *et al.,* 2004). Muscarinic receptor regulation of airway smooth muscle tone is enhanced in asthma by two major mechanisms. One on them is exaggerated release of neuronal acetylcholine due to neuronal mechanisms associated with inflammation. Epithelial damage during allergic airway inflammation plays a key role in asthma and exposes sensory nerve endings in the sub-mucosa to airway lumen, which promotes reflex mechanisms leading to enhanced vagal release of acetylcholine. The second mechanism is, increased expression and enhanced function of signaling molecules essential for muscarinic receptor mediated airway smooth muscle contraction (Gosens and Kolahian, 2012). It is reported that both tracheal chain and strip preparations of small ruminants like sheep and goats are suitable for screening the spasmolytic activity of a drug in respiratory smooth muscle.

In the present study, an attempt was made to study the anticholinergic effect of acetone extracts of culinary spices on sheep tracheal smooth muscle. The anticholinergic activity of the tested culinary spices in the descending order is as follows *Illicium verum* (hook fruit), *Cinnamomum zeylanicum* (bark), *Terminalia chebula*(seed), *Myristica fragarns* (seed), *Pimpinella anisum* (seed). Presence of extracts of *Cuminum cyminum* (seed), *Menthe piperata* (leaf) and *Nigella sativa* (seed) enhanced contractile response of acetylcholine. Among the culinary spices, *Illicium verum* (hook fruit) (0.25 mg/ml in the bath) completely inhibited the acetylcholine-induced contraction and no contractile response (0%) was produced with acetylcholine indicating the strongest anticholinergic activity of the *Illicium verum* (Fig 3), which possibly explains its use in the treatment of upper respiratory tract problems and bronchial asthmatic attacks (Buchman *et al.,* 1987). The contractile response of acetylcholine in presence of *Cinnamomum zeylanicum* (bark) was 12.5% (Fig 4). *Terminalia chebula* (seed) (Fig 5) and *Myristica fragarns* (seed) (Fig 6) allowed a response to 28.57%, which may be considered as the scientific validation for the use of *Terminalia chebula* as a popular folklore medicine for cough, sore throat and asthma in north east India (Khan *et al.,* 2009). *Pimpinella anisum* (seed) (Fig 7), allowed a 47.6% contractile response for acetylcholine indicating moderate anticholinergic activity and this supports use of *Pimpinella anisum* in upper respiratory tract problems (Buchman *et al.,* 1987). It was reported that *Cuminum cyminum* relaxed guinea pig tracheal chain by its β_2 receptor stimulation (Boskabady *et al.,* 2004), but in this study it enhanced the contractile response of acetylcholine, which may be due to usage of propranolol to block the relaxation produced by β_2 receptor stimulation. In the absence of β_2 activity, *Cuminum cyminum* potentiates parasympathetic activity of acetylcholine.

In conclusion, the present study indicates that the acetone extracts of *Illicium verum* (fruit), *Cinnamomum zeylanicum* (bark), *Terminalia chebula* (seed), *Myristica fragarns* (seed) and *Pimpinella anisum* (seed) have considerable anticholinergic property. This particular property can be of use in developing the therapeutic candidates for the treatment of upper respiratory tract problems and bronchial asthmatic attacks.

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References

Al-Sereiti, M.R, Abu-Amer, K.M. and Sen, P. 1999. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J Exp Biol., 37(2): 124-30.

Aqel, M.B. 1991. Relaxant effect of the volatile oil of *Rosmarinus officinalis* on acheal smooth muscle. J Ethnopharmacol., 33(2): 57-62.

Baker, B., Peatfield, A.C. and Richardson, P.S. 1985. Nervous control of mucin secretion into human bronchi. J Physiol., 365:297–305.

Boskabady, M .H., Kiani, S. and Azizi, H. 2004. Relaxant effect of Cuminum cyminum on guinea pig tracheal chains and its possible mechanisms. Indian J Pharmacol., 37(2): 111-115.

Breschi, M.C., Martinotti, E., Apostoliti, F. and Nieri, P. 2002. Protective effect of silymarin in antigen challenge- and histamine-induced bronchoconstriction in *in-vivo* guinea-pigs. Eur J Pharmacology., 437(1-2): 91-95.

Buchman, D.D., (1987). *Herbal Medicine: The Natural Way to Get Well and Stay Well*, Century Hutchinson, London.

Choi J.R., Lee, C.M., Jung, I.D., Lee, J.S., Jeong, Y.I., Chang, J.H., Park, H.J., Choi, I.W., Kim, J.S., Shin, Y.K., Park, S.N. and Park, Y.M., 2009. Apigenin protects ovalbumin-induced asthma through the regulation of GATA-3 gene. Int Immunopharmacol., 9(7-8): 918-924.

Colebatch, H.J.H. and Halmagyi, D.F.J. 1963. Effect of vagotomy and vagal stimulation on lung mechanics and circulation. J Appl Physiol., 18: 881–887.

Das, M., Ram, A. and Ghosh, B., 2003. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. Inflamm Res., 52: 101-106.

Fernandez, J., Reyes, R., Ponce, H., Oropeza, M., Van Calsteren, M. R., Jankowski, C. and Campos, M.G. 2005. Isoquercitrin from *Argemone platyceras* inhibits carbachol and leukotriene D4-induced contraction in guinea-pig airways. Eur J Pharmacol., 522(1-3): 108-115.

Fisher, J.T., Vincent, S. G., Gomeza, J., Yamada, M. and Wess, J. 2004. Loss of vagally mediated bradycardia and bronchoconstriction in mice lacking M2 or M3 muscarinic acetylcholine receptors. The FASEB J., 18(6): 711–713.

Franova, S., Joskova, M., Novakova, E., Adamicova, K., Sutovska, M., and Nosal, S. 2009. Effects of flavin7 on allergen induced hyperreactivity of airways. Eur J Med Res., 14(4): 78-81.

Gosens, R. and Kolahian, S. 2012. Cholinergic Regulation of Airway Inflammation and Remodelling. J Allergy. Article ID 681258.

Holgate, S.T., Roberts, G., Arshad, Howarth, P.H., and Davies, D.E., 2009. The role of the airway epithelium and its interaction with environmental factors in asthma pathogenesis. Proc Am Thorac Soc., 6(8): 655–659.

Joskova, M., Franova, S. and Sadlonova, V., 2011. Acute bronchodilator effect of quercetin in experimental allergic asthma. Bratisl Lek Listy., 112(1): 9-12.

Joskova, M., Franova, S., Nosalova, G., Pechanova, O., Prisenznakova, L., and Sutovska, M. 2009. A beneficial influence of provinol on the reduction of allergen induced hyperreactivity in guinea-pigs. Bratisl Lek Listy., 110(8): 454-458.

Jung, C.H., Lee, J.Y., Cho, C.H. and Kim, C.J., 2007. Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. Arch Pharm Res., 30(12): 1599-1607.

Khan, A., and Gilani, A.H., 2006. Selective bronchidilatory effect of Rooibos tea (*Aspalathus linearis*) and its flavonoid, chrysoeriol. Eur J Nutr., 45(8): 463-469

Khan, M.H., and Yadava, P.S. 2009. Herbal remedies of asthma in Thoubal district of Manipur in north east India. Indian Journal of natural products and resources., 1: 80-84.

Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., Hakulinen, T. and Aromaa, A. 2002. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr., 76(3): 560-568.

Laitinen, L.A., Laitinen, M.V., Widdicombe, J.G., 1987. Parasympathetic nervous control of tracheal vascular resistance in the dog. J Physiol., 385: 135–146.

Leal, L.K.A.M., Costa, M.F., Pitombeira, M., Barroso, V.M., Silveira, E.R., Canuto, K.M., and Viana, G.S.B., 2006. Mechanisms underlying the relaxation induced by isokaempferide from *Amburana cearensis* in the guinea-pig isolated trachea. Life Sci., 79: 98–104.

Lee, M., Kim, S., Kwon, O.K., Oh, S.R., Lee, H.K. and Ahn, K. 2009. Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. Int Immunopharmacol., 9(4): 418-424.

Lin, B.Q., Li, P.B., Wang, Y.G., Peng, W., Wu, Z., Su, W.W., and Ji, H. 2008. The expectorant activity of naringenin. Pulm Pharmacol Ther., 21(2): 259-263.

Liu, B., Yang, J., Wen, Q. and Li, Y. 2008a. Isoliquiritigenin, a flavonoid from licorice, relaxes guinea-pig tracheal smooth muscle *in-vitro* and *in-vivo*:Role of cGMP/PKG pathway. Eur J Pharmacol., 587(1-3): 257-266.

Moon, H., Choi, H.H., and Lee, J.Y., 2008. Quercetin inhalation inhibits the asthmatic responses by exposure to aerosolized-ovalbumin in conscious guinea pigs. Arch Pharmacol Res., 31(6): 771-778.

Mustafa, S., Abdulraheem, A. and Alsughayer A., 2008. Verapamil induces calcium influx in the trachea. J Pharmacol Sci 106: 609-614.

Mustafa, S. and Oriowo, M.A. 1999. Inhibitory effect of capsaicin on cholinergic transmission in ovine airways: evidence for non- cholinergic contractions. Eur J Pharmacol., 385(2-3): 203-208.

Okamura, N., Haraguchi, H., Hashimoto, K., and Yagi, A. 1994. Flavonoids in *Rosmarinus officinalis* leaves. Phytochemistry., 37(5): 1463-1466.

Park, H.J., Lee, C.M., Jung, I.D., Lee, J.S., Jeong, Y.I., Chang, J.H., Chun, S.H., Kim, M.J., Choi, I.W., Ahn, S.C, Shin, Y.K., Yeom, S.R., and Park, Y.M. 2009. Quercetion regulates Th1/Th2 balance in a murine model of asthma. Int Immunopharmacol., 9(3): 261-267.

Rodriguez-Ramos, F., Gonzalez-Andrade, M. and Navarrete, A. 2011. Gnaphaliin A and B relax smooth muscle of guinea-pig trachea and rat aorta via phosphodiesterase inhibition. J Pharm Pharmacol., 63(7): 926-935.

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