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# Potential of black pepper as a functional food for treatment of airways disorders

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ABSTRACT

The potential of black pepper (*Piper nigrum*) to cause bronchodilation was examined using *in vivo* and *in vitro* assays. HPLC fingerprint analysis of the crude extract of *Piper nigrum* (Pn.Cr) and its fractions showed piperine, piperidine, eugenol and catechin as plant constituents. In anaesthetized rats, Pn.Cr and piperine relieved carbachol (CCh)-induced bronchospasm. In isolated guinea-pig trachea, Pn.Cr and piperine inhibited CCh and $K^+$ (80 mM)-induced contractions, potentiated isoprenaline concentration–response curves (CRCs) and suppressed $Ca^{2+}$ CRCs. In guinea-pig atria, Pn.Cr and piperine showed stimulatory and inhibitory effects on rate and force of contraction. Its fractions showed similar activities with varied potency in the *in vivo* and *in vitro* assays. These results suggest that black pepper and piperine cause bronchodilation through dual inhibition of phosphodiesterase enzyme and $Ca^{2+}$ influx, which substantiate its potential as a functional food ingredient for airway disorders.



## 1. Introduction

*Piper nigrum* Linn. belongs to the family *Piperaceae* and its dried unripe fruit is used commonly as "black pepper." This perennial shrub is indigenous to the Western coasts of India and tropical parts of Asia. In Pakistan, it grows mainly in Northern areas, such as Gilgit and Chitral valleys, and is vernacularly known in Urdu as "kali mirch" (Usmanghani, Saeed, & Alam, 1997). Black pepper – the dried unripe fruit of *Piper nigrum* – is used as a condiment in various cuisines worldwide and is a part and parcel of every kitchen. In 2013, the global

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production of black pepper was reported to be nearly 355,000 tonnes (Anonymous, 2008). Due to its widespread consumption, biochemical and pharmacologic properties of black pepper have been investigated extensively (Butt et al., 2013).

Phytochemical studies on black pepper have determined the presence of various minerals, vitamins (β-carotenes, tocopherols, ascorbic acid, thiamine, riboflavin and niacin), polysaccharides (arabinose, rhamnose, galacturic acid), sterols (β-sitosterol, terpenoids, sesquiterpenes), fatty acids (linoleic acid), volatile oils (camphenes, pinenes), alkaloids (piperine, piperidine, piperolein, capsaicin, 2-dihydrocaspaicin), resins (chavicin), organic acids (hexadecanoic acid, octadecanoic acid), amides (pipnoohine, pipyahyine, guineensine, pipericide) and various phenolic compounds (benzamides, gallic acid, kaempferol, coumarins, quercetin) (Duke, 1992; Siddiqui et al., 2004; 2005). Piperine, the active principle of black pepper, has been found to possess anti-inflammatory (Mujumdar, Dhuley, Deshmukh, Raman, & Naik, 1990), anti-convulsant (D'Hooge et al., 1996), hypoglycaemic (Panda & Kar, 2003), immunomodulatory (Sunila & Kuttan, 2004), anti-depressant (Lee et al., 2005), vasomodulatory (Taqvi, Shah, & Gilani, 2008), bioavailability-enhancing (Veda & Srinivasan, 2009), anti-spasmodic (Mehmood & Gilani, 2010), anti-hyperlipidaemic (Chen, Ma, Liang, Peng, & Zuo, 2011), insecticidal (Park, 2012), anti-tumour (Do et al., 2013), cardioprotective (Dutta et al., 2014) and anti-oxidant (Embuscado, 2015) properties. More recently, novel compounds isolated from black peppercorns have been found to possess larvicidal activity against *Aides egypti*, the principle vector of dengue virus (Santiago, Alvero, & Villaseñor, 2015).

Besides being used as a spice, black pepper enjoys folkloric reputation for use in airway disorders including bronchitis and asthma (Duke, Bogenschutz-Godwin, Du Celliar, & Duke, 2002; Kapoor, 1990; Usmanghani et al., 1997). Currently, no scientific evidence is available to support its use in respiratory disorders. We hypothesized that black pepper may contain certain bioactive constituents that can cause bronchodilation. As black pepper is consumed heavily throughout the world, precipitation or relief of bronchospasm by its chemical constituents can have important nutraceutical implications. Moreover, as black pepper is an ingredient of numerous recipes including soups, it may be an attractive option for the development of functional foods and nutraceuticals. Therefore, in the present study, we investigated the effects of black pepper and its active principle – piperine – on tracheal tissues through *in vitro* and *in vivo* experiments.

## 2. Materials and methods

### 2.1. Chemicals

Acetylcholine (ACh) perchlorate, carbachol (CCh), papaverine, isoprenaline hydrochloride, histamine hydrochloride and verapamil hydrochloride were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Piperine, Tween80, thiopental sodium and castor oil were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), Scharlau Chemie S.A. (La Jota, Barcelona, Spain), Abbott Laboratories (Karachi, Sindh, Pakistan) and KCL Pharma (Karachi, Sindh, Pakistan). Chemicals used for making physiological salt solutions were sodium chloride (BDH Laboratory Supplies, Poole, England), potassium chloride (Sigma Chemicals Co.), calcium chloride, glucose, magnesium chloride, magnesium sulphate, sodium bicarbonate, sodium dihydrogen phosphate and potassium dihydrogen phosphate (Merck, Darmstadt, Germany). All chemicals used were of the analytical grade available and dissolved in distilled water, except piperine, which was solubilized by Tween80.

### 2.2. Animals

Sprague–Dawley rats (200–250 g) and guinea-pigs (350–550 g) of either sex were used in this study and housed at the Animal House of the Aga Khan University, maintained at 23–25 °C. Animals were given tap water *ad libitum* and a standard diet. Guinea-pigs had free access to water, but food was withdrawn 24 hours prior to the experiment. Experiments performed on these animals complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethics Committee for Animal Care and Use (ECACU) at Aga Khan University, Karachi, Sindh, Pakistan (approval number 42-ECACU-BBS-14). This study is also the part of the PhD proposal of Mr. Muhammad Hanif, approved by the Board of Advanced Studies and Research (BASR/NO/02535/Pharm), University of Karachi, Karachi, Pakistan.

### 2.3. Plant material

For the purpose of this study, dried fruits of *Piper nigrum* were used, which are commonly known as "kali mirch" in Urdu (native language) and "black pepper" in English. Botanical name of this plant was further confirmed using an online resource (http://www.theplantlist.org) on 25th January, 2014. Dried fruits of *Piper nigrum* were bought from a local market (Jouria Bazaar) in Karachi, Sindh, Pakistan and were authenticated by a qualified botanist (Dr. Altaf A. Dasti, late) from the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Punjab, Pakistan. A sample voucher (Pn-F-06-07-72A) was submitted to the herbarium of Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan.

### 2.4. Preparation of crude extract and its fractionation

After cleaning of adulterant material, 450 g of fruits was crushed and soaked in 3 L of 70% aqueous-methanol for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a Whatman (Maidstone, UK) qualitative grade 1 filter paper (Williamson, Okpako, & Evans, 1996). This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator (model RE-111, Buchi, Flawil, Switzerland) to obtain the crude extract (Pn.Cr) yielding approximately 8.7%.

Activity-guided fractionation was carried out by using solvents of increasing polarity to obtain organic and aqueous fractions. Pn.Cr was dissolved in about 150 mL of distilled water and petroleum ether was added to it with vigorous shaking. This solution was taken in a separating funnel and the layers were allowed to separate. The petroleum ether (upper) layer was removed and petroleum ether was added to the remaining