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Comparative GC Analysis, Bronchodilator Effect and the Detailed Mechanism of Their Main Component—Cinnamaldehyde of Three Cinnamon Species

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Abstract: Cinnamon is one of the most commonly used spices worldwide. In some Arab countries, cinnamon is used with other ingredients to relieve bronchospasm and treatment of airways-related disorders. In the current study, GC, GC-MS and tracheal relaxant effect comparison were performed using the three available types in Saudi Arabia, *Cinnamomum verum* (Ceylon cinnamon), *C. cassia* (Chinese cinnamon) and *C. loureiroi* (Vietnamese cinnamon). The essential oil of *C. verum* was the most potent in the relaxation of guinea pig isolated tracheal muscles against carbachol (CCh, 1 uM)-evoked bronchospasm at the concentration range from 0.03 to 3 mg/mL followed by *C. bureiroi* at 0.03 to 5 mg/mL; whereas, *C. cassia* was the least potent oil. Cinnamaldehyde (**1**), isolated as the main component of the three oils induced complete relaxation of low K^+ (25 mM)-evoked contractions, with mild effect on the contractions evoked by high K^+ (80 mM). Pre-incubation of the tracheal tissues with glibenclamide (10 µM) significantly opposed the relaxation of low K^+ by cinnamaldehyde. The standard drug, cromakalim also inserted glibenclamide-sensitive inhibition of low K^+ without relaxing high K^+. These results indicate that cinnamaldehyde acts predominantly by ATP-specific K^+ channel opening followed by weak Ca^{++} antagonistic effects. The obtained results justify the medicinal value of cinnamon oil in respiratory disorders.

Keywords: *Cinnamon* sp.; bronchoconstriction; cinnamaldehyde; KATP channel activator; guinea pigs

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1. Introduction

Cinnamon is a known spice obtained from the inner bark of several trees belonging to the genus *Cinnamomum*. Cinnamon is used mainly as an aromatic condiment and flavouring additive. The aroma and flavour of cinnamon derive from its essential oil and the principal component, cinnamaldehyde, as well as numerous other minor constituents including eugenol [1–3]. In 2017, four countries accounted for 99% of the world's total cinnamon production: Indonesia, China, Vietnam and Sri Lanka [4]. There are many types of cinnamon, all of which come from the inner bark of several species of evergreen trees belonging to the genus *Cinnamomum*. However, there are two varieties that are most likely commercially available for use in food products: *Cassia* and Ceylon. *Cinnamomum verum*, also called "true cinnamon" or Ceylon cinnamon, is a more expensive and difficult-to-find cinnamon variety. Ceylon has a lighter and sweeter flavour than *Cassia*. Most cinnamon in international commerce is derived from the related species *C. cassia*, known as "Cassia" or Chinese cinnamon [2]. Indonesian cinnamon is derived from *C. burmanni* while Vietnamese

cinnamon is derived from *C. loureiroi* [5]. In addition to the folkloric use of cinnamon as a spice and flavouring agent, it is mixed as a refreshing agent in chewing gums where it protects and stops bad breath [6]. Cinnamon also has a role in protecting the colon by decreasing the risk of colon cancer [7]. It also exerts a coagulant effect playing a role in bleeding prevention [8].

Ten randomized controlled trials including 543 patients have established that cinnamon, when taken in a dose of 0.12 to 6 g/day for a period of approximately 4 months leads to a statistically significant decrease in the level of fasting plasma glucose in addition to an improvement in the lipid profile [9]. Among the multiple reported activities of cinnamon is the diverse antimicrobial effect against various organisms such as bacteria such as *Staphylococcus aureus* and fungi such as *Aspergillus flavus*, *Mucor plumbeus* and *Candida lipolytica* [10]. The methanol extract of cinnamon has been reported to contain maximum anti-oxidant properties compared to ethanol and water extracts [11]. Moreover, cinnamon has been reported to have an anti-inflammatory potential [12], antitermitic [13], nematicidal [14], mosquito larvicidal [15], insecticidal [16], antimycotic, [17] and anticancer activities [18]. One of the primary constituents of the extracted essential oil from *C. zeylanicum* known as (E)-cinnamaldehyde has been reported to have antityrosinase activity [19,20].

Respiratory tract diseases (RTDs) such as bronchoconstriction are associated with microbial infection and inflammation, having a debilitating effect on the health of a large number of people worldwide. Aromatic medicinal plants are characterized by elaborating mixtures of volatile compounds known as essential oils (EOs) with diverse biological activities. Due to the volatile nature of the EOs, they can be administered by inhalational to offer effective treatment in various respiratory disorders as they have easy access to the upper and lower parts of the respiratory tract. For example, Anise oil obtained from the fruits of *Pimpinella anisum* is used traditionally as an expectorant for the treatment of cough associated with the common cold [21]. Peppermint oil obtained from the fresh aerial parts of the flowering plants of *Mentha piperitae* is used for the treatment of coughs and colds in addition to the symptomatic treatment of digestive disorders such as flatulence and irritable bowel syndrome. Inhalation of peppermint oil vapours after the addition of a few drops to hot water is used traditionally for respiratory problems [21]. While *trans*-anethole, anisaldehyde and methyl-cavicol are the main components in Anise oil [22], peppermint oil contains mainly menthol, menthon, isomenthone, menthyl acetate, menthofuran, 1,8-cineole, limonene, pulegone and carvone [21]. We recently reported on the components and bronchodilatory effect of *Achillea fragrantis*. GC study revealed the presence of α-thujone, β- thujone and sabinol as the principal constituents [23]. The bronchodilator activity of the plant EO was evaluated using isolated guinea pig tracheal strips. A mechanistic study indicated that the bronchodilation was mediated by a combination of anti-muscarinic, Ca^{++} channel inhibition and K^{+} channel activation [23]. The essential oils obtained from various *Thymus* species have been reported for their effectiveness against airway disorders [24]. The GC study of the EOs obtained from *Thymus serrulatus* collected from different Ethiopian localities found them to contain thymol, carvacrol, *p*-cymene, γ-terpenene, and rosmarinic acid [25]. The two monoterpenes, thymol and carvacrol were the major constituents of the EOs. Exploring the mechanism of action of the bronchodilator effect of the *T. serrulatus* EO revealed that the oil expressed tracheal relaxants including anticholinergic, Ca^{++} antagonist and PDE inhibitory-like effect. Interestingly, the oil also expressed marked antimicrobial effects against strains involved in bronchial infections [26].

Keeping in view the importance of EOs' therapeutic role in respiratory diseases, in the present research, we provided a comparison of the chemical components of the EOs of three *Cinnamon* species as well as their bronchodilator effects. The detailed pharmacodynamics for the bronchodilator effect of cinnamaldehyde, the main component of the different *Cinnamomum* species, was also explored.

2. Materials and Methods

2.1. General

One- and two-dimensional Nuclear Magnetic Resonance (NMR) data were accumulated on a 500 MHz spectrometer Bruker UltraShield Plus (Billerica, MA, USA). The resonance of the protons is achieved at 500 MHz while carbon-13 atoms resonate at 125 MHz. The reported chemical shifts δ (ppm) were calculated based on the residual C_6D_6 peaks value, while the *J* values were measured in Hertz (Hz). The two-dimensional experiments were recorded under the control of the standard program from Bruker. HRESIMS were determined using UPLC RS Ultimate 3000—Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA). The mass spectrometer was fitted with an (HR/AM) Orbitrap™ detector. All samples were measured by direct injection. Chromatotron (Harrison Research Inc. model 7924, Union, NJ, USA) was used for Centrifugal preparative TLC (CPTLC). Routine TLC analyses were performed using Kiesel gel 60 F254 (Merck, (Merck, Kenilworth, NJ, USA) plates. The spots were detected by means of a UV lamp (entela Model UVGL-25, (CN-15-MC, Vilber Lourmat, Cedex, France) operated at 254 nm.

2.2. Chemicals

Carbachol (CCh) (98%), sodium borohydride (98%), acetic anhydride and glibenclamide (Gb) (99%), alkane standard mixture C7–C40 (49452-U), W228613-Cinnamaldehyde (95%) were obtained from Sigma Chemicals Co, St Louis, MO, USA, while cromakalim (99%) from Tocris, Ellisviille, MO, USA. Potassium chloride, anhydrous sodium sulphate, calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate (Merck, Darmstadt, Germany) and sodium chloride (BDH Laboratory supplies, Poole, UK). Silica gel high-purity grade (Merck Grade 7749) with gypsum binder and fluorescent indicator for TLC was used to prepare the 4 mm disc used in CPTLC.

2.3. Plant Materials

The dried decorticated barks of *Cinnamomum verum*, *C. cassia* and *C. loureiroi* were purchased from the local market in Al-Kharj city, Riyadh Province, Saudi Arabia. The samples were authenticated by Dr Mona Alwahibi, Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia and voucher specimens (# 13754, 13755, 13756) were deposited at the herbarium of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University (KSU), Riyadh, Saudi Arabia.

2.4. Essential Oils Preparation

Samples of 100 g from each cinnamon sample after grinding were used for EOs preparations. The EOs were prepared by hydrodistillation method for 5 h using Clevenger-type apparatus fitted with a round-bottom flask of 2 L capacity. At the end of the process, the oil layers were separated from the water condensates. The condensates were subjected to extraction with diethyl ether. The ethereal layers were added to the oils and dehydrated using anhydrous Na_2SO_4. The ether was then distilled off under a vacuum (350 mbar) to obtain the EOs. The process was run in triplicate for each sample.

2.5. GC/MS Analysis

Aliquots of the oils were diluted with chloroform (1 µL of 10 ppm concentration) and injected into the GC/MS apparatus (Agilent Model 7890 MSD) via autosampler. The temperature program start point was 40 °C, held constant for 2 min, followed by a gradual increase at a constant rate of 5 °C/min to 120 °C and the temperature was held isothermally for 5 min. The temperature was then increased at the rate of 10 °C/min till 290 °C where it was held for 5 min. The used column was an HP5MS column of 30 m length, 0.25 mm i.d. and 0.25 µm thickness. The carrier gas used was helium of 99.999% purity applied with a

flow rate of 1.2 mL/min. Injector temperature was constant at 280 °C. The injected volume was 1 µL, in the splitless mode. The mass spectrometer ionization voltage was 70 eV, the mass scan range was 30–600 mass units, and the ion-source temperature was held at 280 °C.

2.6. GC/FID Analysis

Chromatograms of the analyzed samples were recorded utilising using Agilent 7890B gas chromatography fitted with HP-5 19091J-413 (30 m × 0.25 mm) capillary column and FID detector. Conditions similar to the GC/MS analysis were adopted. The area for each peak was measured automatically to enable peak quantification. Identification of the EOs' chemical compositions was based on a comparison of the compounds' mass spectra with the NIST database and a comparison of their retention indices, relative to a series of *n*-alkanes C7–C40 (49452-U) (RRI), with the reported literature values using NIST 2017 (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.7. Purification of Compounds **1**

About 500 mg of *C. loureiroi* oil was purified by the CPTLC technique. An isocratic system using a mobile phase composed of hexane/ethyl acetate 9:1 was applied for the separation. The first eluted band detected under UV light afforded 315 mg of **1**.

2.8. Synthesis of Compound **2**

About 200 mg of **1** was dissolved in 10 mL methanol and 300 mg of sodium borohydride was added with stirring for 1 h at room temperature. The reaction mixture was mixed with 50 mL of distilled water and then extracted with chloroform (3 × 20 mL). The chloroform layers were combined, dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure to afford chromatographically homogenous product identified as cinnamyl alcohol (**4**) (194 mg) (Supplementary Materials). To 100 mg of cinnamyl alcohol, about 150 µL of acetic anhydride was added and the reaction mixture was kept overnight at room temperature. Distilled water (20 mL) was added to the mixture followed by extraction with chloroform (3 × 10 mL). The organic layers were combined, dried over anhydrous sodium sulphate then the solvent was evaporated under reduced pressure to afford 95 mg of **2**.

2.9. Animals

Guinea pigs with a body weight of about 0.5 kg of either gender were procured from the KSU animal care unit and kept at the Animal Care Unit, College of Pharmacy, Prince Sattam Bin Abdulaziz University (PSAU), KSA under a controlled temperature range of 23–25 °C. Animals were free to access tap water and commercial standard animal diet. All the ex vivo assays conducted qualifies the instructions specified by the Institute of Laboratory Animal Resources, Commission on Life Sciences, NRC [27]. The study protocol has been followed with prior approval of the Bio-Ethical Research Committee (BERC) at PSAU with reference number BERC-001-12-19.

2.10. Guinea Pig Trachea

The trachea of guinea pigs sacrificed by holding their heads in between the middle and index figure in an upright hanging position and where cervical dislocation was made after a strong jerk. The tracheal tube was dissected out and preserved in a suitable physiological buffer (Kreb's solution). The tracheal tube was cut into 7–8 individual tissues where each tissue ring was opened by a longitudinal cut on the cartilage parallel and opposite to the smooth muscle layer. The prepared trachea tissue was mounted in a 10 mL tissue bath containing Kreb's solution, maintained at 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2 (carbogen). The concentration buffer solution was (mM): KCl 4.7, KH_2PO_4 1.3, NaCl 118.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 11.7 with pH 7.4. A constant tension measuring 1 g was applied to each tracheal strip throughout the experiment. An equilibration period of 60 min was elapsed before the addition of any drug to the tissues

with the replacement of the fresh buffer every 15 min. The CCh (1 µM) was used for tissue stabilization until the comparable peak of contractions with 1 uM of CCh concentration was achieved. Constant superimposable contractions were achieved and the relaxant effect of the essential oil and standard drugs were recorded by adding cumulative concentrations to obtain concentration-dependent relaxant responses. An isometric transducer was used to record responses on an isolated organ bath (emkaBATH, France) with iox software (2.10.8.6) installed.

2.11. Statistical Analysis

The data expressed are mean ± standard error of the mean (SEM, n = number of experiments) and median effective concentrations (EC_{50}) with 95% confidence intervals (CI). A difference of $p < 0.05$ was considered statistically significant. Relaxant concentration–response curves were analyzed by non-linear regression using the GraphPad program (GraphPAD, San Diego, CA, USA).

3. Results

3.1. Essential Oils Preparation

The percentage yields of the oils from different cinnamon types are presented in Table 1.

Table 1. The yield of EOs prepared from *C. verum*, *C. cassia* and *C. loureiroi* *.

	C. verum	*C. cassia*	*C. loureiroi*
Sample weight (g)	100	100	100
Oil weight (g)	2.72 ± 0.079	2.2 ± 0.094	2.5 ± 0.082
Total % Yield (*W*/*W*)	2.72%	2.2%	2.5%

* Expressed values are the mean of triplicate determination (n = 3) ± standard deviations.

3.2. GC/MS and GC/FID Analysis

The EO's components were achieved by comparing the MS spectra data with the data of the library of the National Institute of Standards and Technology (NIST 2017). The relative retention index (RRI) was used for peak identification and was calculated using n-Alkanes series C7–C40. The relative percentage of each EO's component was obtained via the computerized peak area measurements following the samples injection using the autosampler (Table 2, Figures 1 and 2).

Table 2. Components of the EOs of three cinnamon species.

Components	**RT (min)**	**Estimated RI**	**Literature RI**	**Area %**		
				Ceylon Cin	**China Cin**	**Vietnam Cin**
(*E*)-Cinnamaldehyde (**1**)	17.9662	1266	1266	94.70	99.99	97.52
Cinnamyl acetate (**2**)	20.7994	1415	1418	1.668	-	-
2-Methoxycinnamaldehyde (**3**)	24.4865	1509	1512	0.493	-	**-**
Total % Yield				**96.864**	**99.99**	**97.52**

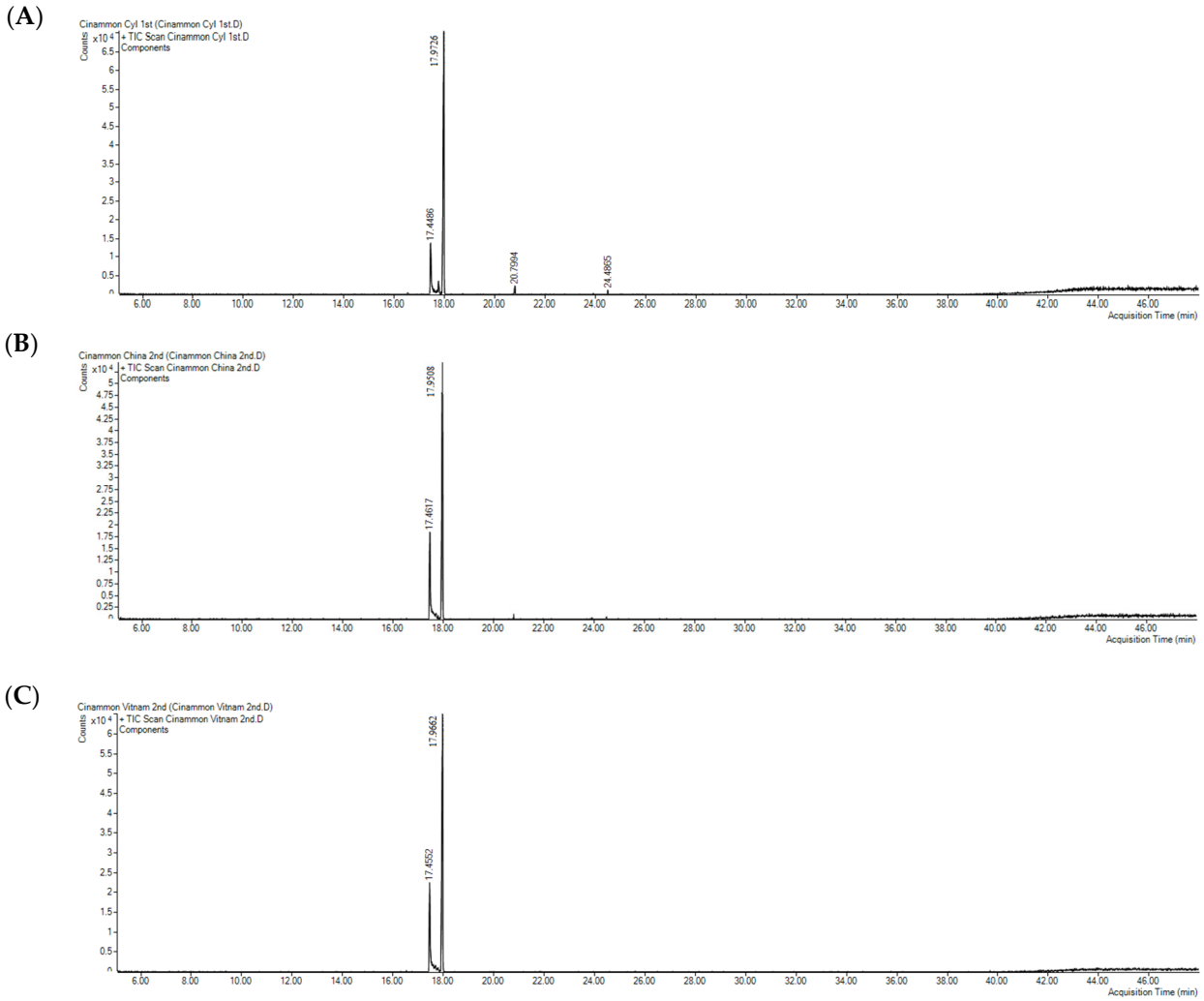
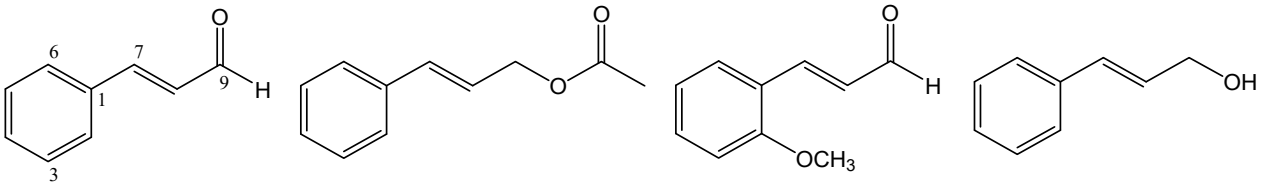


Figure 1. GC chromatograms of the EOs obtained by hydrodistillation from *C. verum* (**A**), *C. cassia* (**B**) and *C. loureiroi* (**C**).



1 **2** **3** **4**

Figure 2. Chemical structure of cinnamon EOs components.

3.3. Effect on Trachea

C. verum caused concentration-dependent relaxation of CCh-mediated contractions of guinea pig isolated tracheal preparations with EC_{50} values of 1.08 mg/mL (0.92–1.24, 95% CI, n = 5) while *C. bureiroi* and *C. cassia* inhibited this contraction with comparable concentrations having EC_{50} values of 3.84 mg/mL (3.42–4.14, 95% CI, n = 5) and 4.12 mg/mL (3.68–4.58, 95% CI, n = 5), respectively (Figure 3).

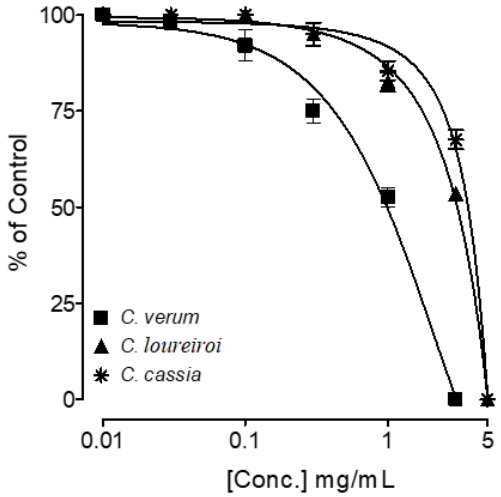


Figure 3. Concentration–response curves showing the comparison of the essential oil *C. verum*, *C. bureiroi* and *C. cassia* for the bronchodilator effect against carbachol (CCh; 1 μM)-induced contractions in guinea pig tracheal strips preparations. Results are expressed as mean ± SEM, n = 4.

Among the pure compounds tested, complete inhibition of CCh-1 uM mediated contractions was observed using **1** with resultant EC_{50} values of 0.40 mg/mL (0.29–0.54; n = 4) while non-significant ($p > 0.05$) inhibition was observed with compound **2** with maximum relaxation of 5% whereas compound **4** was found inactive at the highest tested concentration of 1 mg/mL (Figure 4).

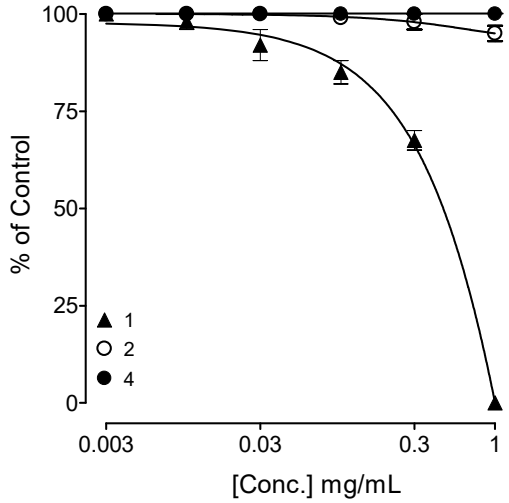


Figure 4. Concentration–response curves showing the comparison of the tracheal relaxation of compounds (**1**), (**2**) and (**4**) against carbachol (CCh; 1 μM)-induced bronchoconstriction in guinea pig tracheal strip preparations. Results are expressed as mean ± SEM, n = 4.

When evaluated on the high K^+ (80 mM)-evoked spasms, **1** showed partial relaxation (14%, n = 4) at the highest tested concentration (1 mg/mL), but the low K^+ (25 mM)-evoked spasm was completely reversed with recorded EC_{50} value of 0.18 mg/mL (0.10–0.26, n = 4) as shown in Figure 3A. Cromakalim also caused selective relaxation of the contractions induced by low K^+ with an EC_{50} value of 6.32 μM (5.82–7.25, n = 4), with no effect on the high K^+ (80 mM)-induced bronchoconstriction (Figure 5B). Pretreatment with the ATP-mediated K+ channel blocker glibenclamide at 10 μM, the bronchodilator effect of cinnamaldehyde against low K^+ (25 mM)-induced bronchoconstriction was significantly reversed (Figure 6A), similar to the suppressive effect resulted from cromakalim on tissues pretreated with glibenclamide (Figure 6B).

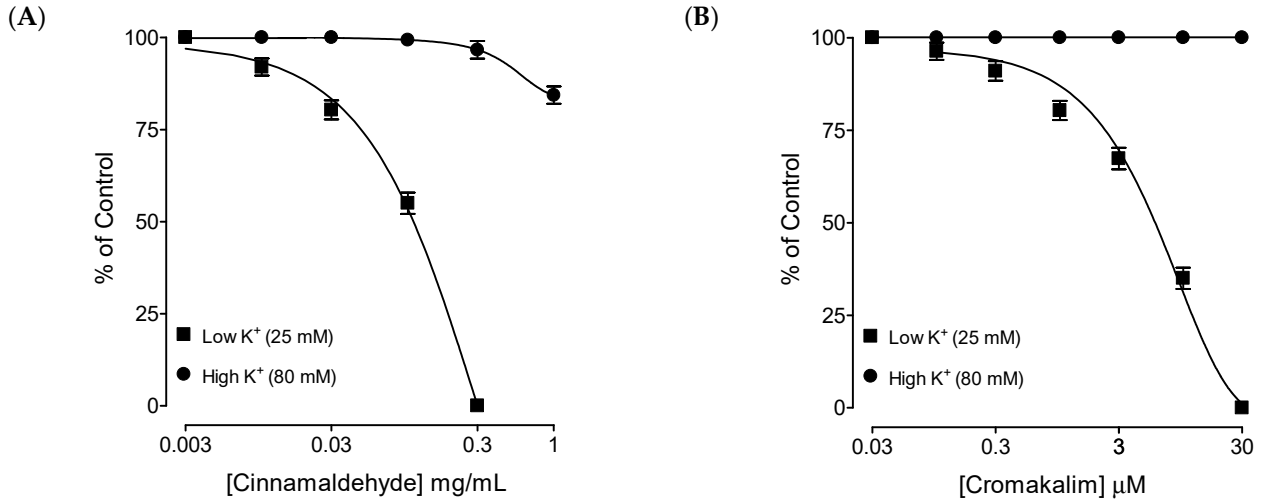


Figure 5. Concentration-dependent bronchodilator effect of (**A**) cinnamaldehyde and (**B**) cromakalim, against low K^+ (25 mM) and high K^+ (80 mM)-induced bronchoconstriction using isolated guinea pig tracheal strip preparations. Results are expressed as mean ± SEM, n = 4.

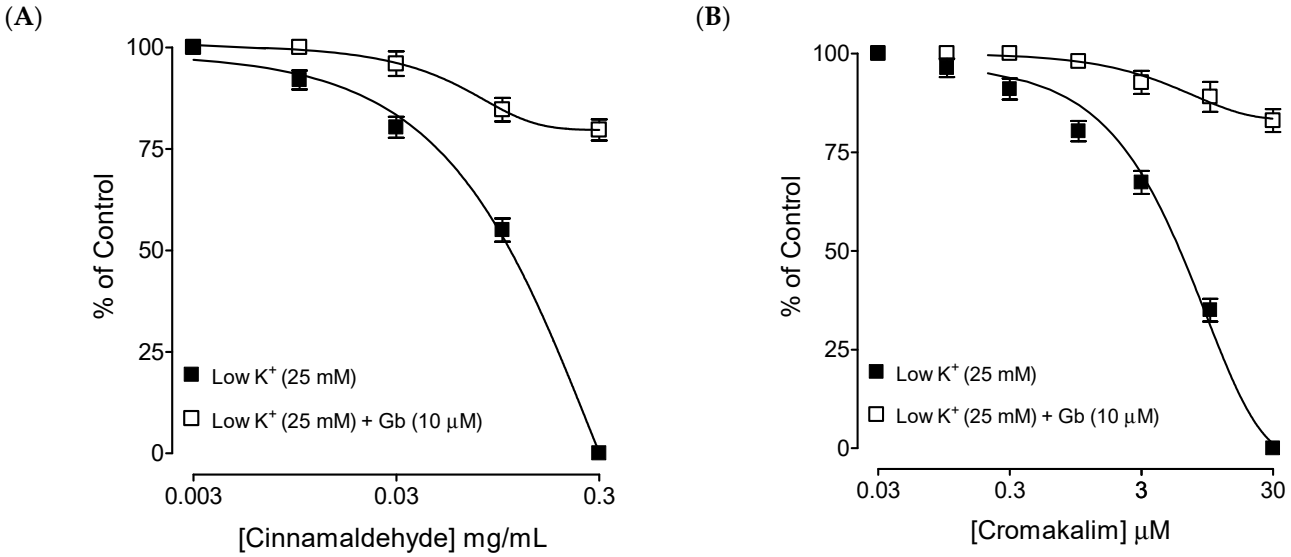


Figure 6. Concentration-dependent inhibitory effect of (**A**) cinnamaldehyde and (**B**) cromakalim, against low K^+ (25 mM) in the absence and pretreatment with glibenclamide (Gb; 10 µM) using isolated guinea pig tracheal strip preparations. Results are expressed as mean ± SEM, n = 4.

4. Discussion

GC-MS and GC evaluations indicated that the three oil samples extracted from different geographical regions, *Cinnamomum verum* (Ceylon cinnamon), *C. cassia* (Chinese cinnamon) and *C. loureiroi* (Vietnamese cinnamon), contain (*E*)-Cinnamaldehyde (**1**) as the predominant component representing more than 94%. While in the case of *C. bureiroi* and *C. cassia,* no other compounds were identified, *C. verum* GC spectrum showed two other peals identified as Cinnamyl acetate (1.668%) (**2**) and 2-methoxycinnamaldehyde (0.493%) (**3**) (Table 2) [28,29]. In view of the medicinal use of cinnamon in bronchoconstriction, these three essential oils were evaluated in isolated guinea pig tracheal smooth muscles for their possible relaxant effects on evoked contractions with multiple agents. *C. verum*, the "true cinnamon" or Ceylon cinnamon, showed a relaxant effect with EC_{50} values of 1.08 mg/mL while the Vietnamese cinnamon, *C. bureiroi* and the Chinese cinnamon, *C. cassia* expressed EC_{50} values of 3.84 mg/mL and 4.12 mg/mL (Figure 3). As *C. verum* oil was found to be more active than the other two oils although it has less percentage of **1**, the impact of the

minor components was explored. Compound **1** was isolated by CPTLC and identified via its spectral data (Supplementary Materials). The most characteristic ^{1}HNMR features of **1** were the propan-1-al moiety signals. The 16 Hz *J* value of the doublet at δ_H 7.49 ppm assigned to H-7 was diagnostic for the *trans* orientation of the **1**, while the appearance of the aldehyde signal as a doublet at δ_H 7.49 ppm 4.99 ppm (*J* = 6.3 Hz) further supported the structure of **1**. The aldehyde carbon was observed at δ_c 194.49 ppm in the ^{13}CNMR spectrum of **1** [30]. Compound **1** was reduced to the corresponding alcohol using sodium borohydride [31]. The reaction yielded single product **4** characterized by the replacement of the aldehyde signals by CH_2OH signals at δ_H 4.23 (d, *J* = 6.3) and δ_c 62.39 ppm [32] and identified as *trans*-cinnamyl alcohol. Compound **4** was not detected in any of the three oils in the current study. In turn, **4** was esterified with acetic anhydride in the presence of pyridine to yield **2** (Supplementary Materials) [33]. The spectra of **2** showed a *downfield* shift of the CH_2 proton signal to δ_H 4.66 (d, *J* = 6.3 Hz) and δ_c 64.81 ppm compared with **4**. The acetyl signals appeared at δ_H 2.03, δ_C 19.76 and, δ_C 171.13 ppm [34]. Testing the pure compounds **1**, **2** and **4** for their bronchodilator potential in isolated trachea indicated that **1** is the most active with EC_{50} = 0.40 mg/mL, **2** expressed a very weak non-significant effect ($p > 0.05$) while **4** which is not present in the oils was found inactive. These critical interpretations of the results indicated that the presence of the α, β unsaturated carbonyl group is essential for the observed activity. This finding can be supported by our earlier demonstration of the bronchodilator activity of some phenylpropanoid glycosides all with α, β unsaturated carbonyl. Interestingly, this study revealed that 2-mono-oxygenated compounds were more active than the 1, 4-di-oxygenated ones [35]. These results indicate that the stronger effect of *C. verum* oil is most likely due to the 2-mono-oxygenated derivative **3**. However, this assumption requires further investigation with more detailed experimental support.

The detailed pharmacodynamics for the tracheal relaxation was explored in the major active phytoconstituent **1** commonly possessed by these species. In our earlier reported studies, it was noted that the common spasmolytic effect of the medicinal herbs in guinea pig trachea is usually mediated through a combination of K^+ channel activation [36] and/or Ca^{++} antagonism [23]. Therefore, to explore the involvement of similar pharmacodynamics in the bronchodilator effect of **1**, it was tested (in a concentration-dependent way) on K^+-evoked contractions. However, its partial relaxation against high K^+ is an indication of some alternate components' involvement other than the CCB-like effect. Hence, it was tested against the evoked contractions by low K^+ where interestingly **1** caused complete inhibition. It is well known that a substance that is selectively efficacious in relaxing the low K^+ is labelled as a K+ channel opener whereas CCBs show comparable potency and efficacy against both types of K+-evoked spasms. Thus, these assays distinguish K^+ channel openers from CCBs [37–39]. The experimental protocols were further extended to identify the specific subtype of the K^+ channel involved in the tracheal relaxation was explored to be mediated via ATP-dependent K^+ channels when the relaxation of low K^+-evoked spasms was prevented in the pre-incubated strips with a specific blocker of ATP-dependent K^+ channels (KATP), known as glibenclamide [40,41]. Similar to cinnamaldehyde, selectively low K relaxation was reproduced with cromakalim, a prototypical KATP channel opener [42–44]. These findings indicate that the tracheal smooth muscle antispasmodic effect observed with cinnamaldehyde is inserted by a dual components involvement, the predominant KATP channel activation and followed by a weak CCB-like effect. Multiple earlier reports have shown that K^+ channel openers exert a wide range of therapeutic activities against multiple health-related issues such as asthma, GIT upsets, high BP and urinary system problems [45–47]. These agents by their specific action of K^+ channels activation, result in hyperpolarization through the increase in K^+ outflux, which ultimately decreases the intracellular free Ca^{++} and thus leads to generalized smooth muscle relaxation [48].

Cinnamon, whether grown wildly or cultivated, requires a temperature between 20–30 °C, annual rainfall from 1250 mm to 2500 mm and an altitude of 300–350 m from sea level [49]. In all the producing countries of the three species used in our study, the plants

are grown in tropical areas with a temperature range of 25–32 °C [49,50]. It seems that the major factor in the minor differences between the three types is the biosynthetic process of the plants rather than the weather conditions that express minor variations. *C. verum* was the only species that could produce the 2-mono-oxygenated derivative **3.**

5. Conclusions

The essential oil components of three cinnamon species were studied by GC and GC-MS. Cinnamaldehyde was found the major component with more than 94% yield in the three evaluated species. The bronchodilator effect of the three oils against CCh-evoked tracheal spams revealed that cinnamon oils of different species of diverse geographical locations possess tracheal smooth muscle relaxant activities with specie obtained from Ceylon, *C. verum*, as the most potent followed by the comparable potencies observed with Vietnamese *C. bureiroi* and the Chinese cinnamon, *C. cassia*. Individual components were tested for their bronchodilator effects and their findings indicate that the α, β unsaturated carbonyl structure is essential for the observed bronchodilatory activities. The possible mechanism(s) explored in the major common component of these species, cinnamaldehyde, is a combination of two pathways, predominantly by KATP channel activation followed by weak CCB mechanisms. Thus, the present study provides detailed pharmacodynamic background to the medicinal use of cinnamon and its phytoconstituent cinnamaldehyde in hyperactive respiratory disorders and is a useful step towards the understanding of the role of phytomedicine in the current medicinal uses.

Supplementary Materials: The provided supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10030198/s1, Table S1: 1H and 13C-NMR data in ppm (multiplicity, *J* in parentheses in Hz) of **1**, **2** and **4** in CD3OD; Figures S1–S22: Spectral data of compounds **1**, **2** and **4**.

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