A C G publications

Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Bronchodilator Monoterpenes from the Fruits of *Trachyspermum ammi* L.

Abdulaziz S. Saeedan [1*], Najeeb Ur Rehman [1], Faisal K. Alkholifi [1], Yousef A. Alanzi [2], Anzarul Haque [3], Omar K. M. Al-Rahimi [2] and Maged S. Abdel-Kader [4,5*]

[1]*Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia*

[2]*College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia*

[3]*Central Laboratories Unit, Qatar University, Doha 2713, Qatar*

[4]*Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia*

[5]*Department of Pharmacology, Faculty of Pharmacy, Alexandria University, Alexandria 21215, Egypt*

(Received August 09, 2024; Revised September 16, 2024; Accepted September 20, 2024)

Abstract: *Trachyspermum ammi* is widely used among the people in the Arabian Peninsula to treat general respiratory problems including bronchoconstriction. In this study, the fruit extracts and its fractions and purely isolated components obtained from the *T. ammi* were investigated for their possible bronchodilator potential in an ex vivo model using guinea pig trachea. It was observed that the ethanol extract (TAT) of the plant and its hexane (TAH) and chloroform (TAC) extract fractions completely inhibited carbachol (CCh, 1 µM)-induced contractions at a concentration of 1 mg/mL. As a result of the biological activity-guided purification studies of the chloroform extract (TAC), in which the highest activity was observed, eight sub-fractions were obtained between A-H. Of these, fractions A, D, G were found to have significant activity for tracheal relaxation with 100%, 55 ± 5% and 31 ± 3%, respectively, while the other five fractions were found not to have any activity. Three monoterpene compounds were isolated from the fractions with high activity and their structures were determined by 1D and 2D NMR and mass spectrometric techniques as well as by simple chemical derivatization. In this study, it was determined that the bronchodilator effect of compound **1** (thymol) was demonstrated by the activation of different subtypes of K^+ channels. Reported data herein, demonstrated the scientific justification for the traditional use of *T. ammi* species against asthma and bronchitis.

Keywords: *Trachyspermum ammi* L.; thymol; bronchodilator; K^+ channel opener; *ex-vivo*.

1. Introduction

The Apiaceae family, previously called Umbelliferae, consists mostly of aromatic plants. Approximately 440 genera and 3800 species have been identified and reported as members of this family

* Corresponding author: mpharm101@hotmail.com (M.S. Abdel Kader); a.binsaeedan@psau.edu.sa (A.S. Saeedan)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.478.24.08.3285
Available online: October 11, 2024

worldwide [1]. Many members of the family have been used as folk medicine in different geographies for the treatment of respiratory, digestive and reproductive system problems from past to present [2]. Apiaceae plants, especially fennel and cumin, have been reported to be used traditionally for their antiseptic, antispasmodic, carminative and antiulcer effects [3–5]. The essential oils of the Apiaceae family members are widely used as flavoring agents, perfumery, and for the coloring of soaps and detergents [6]. Twenty-two species of the Apiaceae family are registered in the Chinese Pharmacopoeia [7].

Trachyspermum is a genus consisting of 14 recorded species worldwide [8]. The species *Trachyspermum ammi* L. (Carum ajowan) has been reported to be used in herbal medicine or drug composition to treat various ailments such as travel sickness, anorexia, abdominal bloating, nausea and vomiting [9]. In addition, it is claimed to have laxative, stomachic and anthelmintic properties [10]. It has also been reported, albeit limitedly, to relieve abdominal pain, haemorrhoids and abdominal tumours [11]. In fact, the plant is widely used for the management of asthma. The volatile oil of *T. ammi* contains up to 50% thymol together with other terpenes [12–15]. The antifungal activity of the essential oil of the plant has been observed in a wide spectrum of fungi and this activity has been associated with the high thymol content [15]. In addition, highly oxygenated monoterpenes and monoterpene glycosides have been reported from polar fractions of *T. ammi* fruits as well as from other family members [16].

Current medications used as bronchodilators in the treatment of chronic obstructive pulmonary disease (COPD) mainly fall into three categories. These are anticholinergic agents, β2 adrenergic agonists and xanthine derivatives that target receptors, such as cGMP-inhibited 3',5'-cyclic phosphodiesterase A, Adenosine receptors, such as theophylline and Oxtriphylline. The former have been replaced by β2 adrenergic agonists due to their various side effects such as dry mouth, blurred vision, nausea, decreased intestinal motility, urinary retention and tachycardia [17]. However, the development of semisynthetic analogues of tropane alkaloids with fewer side effects, such as ipratropium and tiotropium bromide, has renewed interest in the use of anticholinergic drugs [18]. The third place is taken by xanthine derivatives, namely theophylline and Oxtriphylline [19]. The most common side effects of theophylline include anorexia, nausea, headache, sleep disturbance and cardiac arrhythmias [20]. There is ongoing interest in developing new classes of bronchodilators to overcome these limitations and side effects that characterize existing classes.

In the current study phytochemical investigation directed by bronchodilator activity was conducted and led to the isolation of three active monoterpenes. Detailed mechanistic study for the effect was performed.

2. Materials and Methods

2.1. General

Optical rotation measurements were carried out by using a Jasco P-2000 Polarimeter. ^{1}H, ^{13}C-NMR and 2D-NMR experiments were performed on a Bruker UltraShield Plus 500 MHz spectrometer. HRMS were determined on Thermo Scientific UPLC RS Ultimate 3000 - Q Exactive hybrid quadrupole-Orbitrap mass spectrometer via direct injection.

2.2. Plant Material

The fruits of *Trachyspermum ammi* L. were provided from Riyadh city local market, Saudi Arabia. The plant's identity was confirmed by Mona Alwahibi, PhD., Department of Botany and Microbiology, College of Science at KSU, Riyadh. MSA 10832 was voucher number for conserving the plant material at the Department of Pharmacognosy at the College of Pharmacy, PSAU.

2.3. Extraction and Isolation

The powdered fruits of species (850 g) were extracted thoroughly using 95% ethyl alcohol (6 L) by maceration at room temperature for 3 days. The solvent was filtered through filter paper and the filtrates were combined in a beaker, then evaporated using a rotary evaporator to obtain 60.25 grams of ethanolic extract (TAT). The extract (TAT) was then dissolved with 40% aqueous ethanol and its fractions were obtained from apolar to

polar using the following solvents: hexane (3 × 400 mL) to obtain 14.21 g of the hexane fraction (TAH); $CHCl_3$ (4 × 400 mL) to obtain 7.56 g of TAC, EtOAc (3 × 400 mL) to obtain 11.97 g of TAE, and the remaining aqueous phase was freeze-dried to give 25.75 g (TAW).

Part of TAC (5 g) were purified on column (100 × 3 cm id, 125 g silica gel) using $CHCl_3$ followed by $CHCl_3$/MeOH mixtures in a gradient elution system. Fractions of 150 mL were collected and after TLC examination they were and combined giving eight subfractions A-H.

Fraction A (1.45 g) afforded 700 mg of **1** after crystallization from MeOH.

Fraction D (0.210 g) was further fractionated on silica gel column (50 × 1 cm id, 20 g silica gel) eluting with $CHCl_3$ followed by gradient of $CHCl_3$/MeOH. Fractions eluted with 5% MeOH afforded 35 mg of compound **2**.

Fraction **G** (0.311 g) was subjected to VLC using RP18 silica gel (i.d. 3 cm, 30 g) and H_2O followed by H_2O/MeOH mixtures as eluents. Fractions eluted with 40% MeOH in H_2O afforded 38 mg of **3**.

2.4. Characterization of the Isolated Compounds

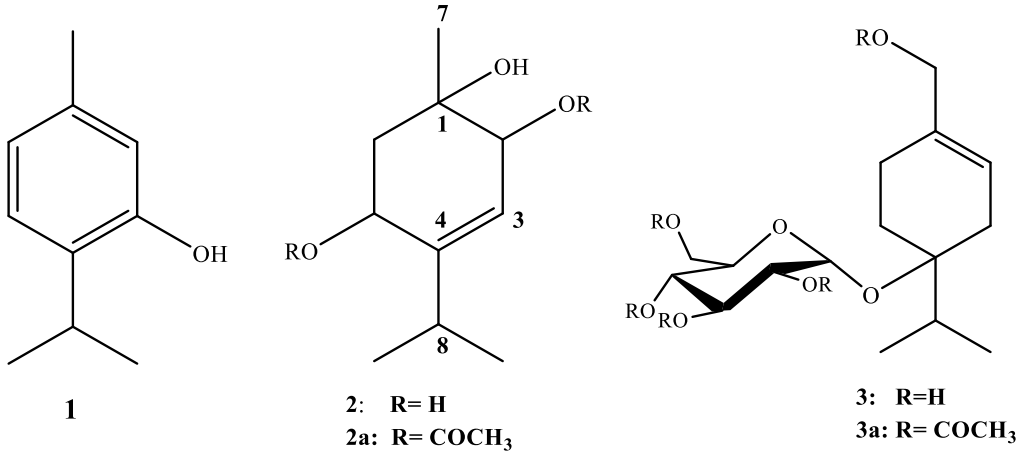
Thymol (**1**): Colouless crystals; ^1H and ^{13}C NMR spectral data [28]. are given in Table S1 and Figures S1-S8 in supporting information file.

p-Menth-3-ene-1β,2β,5β-triol (**2**): Amorphous; $[\alpha]^{25}_D$ = + 18 (c 0.9, MeOH); ^1H and ^{13}C NMR spectral data (see Table S1, Figures S9-S16); HRESIMS $[M-H]^+$ *m/z* 185.1173 (calcd. for $C_{10}H_{17}O_3$, 185.1178) (Figure S17), $[M+Na]^+$ *m/z* 209.1144 (calcd. for $C_{10}H_{18}O_3Na$, 209.1154) (Figure S18).

Synthesis of p-Menth-3-ene-1β,2β,5β-triol diacetate (**2a**): About 7 mg of **2** were dissolved in 0.2 mL pyridine and few drops of acetic anhydride were added. The reaction mixture was left at room temperature for 24 hrs. The solution was dried under stream of nitrogen to leave chromatographically homogenous solid of **2a**. ^1H and ^{13}C NMR spectral data (see Table S1, Figures S19-S25); HRESIMS $[M+Na]^+$ *m/z* 293.1360 (calcd. for $C_{14}H_{22}O_5Na$ 293.1365) (Figure S26).

(4S)-p-Menth-1-ene-4,7-diol 4-O-β-D-Glucopyranoside (**3**): Colorless gum; $[\alpha]^{25}_D$ -66 (c 0.5, MeOH); ^1H and ^{13}C NMR spectral data (see Table S1, Figures S27-S40); HRESIMS $[M-H]^+$ m/z 331.1765 (calcd for $C_{16}H_{27}O_7$, 331.1765) (Figure S41), $[M+Na]^+$ *m/z* 355.1721 (calcd. for $C_{16}H_{28}O_7Na$, 355.1733) (Figure S42).

(4S)-p-Menth-1-ene-4,7-diol 4-O-β-D-Glucopyranoside (**3a**): About 6 mg of **3** were treated similar to **2** to obtaine chromatographically homogenous solid of **3a**. ^1H and ^{13}C NMR spectral data (see Table S1, Figures S43-S49); HRESIMS $[M+Na]^+$ *m/z* 565.2255 (calcd. for $C_{26}H_{38}O_{12}Na$, 565.2261) (Figure S50).



1

2: **R= H**
2a: R= COCH$_3$

3: R=H
3a: R= COCH$_3$

Figure 1. Structures of monoterpenes **1- 3**

2.5. Animals and Guinea Pig Tracheal Muscles Preparation

All the *ex vivo* experiments performed adhere to the guidelines established by the Institute of Laboratory Animal Resources, Commission on Life Sciences, NRC. The PSAU Bio-Ethical Research Committee (BERC) approved the study protocol under the reference number BERC-001-12-19. Guinea-pigs were mercy killed by cervical dislocation later on the separated trachea was treated in ice-cold Krebs solution. Carbogen gas (95 % O_2 and 5 % CO_2 mixture) at 37 °C [21] was passed into the solution following separation of the tracheal tissues from adherent tissues. The tracheal tissues were cut into 2-3 mm width rings. The rings were opened opposite the tracheal muscle, connected together forming a tracheal chain, and then placed in 20 mL organ bath filled with Kreb's solution. Each tracheal strip put under constant tension of 1 g throughout the experiment time. A minimum 60-minute equilibration time was allowed before employing CCh (1 µM) to induce contraction. The emkaBATH data access software (France) was in charge of the system. The plant materials were then introduced to the bath in a concentration-dependent manner utilizing the cumulative approach to assess their bronchodilator activity once a stable tonic contraction was reached.

2.6. Determination of the Mechanism of the Bronchodilator's Effect

The tracheal relaxation of the test samples was explored against low (25 mM) and high K^+ (80 mM)-mediated contraction to evaluate the involvement of K^+ channel opening and Ca^{++} channel blocking-like effects [21,22]. Following a sustained contraction in response to low and high K^+, the test materials were added in a cumulative fashion to obtain the concentration-dependent inhibitory responses. The inhibitory effects of the test materials were examined against low K^+-mediated contractions in the absence and presence of different antagonists of K^+ channels such as tetraethylammonium (TEA, 1 mM); a nonselective K^+ channel blocker [23], 4-aminopyridine (4-AP, 100 µM); a selective blocker of voltage sensitive K^+ channels [24], quinine (30 µM), a non-selective blocker of Ca^{++} activated K^+ channels [25], iberiotoxin (IbTX, 180 nM); a selective blocker of large Ca^{++} sensitive K^+ channels [26], apamine (100 nM), a small conductance calcium-activated potassium channel blocker [27] and glibenclamide (Gb, 10 µM); a selective blocker of ATP-dependent K^+ channels [28] to determine the K^+ channels subtype involved in the activity.

2.7. Statistical Analyses

The reported results are given as the mean ± standard error of the mean (SEM, where n represents the number of experiments). The EC50 value represents the median effective power of the data with 95% confidence intervals (CI), while any p-value > 0.05 obtained in the study was considered significant. Relaxation concentration response curves (CRCs) were generated using nonlinear regression with the help of the GraphPad program (GraphPAD, San Diego, CA, USA).

3. Results and Discussion

The TAT, its TAH and TAC fractions all inhibited completely the carbachol (CCh, 1 µM)-induced contractions at 1 mg/mL while TAE showed partial efficacy (55% ± 5) whereas TAW did not show inhibitory effect ($p > 0.05$) as shown in Figure 2. The TAC-subfractions at 0.5 mg/mL, A, D, G were found active significantly for their tracheal relaxation with 100%, 55% ± 5 and 31% ± 3, respectively whereas the subfractions B, C, E, F and H were found inactive for relaxation of CCh-induced contraction (Figure 3).

Pure isolates when tested for the tracheal relaxation, compound **1** (Figure 4) was found more efficacious (100% ± 0) compared to other two isolated compounds **2** and **3** which were found partially active at 0.3 mg/mL with respective inhibition of 61% ± 4.24 and 29.5% ± 3.53 (Figure 4). The **1** was further subjected for detailed mechanistic evaluation where it selectively inhibited low K^+ in concentration-dependent manner with resultant EC50 values of 0.12 mg/mL (0.09 – 0.18, n=5) whereas it was completely inactive against high K^+-induced contractions (Figure 5A).

The biological activity-guided phytochemical isolation study of TAT allowed the identification of three monocyclic monoterpenes with high activity from the chloroform subfraction. Of these, compound **1** was obtained in high yield and was identified as thymol by evaluation of NMR and mass spectral data (see Figures S1-S8) and comparison with the authentic sample [29].

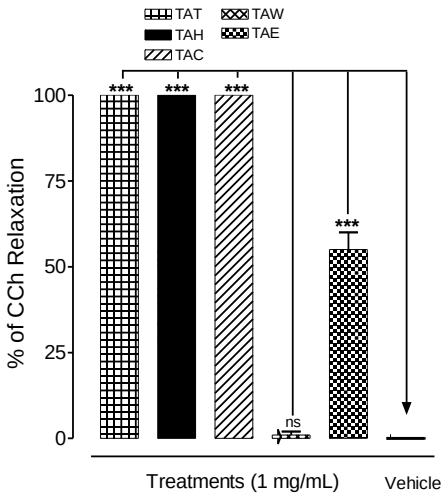


Figure 2. Inhibitory effects of the fruit extract (TAT), and its hexane (TAH), chloroform (TAC), aqueous (TAW) and ethyl acetate (TAE) fractions against carbachol (1 µM)-mediated contractions in isolated guinea-pig tracheal tissues. Symbols represent mean ± SEM; n= 4-5. $^{ns}P>0.05$, $^{***}P<0.001$vs. control (vehicle), one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

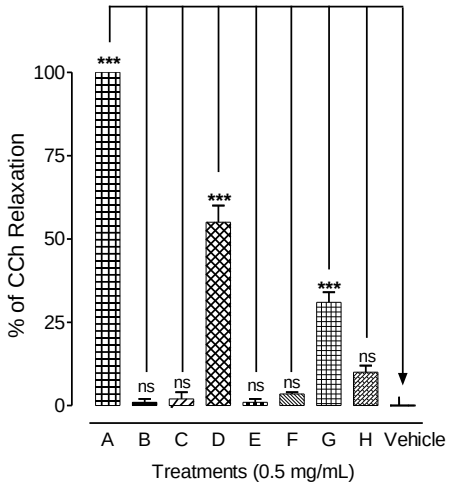


Figure 3. Inhibitory effects of the subfractions A to H of the chloroform fraction of the fruit extract against carbachol (1 µM)-mediated contractions in isolated guinea-pig tracheal tissues. Symbols represent mean ± SEM; n= 4-5. $^{ns}P>0.05$, $^{***}P<0.001$vs. control (vehicle), one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

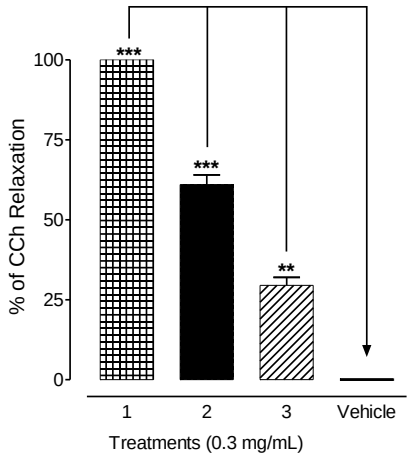


Figure 4. Comparison of the Inhibitory effects of the pure isolates 1, 2 and 3 derived from subfractions A of the fruit extract against carbachol (1 µM)-mediated contractions in isolated guinea-pig tracheal tissues. Symbols represent mean ± SEM; n= 4-5. $^{**}P<0.01$, $^{***}P<0.001$vs. control (vehicle), one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

Compound **2** was isolated from the more polar fractions of the TAC. ^{13}C-NMR spectrum of it (Table S1, Figure S12) revealed 10 carbon resonances sorted by DEPT135 experiment (Figure S13) into 3 X CH_3, 1 X CH_2, 4 X CH and 2 quaternary carbons including one olefinic at δ_C 147.16 and one oxygenated at δ_C 73.19 ppm. Two of the CH were oxygenated as indicated by their chemical shift at δ_H 4.63 (t *J*= 5.0 Hz), δ_C 67.29 and δ_H 4.71 s, δ_C 74.60 ppm. These data were evaluated as evidence for the structure of trioxygenated p-ment-3-ene. To exclude the possibility of having epoxy function between two hydroxyls, compound **2** was subjected to acetylation with acetic anhydride/pyridine to give a diacetyl derivative of compound **2** (**2a**) as indicated from the NMR data where two protons were shifted to down field and they observed at δ_H 5.34 ppm and 5.43 ppm (Table S1, Figures S19-S25, in supporting information). The third hydroxyl on the quaternary carbon did not react under such conditions [30]. As the tertiary hydroxyl group can easily eliminated under EIMS conditions [31] both **2** and **2a** where

measured using HR-ESI/MS. HR-ESI/MS of **2** showed an $[M-H]^+$ at *m/z* 185.1173 (calcd. for $C_{10}H_{17}O_3$, 185.1178) (Figure S17), $[M+Na]^+$ *m/z* 209.1144 (calcd for $C_{10}H_{18}O_3$+Na, 209.1154) (Figure S18) supporting the presence of 3 hydroxyl groups rather than epoxy moiety. HRESIMS of **2a** revealed an $[M+Na]^+$ at *m/z* 293.1360 (calcd. for $C_{14}H_{22}O_5$+Na, 293.1365) (Figure S26) further supporting a triol derivative. The data of **2** was quite similar to those reported for *p*-menth-3-ene-1β,2β,5β-triol [32].

The NMR data of **3** showed in addition to the 10 carbon resonances of a monterpene skeleton six oxygenated carbon signals assigned for glucopyranosyl moiety (Table S1, Figures S30-32). The ^{1}HNMR signal at δ_H 4.27 (s) (Figure S27, S29) correlated with ^{13}CNMR signal at δ_C 66.03 ppm (Figure S35, S37) replacing the singlet for CH_3 in the menthene skeleton as well as the quaternary carbon signal at δ_C 79.58 ppm were diagnostic for glycosylated 4,7-diol derivative. HREIMS supported such assignment with the $[M-H]^+$ at *m/z* 331.1765 (Figure S41) and $[M+Na]^+$ at *m/z* 355.1721(Figure S42). Such data could represent (*4S*)-*p*-menth-1-ene-4,7-diol 4-O-*β* -D-Glucopyranoside [33] or eleuhenryiside C isolated from *Eleutherococcus henryi* family Araliaceae [34]. To determine the site of glycosylation **3** was acetylated and the resulted **3a** expressed the addition of 5 acetyl groups as indicated from the HRESIMS that showed a quasi-molecular ion at *m/z* 565.2255 for $C_{26}H_{38}O_{12}$+Na (Figure S50). The H-7 singlet at δ_H 4.27 appeared as two doublets and, shifted to down field at δ_H 4.38 and 4.45 (*J*= 11.8 Hz) (Figure S43). These spectral evidences enable to elucidate its structure as (*4S*)-*p*-menth-1-ene-4,7-diol 4-O-*β* -D-Glucopyranoside (**3**) [35].

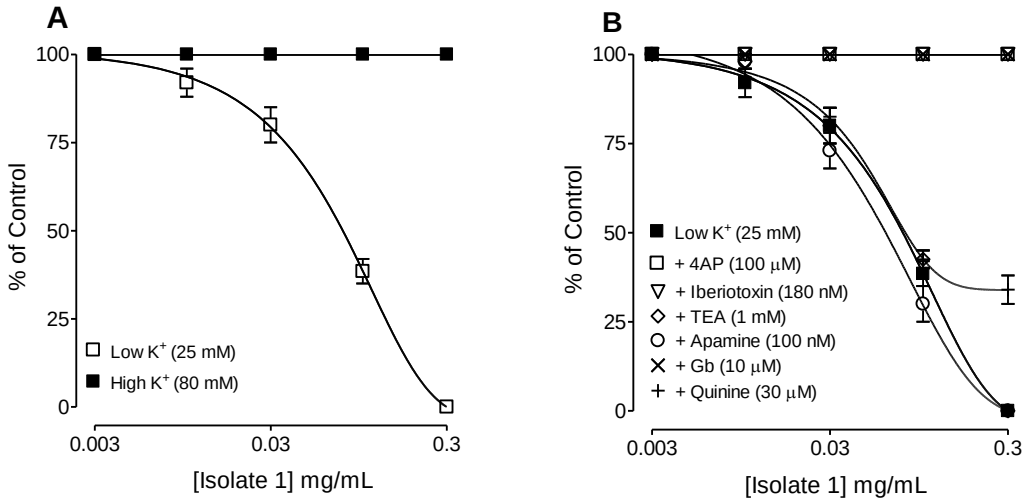


Figure 5. (A): Inhibitory effects of pure isolates **1** against low and high K^+-induced contractions. **(B):** Inhibitory effects of pure isolates **1** in the absence and presence of different K^+ channels blockers against low K^+ (B) in isolated guinea-pig tracheal tissues. Symbols represent mean ± SEM; n= 4-5.

This study showed that *T. ammi* fruit extracts, especially TAC (chloroform extract) obtained from TAT, total, subfractions and pure isolates have bronchodilator activity. Compound **1** was found to be more effective (100%) than the other two isolated compounds **2** and **3**, which were partially active at 0.3 mg/mL with 61% and 29% inhibition, respectively. In order to investigate its K^+ channel opening-like effects, compound **1** was subjected to detailed mechanistic evaluation where it selectively inhibited low K^+ mediated tracheal relaxation compared to high K^+ mediated tracheal relaxation. Compound **1** showed data indicating K^+ channel opening-like effects by selectively inhibiting contractions induced by K^+ (25 mM), which is consistent with literature [21]. This model effectively discriminates whether the test samples act by opening K+ channels or blocking Ca^{++} channels. K^+ channel openers have a wide scope of therapeutic applications, including asthma, gastrointestinal spasms, hypertension, and urinary permeability [33,34]. These compounds relax the smooth muscles via opening the K+ channels causing increase in K^+ efflux leading to decrease in the free intracellular Ca^{++} [35,36]. The contributions of different types of K^+ channels were demonstrated using the major compound **1.** The relaxant effect of thymol **1** against low K^+ was reproduced in tissues pre-treated with six different K^+ channel antagonists. These were tetraethylammonium (TEA, 1 mM); a nonselective K^+ channel blocker [23], 4-

aminopyridine (4-AP, 100 µM); a selective blocker of voltage sensitive K^+ channels [24], quinine (30 µM), a non-selective blocker of Ca^{++} activated K^+ channels [25], iberiotoxin (IbTX, 180 nM); a selective blocker of large Ca^{++} sensitive K^+ channels [26], apamine (100 nM), a small conductance calcium-activated potassium channel blocker [27] and glibenclamide (Gb, 10 µM); a selective blocker of ATP-dependent K^+ channels [33]. Interestingly, 4-aminopyridine (4-AP, 100 uM), glibenclamide (Gb, 10 uM) and iberiotoxin (180 nM) completely resist the tracheal relaxation against low K^+ thus showing the involvement of the activation of voltage sensitive K^+ channels, ATP-dependent K^+ channels and large conductance Ca^{++}-activated K^+ channels in the bronchodilator effect of thymol **1.** On the other hand, partial involvement of non-selective Ca^{++} activated K^+ channels is involved as quinine pre-incubation reversed partially the tracheal relaxation of **1** whereas involvement of nonselective K^+ channels and small conductance Ca^{++} activated K^+ channels is excluded due to ineffectiveness of TEA and apamine on the tracheal relaxant effect of **1** against low K^+.

4. Conclusion

T. ammi fruit extract was subjected to phytochemical study directed by *ex vivo* bronchodilator activity using Guinea Pig tracheal muscles. Three known monoterpenes were isolated from the most active chloroform sub-fraction and were identified by various spectroscopic methods and simple chemical derivatization. The most active compound **1** identified as thymol was subjected to detailed mechanistic study. The bronchodilator effect of **1** found to be mediated by activation of different subtypes of K^+ channels. The study gives scientific evidence for the traditional use of *T. ammi* fruit for the management of asthma.

Acknowledgments

The authors express their sincere gratitude to Prince Sattam bin Abdulaziz University for financially supporting this research work, project number (PSAU/2023/03/26796).

Funding

The project was funded by Prince Sattam bin Abdulaziz University through the project number (PSAU/2023/03/26796).

ORCID
Abdulaziz S. Saeedan: 0000-0003-1766-8480
Najeeb Ur Rehman: 0000-0001-6925-7697
Faisal K. Alkholifi: 0000-0003-3150-3439
Yousef A. Alanzi: 0009-0003-6900-2127
Anzarul Haque: 0000-0001-9517-7404
Omar K. M. Al-Rahimi: 0009-0006-5555-1573
Maged S. Abdel-Kader: 0000-0002-5128-2656

References

[1] P. F. Stevens and H. Davis (2001). Angiosperm phylogeny website, Missouri Botanical Garden St. Louis.
[2] M. G. Aćimović, L. M. Kostadinović, S. J. Popović and N. S. Dojčinović (2015). Apiaceae seeds as functional food, *J. Agric. Sci. (Belgr.)* **60**, 237–246.
[3] R. Fang, C. H. Jiang, X. Y. Wang, H. M. Zhang, Z. L. Liu, L. Zhou, S. S. Du and Z. W. Deng (2010). Insecticidal activity of essential oil of *Carum carvi* fruits from China and its main components against two grain storage insects, *Molecules* **15**, 9391–9402.
[4] H. Hajlaoui, H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri and A. Bakhrouf (2010). Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against Vibrio spp. Strains, *Food Chem. Toxicol.* **48**, 2186–2192.

[5] S. Koppula and D. K. Choi (2011). *Cuminum cyminum* extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: a noninvasive biochemical approach, *Pharm. Biol*. **49**, 702–708.

[6] A. S. Sardoei, M. Shahdadneghad, A. Arsalani and T. Sadeghi (2014). The effect of solopotasse fertilizer on yield and essential oil of cumin (*Cuminum Cyminum* L.), *Intl. J. Adv. Biol. Biomed. Res*. **2**, 2529–2533.

[7] M. Li, M. Li, L. Wang, M. Li and J. Wei (2023). Apiaceae medicinal plants in China: A review of traditional uses, phytochemistry, bolting and flowering (BF), and BF control methods, *Molecules* **28**, 4384.

[8] C. L. Schoch, S. Ciufo, M. Domrachev, C. L. Hotton, S. Kannan, R. Khovanskaya, D. Leipe, R. Mcveigh, K. O'Neill and B. Robbertse (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools, *Database*.

[9] K. Nabi, Imanshu, S. Swarup, D. Bhatia, M. Bhatti and L. Singh (2023). Updated Detailed Review of *Trachyspermum ammi*: Composition, Applications and Pharmacological Profile: Pharmaceutical Science-Pharmacy, *Int. J. Life Sci. Pharma. Res*. **13**, P221–P238.

[10] R. Bairwa, R. S. Sodha and B. S. Rajawat (2012). *Trachyspermum ammi*, *Pharmacogn. Rev*. **6**, 56–60.

[11] V. Krishnamoorthy and M. B. Madalageri (1999). Bishop weed (*Trachyspermum ammi*): an essential crop for north Karnataka, *J. Medicin. Arom. Plant Sci.* **21**, 996-998.

[12] K. K. Chahal, K. Dhaiwal, A. Kumar, D. Kataria and N. Singla (2017). Chemical composition of *Trachyspermum ammi* L. and its biological properties: A review, *J. Pharmacogn. Phytochem*. **6**, 131–140.

[13] I.-M. Chung, T. D. Khanh, O.-K. Lee and A. Ahmad (2010). Chemical constituents from ajwain seeds (*Trachyspermum ammi*) and inhibitory activity of thymol, lupeol and fatty acids on barnyardgrass and radish seeds, *Asian J. Chem.* **19**, 1524–1534.

[14] S. Dubey and P. Kashyap (2015). *Trachyspermum ammi*: a review on its multidimensional uses in Indian folklore medicines, *Res. J. Med. Plant* **9**, 368–374.

[15] G. Singh, S. Maurya, C. Catalan and M. P. De Lampasona (2004). Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract, *J. Agric. Food Chem*. **52**, 3292–3296.

[16] T. Ishikawa, Y. Sega and J. Kitajima (2001). Water-soluble constituents of ajowan, *Chem. Pharm. Bull. (Tokyo)* **49**, 840–844.

[17] T. T. Hansel and P. J. Barnes (2003). An atlas of chronic obstructive pulmonary disease, CRC Press.

[18] G. J. Rodrigo and C. Rodrigo (2002). The role of anticholinergics in acute asthma treatment: an evidence-based evaluation, *Chest* **121**, 1977–1987.

[19] A. J. M. Ward, M. Mckenniff, J. M. Evans, C. P. Page and J. F. Costello (1993). Theophylline—an immunomodulatory role in asthma? *Am. Rev. Respir. Dis.* **147**, 518–523.

[20] P. R. Bender, J. Brent and K. Kulig (1991). Cardiac arrhythmias during theophylline toxicity, *Chest* **100**, 884–885.

[21] M. S. Abdel-Kader, N. Ur Rehman, M. A. Alghafis and M. A. Almatri (2022). Brochodilator phenylpropanoid glycosides from the seeds of *Prunus mahaleb* L, *Rec. Nat. Prod*. **16**, 443–453.

[22] F. F. Bin Dayel, N. Ur Rehman, M. A. Salkini and M.S. Abdel-Kader (2024). Effect of Drying on the Quantity and Composition of *Artemisia monosperma* Essential oil and exploring the bronchodilator effect using guinea pig tracheal muscles, *Rec. Nat. Prod*. **18**, 468-473.

[23] N. S. Cook (1988). The pharmacology of potassium channels and their therapeutic potential, *Trends Pharmacol. Sci*. **9**, 21–28.

[24] N. Satake, M. Shibata and S. Shibata (1996). The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by β1- and β2-adrenoceptor activation in rat aortic rings, *Br. J. Pharmacol*. **119**, 505–510.

[25] I. Findlay, M. J. Dunne, S. Ullrich, C. B. Wollheim and O. H. Petersen (1985). Quinine inhibits Ca^{2+}-independent K+ channels whereas tetraethylammonium inhibits Ca^{2+}-activated K^+ channels in insulin-secreting cells, *FEBS Lett*. **185**, 4–8.

[26] F. Laurent, A. Michel, P. A. Bonnet, J. Bompart, J. P. Chapat and M. Boucard (1993). Effects of toxins, apamine, charybdotoxin and iberiotoxin on the relaxation of the smooth muscular fiber induced by imidazo (1, 2-a) pyrazine derivative, *C. R. Seances Soc. Biol. Fil*. **187**, 526–535.

[27] Y. Yagi, M. Kuwahara and H. Tsubone (2002). Ca2+-activated K+ channel blockers induce PKC modulated oscillatory contractions in guinea pig trachea, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **131**, 511–519.

[28] H. Franck, A. Puschmann, V. Schusdziarra and H.-D. Allescher (1994). Functional evidence for a glibenclamide-sensitive K+ channel in rat ileal smooth muscle, *Eur. J. Pharmacol*. **271**, 379–386.

[29] A. Kumar, S. P. Singh and S. S. Chhokar (2008). Thymol and its derivatives as antimicrobial agents, *Nat. Prod. Commun*. **3**, 823-828.

[30] J. R. Anderson, K. G. O'Brien and F. H. Reuter (1952). The esterification of tertiary alcohols with boric acid, *J. Appl. Chem.* **2**, 241–244.

[31] D. J. Ward, D. J. Saccomando, G. Walker and S. M. Mansell (2023). Sustainable routes to alkenes: applications of homogeneous catalysis to the dehydration of alcohols to alkenes, *Catal. Sci. Technol*. **13**, 2638–2647.

[32] X.-J. Li, K.-W. Kim, D.-C. Kim, H. Oh, X.-Q. Liu and Y.-C. Kim (2021). Three novel monoterpenoid glycosides from fruits of *Eleutherococcus henryi*, *Nat. Prod. Res*. **35**, 1299–1306.

[33] J. R. Empfield (1995). Potassium channel openers: Therapeutic possibilities, *Pharmacol. News* **2**, 23–27.

[34] R. Mannhold (2004). KATP channel openers: Structure-activity relationships and therapeutic potential, *Med. Res. Rev*. **24**, 213–266.

[35] U. Quast (1992). Potassium channel openers: pharmacological and clinical aspects, *Fundam. Clin. Pharmacol*. **6**, 279–293.

[36] T. Lenz and G. Wagner (1995). Potential role of potassium channel openers for the treatment of cardiovascular disease, In: Hypertension: Pathophysiology, Diagnosis and Management. J. H. Laragh, B. M. Brenner (Eds). **1,2** *New York, Raven Press*, 2953–2968.