Citation/Atıf: Vural N, Kaymak S, Yüce O. Exploring bee venom volatiles: a promising avenue for cystic fibrosis. U. Bee J. – U. Arı. D. 2024;24 (2): 267-284. DOI: 10.31467/uluaricilik.1520769

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

EXPLORING BEE VENOM VOL ATILES: A PROMISING AVENUE FOR CYSTIC FIBROSIS

Arı Zehri Uçucu Maddelerini Keşfetmek: Kistik Fibrozis İçin Umut Veren Bir Çözüm

Nilüfer VURAL[1,2], Sibel KAYMAK[1], Oguz YÜCE[1,3]

[1]Department of Traditional, Complementary and Integrative Medicine, Biotherapeutic Products Research and Development Program, Institute of Public Health, Ankara Yıldırım Beyazit University, Ankara, TÜRKİYE, E-mail: sibelkaymak@aybu.edu.tr, ORCID No: 0000-0002-6523-7637

[2]Department of Food Processing, Vocational School of Technical Sciences, Ankara Yıldırım Beyazit University, Ankara, TÜRKİYE, Corresponding author / Yazışma yazarı E-mail: nilufervural@aybu.edu.tr, ORCID No: 0000-0003-3047-3004

[3]Department of Medicinal and Aromatic Plants, Vocational School of Technical Sciences, Ankara Yıldırım Beyazit University, Ankara, TÜRKİYE, E-mail: oguzyuce@aybu.edu.tr, ORCID No: 0000-0001-5623-8518

Received / Geliş: 23.07.2024 Accepted / Kabul: 04.10.2024 DOI: 10.31467/uluaricilik.1520769

ABSTRACT

Bee venom, a complex mixture of bioactive compounds, has demonstrated anti-inflammatory, antimicrobial, and immunomodulatory properties. Notably, the volatiles released by bee venom components have garnered attention for their potential in respiratory-related disease conditions. Cystic fibrosis (CF) is a challenging disorder, characterized by a genetic mutation affecting the CFTR protein, leading to the production of thick and sticky mucus in various organs, particularly the lungs and digestive system, and necessitating innovative therapeutic approaches. This research explored both bee venom volatiles' chemical composition and the effects on airway inflammation and mucus viscosity in CF patients by *in silico* methods. GC/MS analyses with various SPME fibers have conducted the identification of 67 distinct components in volatile compounds of bee venom. For CW/DVB, CAR-PDMS, and DVB-PDMS fibers, the compounds identified in the highest amounts were perilla alcohol (42.21%), tetradecane (11.48%), and 1,2-benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester (39.98%), respectively. *In silico* analyses subsequently indicated that these components exhibit anti-inflammatory effects by modulating key cytokines and reducing inflammatory markers in CF airways. This research highlights the potential of bee venom volatiles as a novel therapeutic avenue for managing CF symptoms. Harnessing the unique properties of bee venom may offer new perspectives in the development of targeted therapies for individuals affected by cystic fibrosis.

Keywords: Bee venom volatiles, Cystic fibrosis, SPME fibers, *In silico*

ÖZ

Biyoaktif bileşiklerin karmaşık bir karışımı olan arı zehri, anti-inflamatuar, antimikrobiyal ve immünomodülatör özellikler göstermektedir. Özellikle arı zehri bileşenleri tarafından salınan uçucu bileşenler, solunum hastalıklarındaki potansiyelleri nedeniyle dikkat çekmektedir. Kistik fibroz (CF), CFTR proteinini etkileyen genetik bir mutasyonla karakterize, başta akciğerler ve sindirim sistemi olmak üzere çeşitli organlarda kalın ve yapışkan mukus üretimine yol açan ve yenilikçi tedavi yaklaşımları gerektiren zorlu bir hastalıktır. Bu araştırma, hem arı zehri uçucularının kimyasal bileşimini hem de CF hastalarında hava yolu inflamasyonu ve mukus viskozitesi üzerindeki etkilerini kimyasal ve

***in silico* yöntemlerle araştırmıştır. Çeşitli SPME fiberleri ile yapılan GC/MS analizleri, arı zehrinin uçucu bileşiklerinde 67 farklı bileşenin tanımlanmasını sağlamıştır. CW/DVB, CAR-PDMS ve DVB-PDMS fiberleri için sırasıyla perilla alcohol (%42.21), tetradecane (%11.48) ve 1,2-benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester (39.98) bileşenleri en yüksek miktarda belirlenmiştir. Daha sonra yapılan *in silico* analizler, bu bileşenlerin, temel sitokinleri modüle ederek ve CF hastalarının hava yollarındaki inflamatuar belirteçleri azaltarak anti-inflamatuar etkiler sergilediğini gösterdi. Bu araştırma, arı zehri uçucularının CF semptomlarını yönetmek için yeni bir terapötik yol olarak potansiyelini vurgulamaktadır. Arı zehrinin benzersiz özelliklerinden yararlanmak, kistik fibrozdan etkilenen bireylere yönelik hedefe yönelik tedavilerin geliştirilmesinde yeni bakış açıları sunabilir.**

Anahtar Kelimeler: Arı zehri uçucu maddeleri, Kistik fibroz, SPME fiberi, *In silico*

GENİŞLETİLMİŞ ÖZET

Amaç: Bal arısı (*Apis mellifera*) zehri, arıların karın boşluklarında bulunan zehir bezleri tarafından üretilen ve zararlılara karşı koruma amaçlı iğneleri aracılı zerk ettikleri yüksek protein yapılı bir karışımdır. Arı zehri yüzyıllardır integratif tıp uygulamalarında medikal ve kozmetik olarak kullanılmakta olup son yıllarda da ilaç geliştirme çalışmalarında ve ticari ürünlerin üretiminde de yaygın olarak kullanılmaktadır. Arı zehrinin büyük biyoterapötik potansiyel barındıran uçucu bileşenleri kovan havası solunmasına benzer olarak solunabilir mi sorusunun cevaplanabilmesi ve arı zehrinin barındırdığı potansiyelin keşfedilmesi amacıyla bu çalışma gerçekleştirilmiştir.

Gereç ve Yöntem: Çalışma kapsamında uçucu bileşenlerin analiz edilebilmesi amacıyla bir kovan düzeneği kurulmuş ve zehir elektrik stimülasyonu yöntemiyle toplanmıştır. Kovan düzeneğinin üst kısmında yer alan boşluğa 3 farklı SPME (Katı Faz Mikro Ekstraksiyon) fiberinin (CW/DVB, CAR-PDMS, DVB-PDMS farklı polarite ve yapıda adsorbanlar içerikli 3 fiber) yerleştirilmesi ile uçucu bileşenler fibere adsorplanmış ve GC/MS cihazı aracılığıyla analizler gerçekleştirilmiştir. Sonrasında elde edilen kimyasal bileşenler tek tek kistik fibrozda önemli biyobelirteçler olan ve inhibisyon mekanizmaları çalışılan CFTR, MUC5AC, IL-13 ile moleküler yerleştirme analizlerine tabii tutulmuş ve diğer farmakolojik özellikleri (ADMET, mutajenite ve toksisite) *in silico* olarak incelenmiştir.

Bulgular: Elde edilen arı zehri uçucu bileşen analiz sonuçları 3 farklı fiber için toplam 67 uçucu bileşen ortaya koymuştur. Biyobelirteçlerin bağlanması ve inhibisyonu noktasında izobutil ftalat en aktif bileşen olmuş ve -7.4 bağlanma afinitesi skoru vermiştir. 67 bileşen için gerçekleştirilen toksisite analizi sonrasında bileşenlerin toksik olmadığı belirlenmiştir. Arı zehirinin uçucu bileşikleri üzerine yaptığımız araştırma, kistik fibrozis semptomlarında umut verici potansiyel sergileyen bir dizi biyoaktif bileşeni ortaya çıkarıyor. SPME-GC/MS analizi aracılığıyla, arı zehirinin uçucu kimyasal profilinin ayrıntılı bir şekilde anlaşılması için temel oluşturan 67 farklı uçucu bileşik belirlenmiştir. Burada sunulan bulgular, bu keşifleri uygulanabilir klinik uygulamalara dönüştürmeyi amaçlayan gelecekteki araştırma girişimleri için bir temel oluşturmaktadır. Bu çalışma, solunum yolu hastalıkları yönetiminin geleceğini şekillendirmede arı zehiri uçucularının potansiyelinin daha derinlemesine araştırılmasına zemin hazırlamaktadır.

Sonuç: Arı kovanları, baldan propolise ve arı feromonlarına kadar çok çeşitli uçucu bileşenleri kapsar ve toplu olarak sinerjik bir etki gösterir. Bu çalışmanın bulguları doğrultusunda, önerilen arı zehiri uçucularının inhalasyonu, benzer şekilde, arı zehiri toplama işlemi sırasında kovan aparatından çıkarılan bir inhaler yoluyla uygulamayı içermektedir. Aradaki fark, ortamda yalnızca arı feromonlarının ve arı zehiri uçucu maddelerinin bulunmasında yatmaktadır. Araştırma kistik fibroz perspektifini benimserken, benzer kategorideki solunum bozukluklarında genel rahatlama ve olumlu etkiler potansiyeli barındırmaktadır. Elde edilen sonuçlar arı zehri uçucu bileşenlerin toksik olmadığını ve soluma yoluyla kistik fibroz hastalarında kullanılması durumunda genel şikayetlerde ve semptomlarda rahatlama sağlanabileceğini vurgulamaktadır.

INTRODUCTION

Apitherapy is a complementary medical technique rooted in the historical use of bee products to address various diseases worldwide (Weis et al., 2022). While it is not employed as a standalone treatment, apitherapeutic applications play a crucial role in multidisciplinary treatment approaches

(Fratellone et al., 2016). The integration of complementary therapies with innovative methods holds significant importance in understanding disease mechanisms and exploring novel drugs and treatment modalities. Bee products include a variety of substances derived from bees and hives, such as bee venom, honey, propolis, royal jelly, bee bread, and pollen (Habryka et al., 2016). Bee venom, produced by glands in the abdominal cavity of bees, undergoes rapid crystallization upon contact with air, resulting in a yellow color. Honeybee venom comprises enzymes (such as hyaluronidase and phospholipase), small molecules (including histamine and dopamine), and proteins and peptides, notably melittin, apamin, and mast cell degranulation (Wehbe et al., 2019). Bee venom has historically been associated with traditional applications due to its anti-inflammatory, skin condition-relieving, and joint pain-alleviating biotherapeutic effects. However, since the late 19th century, the neuroprotective, anti-cholesterol, respiratory disease-treating, and antifungal properties of bee venom have been discovered, and these biotherapeutic effects continue to be actively researched today (Ullah et al., 2023). Modern venom research has enabled the discovery of venom components proven to be of pharmacological significance, paving the way for optimizing therapeutic strategies through the use of active compounds like melittin and apamin. Subsequently, the application scope of bee venom has expanded from its traditional antinociceptive effects to addressing degenerative diseases of the nervous system. This is attributed to the natural stability of venom enzymes and peptides as injectable solutes and their efficacy in reaching target tissues (Stela et al., 2024).

Literature reports indicate the positive impact of bee venom and bee venom therapy on respiratory and related diseases (Choi et al., 2018). Cystic fibrosis (CF) is an autosomal recessive disorder stemming from mutations in the gene encoding the cystic fibrosis transmembrane regulator protein (CFTR), a chloride (Cl) channel in the cell membrane (Castellani et al., 2017). Disease incidence varies among ethnic groups, and despite an officially reported incidence of 1/3000 in Türkiye, consanguineous marriages likely contribute to an underestimation (Dogru et al., 2020). The most prevalent mutation in the CFTR gene is F508del, characterized by the deletion of the 508th codon that encodes the amino acid phenylalanine, with a high frequency in Türkiye (Cutting, 2015). The CFTR protein is present in numerous cell types, including airway epithelium, submucosal glands, pancreas, liver, sweat glands, and reproductive organs. Genetic defects leading to CF disrupt ion transport from epithelial surfaces, diminishing the ability of epithelial cells to secrete chloride and absorb sodium in response to c-AMP agonists (Fraser-Pitt and O'Neil, 2015). Consequently, the defective ion transport results in the failure of chloride and fluid secretions in respiratory epithelium, causing mucus to dry, impairing mucociliary clearance, and ultimately contributing to lung disorders observed in CF patients. Patients with CF commonly experience excessive phlegm, persistent coughing, and frequent lung infections due to the obstruction of airways by thick and sticky mucus secretion in both the lungs and upper respiratory system (Naehrig et al., 2017).

Currently, no cure yet discovered for CF that ensures complete recovery. Instead, treatment aims to alleviate symptoms and enhance the overall quality of life (Graeber and Mall, 2023). Current cystic fibrosis treatments typically include mucolytic agents, bronchodilators, and anti-inflammatory medications. Inhaled therapies and mucolytic agents are frequently used to reduce mucus accumulation in patients' airways. Long-term antibiotic therapies play a crucial role in combating chronic infections; however, this can lead to antibiotic resistance. Recently developed CFTR modulators have shown a potential to alleviate disease symptoms by correcting mutation-related protein defects, highlighting the importance of researching targeted bioactive components and uncovering their potential (Graeber and Mall, 2023). Due to the complexity of CF and its varying impact on individual patients, seeking treatment at a specialized center for the disease is highly advantageous. The primary objectives of CF treatment include (i) Prevention and control of lung infections, (ii) Removal of mucus from the lungs, and (iii) Provision of adequate and balanced nutrition, with a focus on prevention and treatment (Allen et al., 2023).

The inhalation of bee venom, as investigated in this study, could serve as a natural therapeutic method, providing synergistic benefits to existing treatment protocols by specifically targeting pathophysiological processes such as chronic inflammation and mucus accumulation. These methods may deliver localized therapeutic effects directly to the lungs while minimizing systemic toxicity. Additionally, natural

components offer high bioavailability and may slow disease progression through the inhibition of pro-inflammatory cytokines and mucolytic effects. Integrating the inhalation of the bee venom volatiles into current treatments is also considered to reduce the risk of pulmonary complications by offering antibacterial protection, especially against resistant bacteria. This study aimed to explore new treatment potentials and discover various biotherapeutic products for investigation. Fresh bee venom samples were analyzed by SPME/GC-MS, volatile composition investigated and the effects on CF were studied by *in silico* methods.

MATERIALS and METHODS

Bee Venom Harvesting

For bee venom sampling, bees of the *Apis mellifera anatolica* race, selected from a specific hive from the Ankara Yıldırım Beyazıt University GETAT Center research apiary, were studied. A special hive system was created to collect bee venom (Figure 1). This system consists of different compartments; a glass front part has been provided for observing the bees, and a gap has been created at the top for the SPME fiber to collect volatile bioactive compounds. A glass plate was placed under the hive to collect bee venom, and bee venom secretion was ensured through Electrical impulses applied to the hive at certain intervals by the standard electrical stimulation method (de Graaf et al., 2021). Meanwhile, volatile components were adsorbed onto the SPME fiber conditioned and placed in the gap on the hive. Electrical impulse was generated with frequency from 50 to 1000 Hz, duration of 2–3 s, and pauses of 3–6 s. This hive setup method was implemented as an alternative to narrow systems like jars (Hasan et al., 2023), which are commonly used in the literature for bee venom extraction. The aim was to minimize the stress that bees might experience due to environmental conditions and to prevent any potential impact on the volatile component profile.

Figure 1. Special hive system for bee venom volatile analysis

Volatile Analysis of Bee Venom

The bee venom HS-SPME volatile analysis was carried out as described with slight modifications (Abd El-Wahed et al., 2021). The different fibers selected in this study were chosen to determine the chemical profiles of bee venom volatiles through various adsorbent materials, highlighting the importance of using different fibers during volatile component analysis and their impact on the results. SPME fibers of StableFlexTM coated with carbowax/divinylbenzene (CW/DMS, 65 µm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 µm), and divinylbenzene/polydimethylsiloxane (DVB/PDMS, 65 µm) were purchased from Supelco (Oakville, ON, Canada). Bee venom was collected as described and SPME fibers were placed on the beehive. The fibers were subsequently withdrawn into a needle and then sampled into the GC–MS port. GC–MS analysis was performed on a Shimadzu GC-2010 gas chromatogram equipped with Flame Ionization Detector and DB-5 column (30 m, 0.25 mm, 0.25 mm film thickness; Supelco) coupled with a Shimadzu QP2010-Plus mass

spectrometer. The injector and the interface temperatures were both set at 250 °C and for the ion source at 220 °C. A gradient temperature program was applied for volatiles analysis. The oven temperature was initially held at 40 °C for 2 min and then was increased to 200 °C at a rate of 3 °C /min held for 10 min, then ramped to 5 °C/min at 250 °C held for 5 min, and finally ramped to 5 °C/min at 275 °C. The Split injection mode was used for 1/50. The SPME fiber was prepared for the next analysis by placing it in the injection port for 10 min at 220 °C to ensure complete elution of volatiles. Blank runs were performed during the sample analyses. The FID temperature was 300 °C and the quadruple mass spectrometer was operated in EI mode at 70 eV, and the scan range was set at m/z 40–700.

GC–MS Data Processing

The identification of volatile compounds in fresh bee venom involved comparing retention times and spectra with standards. The compounds were identified by comparing their relative retention times to a C8–C32 n-alkanes mixture and mass spectra, utilizing NBS75K, Wiley 7, NIST7MS search 2.0 library data from the GC-MS system, literature data, and standards of the primary components. Additionally, the results were validated by comparing compound elution orders with their relative retention indices on a DB-5 column. All analyses were conducted in triplicate for consistency.

Molecular Docking Analysis

Structures of different target proteins were retrieved from the Protein Data Bank (Burley et al., 2017) and given in Table 1.

Table 1. Proteins and other molecular docking parameters

Structure Name	PDB ID	Target Activity	Grid Box Center Coordinates	Grid Box Size
CFTR	5TF7	Cystic Fibrosis Activity	center_x = -49 center_y = 25 center_z = -10	size_x = 21 size_y = 21 size_z = 21
MUC5AC	5AJN	Mucus Activity	center_x = 38 center_y = 32 center_z = -8	size_x = 35 size_y = 35 size_z = 35
IL-13	4I77	Cytokine Activity	center_x = 2 center_y = 16 center_z = -32	size_x = 22 size_y = 22 size_z = 22

To prepare suitable protein targets, Amber's Antechamber module was utilized with Chimera v1.6 (Pettersen et al., 2004). The docking preparation for the target proteins involved the following steps: (a) conducting energy minimization with 100 steepest descent steps, a .02 Å step size, and an update interval of 10; (b) removal of water molecules and co-crystallized ligands; (c) deletion of solvent and non-complex ions; and (d) addition of polar hydrogen atoms and AM1-BCC charges. Ligand structures corresponding to compounds identified in all four plant species were obtained from the PubChem database (Kim et al., 2016) in (.sdf) format. Furthermore, the energies of all ligand structures were minimized, and Gasteiger charges were added. Subsequently, rigid molecular docking was performed using Chimera v1.6, employing Autodock Vina's scoring function. The top poses with the minimum binding energy for each protein were visualized using Discovery Studio Visualizer (Studio, 2008).

Pharmacophore Modeling

Pharmacophore models were predicted using the LigandScout version 4.5 (Wolber et al., 2005). Molecular structures datasets were inputted into program with .sdf formats, allowing the identification of key pharmacophoric features such as hydrogen bond donors, acceptors, hydrophobic regions, and aromatic rings. Advanced algorithms within the program aligned and superimposed the ligands, extracting consensus pharmacophore hypotheses representing common structural elements essential for biological activity. Validation of the models involved assessing their ability to reproduce known bioactivity or predict the activity of new compounds.

ADMET Analysis

An ADME/T (absorption, distribution, metabolism, excretion, and toxicity) analysis was carried out, and

the results of this analysis present promising initial data inputs for subsequent *in vitro* and *in vivo* experiments. These predictions were obtained using both the SwissADME web tool and relevant literature data (Daina et al., 2017).

Toxicity Analysis

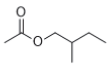
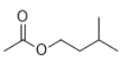
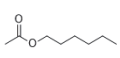
The LAZAR web tool was employed for the *in silico* prediction of the toxicity of volatile compounds presents in the bee venom (Maunz et al., 2013). The LAZAR web tool, an established platform for predictive toxicology assessments, utilizes a range of computational algorithms to evaluate the potential toxicity of chemical compounds. Input data on the bee venom volatile compounds were subjected to the LAZAR webtool's predictive models, allowing for the assessment of acute toxicity levels. The analysis focused on identifying potential toxic effects and elucidating the intricate relationships between the chemical structure of the bee venom volatile compounds and their predicted toxicological outcomes.

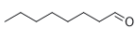
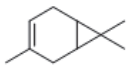
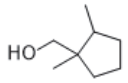
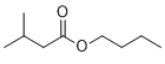
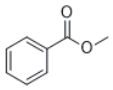
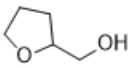
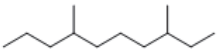
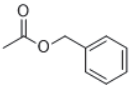
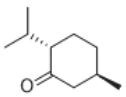
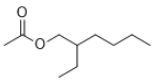
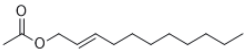
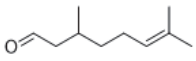
RESULTS

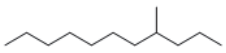
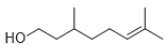
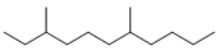
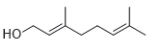
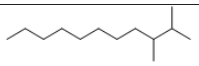
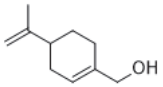
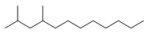
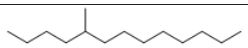
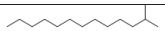
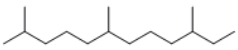
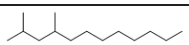
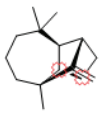
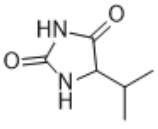
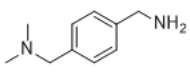
Chemical Composition of Bee Venom Volatile Compounds

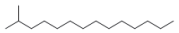
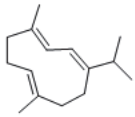
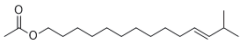
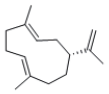
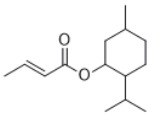
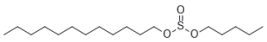
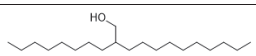
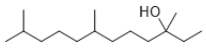
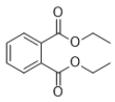
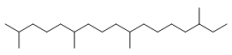
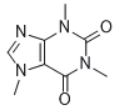
In this study, freshly extracted bee venom volatiles were analyzed by the SPME/GC-MS method, and the results are given in Table 2. Notably, it has been determined that the volatile secretions of fresh bee venom consist of 67 different volatile organic compounds.

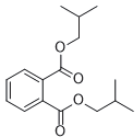
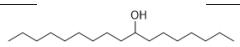
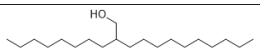
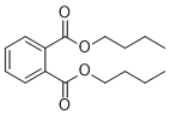
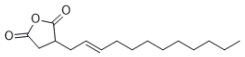
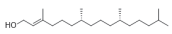
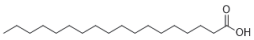
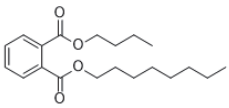
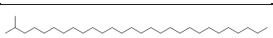
Table 2. Composition of volatiles from fresh honeybee venoms

Volatile Compounds of Bee Venom				**Type of SPME fiber**			
				CW/DVB	**CAR-PDMS**	**DVB-PDMS**	
LRI (cal)	**LRI (lit)**	**Compound**	**Chemical structures of compounds**	**%**	**%**	**%**	**IM**
496	495	Acetaldehyde		0,88±0,05	0,07±0,01	-	a
652	653	Acetic acid		-	0,12±0,01	-	a
667	665	Propanoic acid		0,42±0,02	0,11±0,00	0,98±0,05	a
671	670	2-Propyn-1-ol, acetate		-	0,08±0,01	-	a
870	871	2-Methyl butyl acetate		-	-	0,61±0,07	a
884	885	1-Butanol, 3-methyl-, acetate		-	-	11,13±0,49	a,b
911	912	Cyclobutanol		0,53±0,05	-	-	a
986	988	Acetic acid, hexyl ester		-	-	1,35±0,02	a

992	991	Cyclohexane, 1-isopropyl-1-methyl-		-	2,75±0,08	-	a
998	1001	Octanal		-	0,30±0,05	-	a, b
1007	1008	3-Carene		-	1,45±0,03	-	a
1037	1038	Cyclopentane, 1-hydroxymethyl-1,3-dimethyl-		-	0,21±0,03	-	a
1038	1039	Butanoic acid, 3-methyl-, butyl ester		-	-	1,78±0,22	a
1052	1051	1-Octanol		-	-	0,73±0,08	a,b
1054	1055	2-Hepten-1-ol		-	0,12±0,01	-	a
1077	1076	Methyl benzoate		-	-	0,97±0,07	a
1101	1102	Tetrahydrofurfuryl alcohol		2,44±0,22	-	-	a
1126	1127	Decane, 3,7-Dimethyl		-	0,59±0,04	-	a
1136	1135	Acetic acid, phenylmethyl ester		-	0,06±0,00	0,27±0,02	a
1141	1142	Menthone		-	-	0,29±0,03	a,b
1144	1144	Acetic acid, 2-ethylhexyl ester		-	-	1,84±0,32	a
1149	1151	Acetic acid, undec-2-enyl ester		-	-	0,51±0,04	a
1156	1157	3-Pentanol, 2,4-dimethyl-		-	-	0,41±0,06	a
1158	1158	Citronella		-	-	0,25±0,06	a

1161	1160	4-Methylundecane		-	0,16±0,01	-	a
1170	1171	Menthol		-	-	0,10±0,01	a,b
1208	1208	beta -Citronellol		-	-	0,18±0,01	a
1220	1221	Undecane, 3,7-dimethyl-		-	0,81±0,07		a
1232	1233	trans-Geraniol		-	-	0,43±0,07	a
1251	1250	Undecane, 2,3-dimethyl-		-	0,57±0,07	-	a
1274	1275	Tridecane		-	3,58±0,10	-	a
1296	1297	1-Cyclohexene-1-methanol, 4-(1-methyl ethenyl)-/ Perilla alcohol		42,21±0,12	-	-	a,b
1319	1318	Tetradecane		-	11,48±0,45	-	a,b
1321	1320	2,4-Dimethyldodecane		-	1,12±0,10	-	a
1355	1354	Tridecane, 5-methyl-		-	3,21±0,08	-	a
1362	1363	2-Methyltridecane		-	6,57±0,11	-	
1366	1365	Dodecane, 2,6,10-trimethyl		-	3,46±0,06	0,53±0,02	a
1383	1384	2,4-Dimethyldodecane		-	-	0,31±0,03	a
1426	1427	Junipene		-	-	0,51±0,02	a
1431	1430	5-Isopropyl-2,4-imidazolidinedione		3,45±0,05		-	a
1435	1434	4-Dimethylamino methylbenzylamine		-	0,53±0,04	-	a

1465	1466	Tetradecane, 2-methyl-		-	3,69±0,22		a
1475	1477	Germacrene-C		-	1,87±0,00	-	a
1479	1480	E-11(13-Methyl) tetradecen-1-ol acetate		-	0,97±0,08	-	a
1490	1489	Germacrene-A		-	0,93±0,03	-	a,b
1494	1495	Crotonic acid, menthyl ester		-	1,45±0,11	-	a
1501	1502	Sulfurous acid, dodecyl pentyl ester		-	1,21±0,15	-	a
1534	1535	2-Octyldodecan-1-ol		-	0,33±0,01	-	a
1536	1537	3-Dodecanol, 3,7,11-trimethyl		-	-	0,18±0,00	a
1551	1550	Diethyl Phthalate		-	-	0,23±0,01	a
1620	1620	Hexadecane		-	2,69±0,11	0,43±0,02	a,b
1634	1635	Heptadecane		-	1,88±0,03	-	a,b
1657	1658	Octane, 1,1'-oxybis-		-	0,93±0,04	-	a
1661	1660	Heptadecane, 2,6,10,15-tetramethyl-		-	-	0,17±0,02	a
1745	1746	Dodecane		-	4,75±0,21	-	a,b
1811	1810	Octadecane		-	0,83±0,07	0,25±0,01	a
1839	1840	Caffeine		0,95±0,11	-	-	a,b

1849	1847	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester		-	-	38,98±0,62	a
1872	1873	8-Heptadecanol		-	0,19±0,01	-	a
1884	1885	2-Octyldodecan-1-ol		-	0,11±0,01	-	a
1890	1891	1-Nonadecene		-	-	0,32±0,00	a
1935	1937	1,2-Benzenedicarboxylic acid, dibutyl ester		0,10±0,01	-	0,17±0,01	a
1967	1966	2-Dodecen-1-yl(-)succinic anhydride		-	-	0,67±0,12	a
2121	2122	Phytol		-	1,23±0,07	-	a
2156	2158	Octadecanoic acid		0,25±0,05	-	-	a,b
2315	2317	1,2-Benzenedicarboxylic acid, butyl octyl ester		0,24±0,04	-	-	a
2861	2863	2-methyloctacosane		-	-	0,23±0,01	a

[a] Compounds listed in order of elution from a DB-5 column. b Identification of components based on standard compounds; All values are mean ± standard deviation of triplicates; significant at the $p<0.05$ level

LRI (cal): Linear retention indices (DB-5 column) calculated against n-alkanes. % calculated from FID data with standard LRI (lit): https://pubchem. ncbi.nlm.nih.gov; IM: Identification Method

In Silico Molecular Docking Results of Bee Venom Volatile Compounds

The relevant ligands and analysis results were presented in the heatmap clustering provided in Figure 2. The heatmap was plotted by http://www.bioinformatics.com.cn/srplot, an online platform for data analysis and visualization.

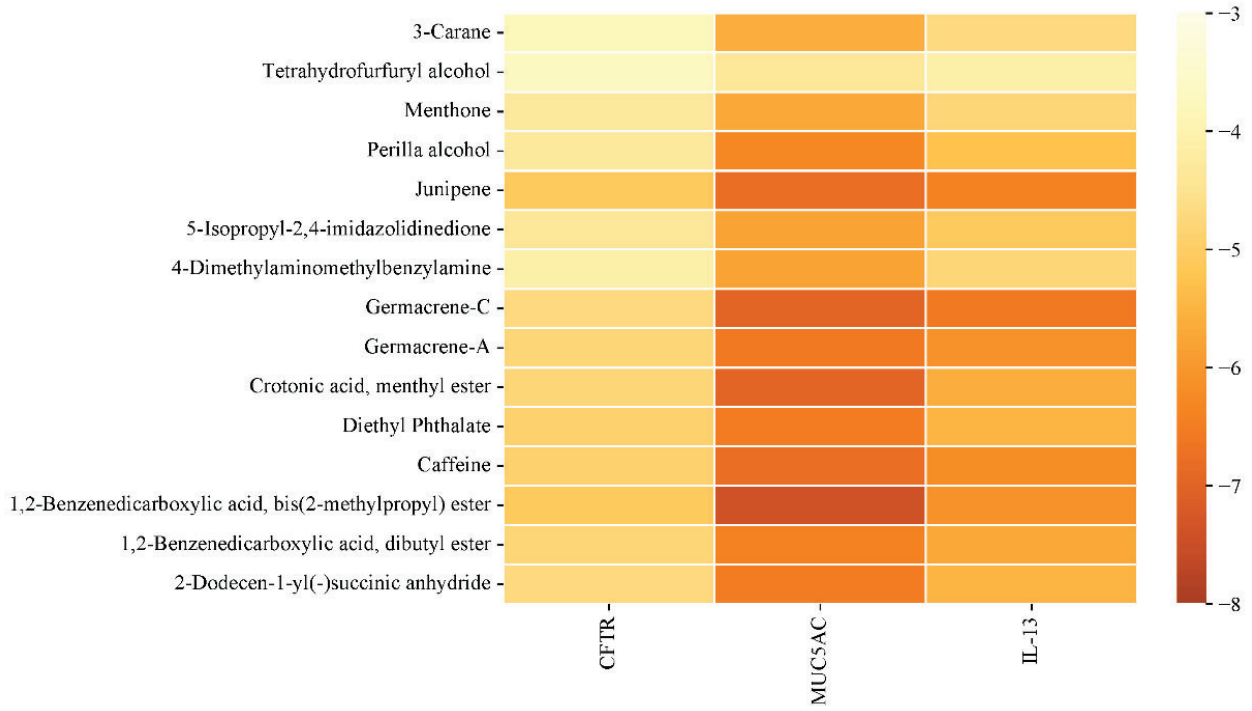


Figure 2. Clustered hierarchical heatmap showing quantified volatiles compounds from bee venom (The binding affinities provided in the chart on the right).

As inferred from the results, volatile components exhibited high inhibitory activity against the MUC5AC structure. The highest binding score was -7.4 for 1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester as known as isobutyl phthalate, followed by -7 for crotonic acid, menthyl ester. Subsequently, other volatile components such as Junipene and Germacrene-C have been found to exhibit high IL-13 suppressor activities. Upon examining the pharmacophore analyses of both structures with the highest binding affinities, in addition to hydrophobic binding regions, the H-bond acceptor ends are observed in the isobutyl phthalate binding site (Figure 3a). On the other hand, the same interaction profile was observed with fewer van der Waals interactions for the crotonic acid, menthyl ester (Figure 3b). It is suggested that the direct influence of a high number of Van der Waals interactions contributes to the high binding affinity.

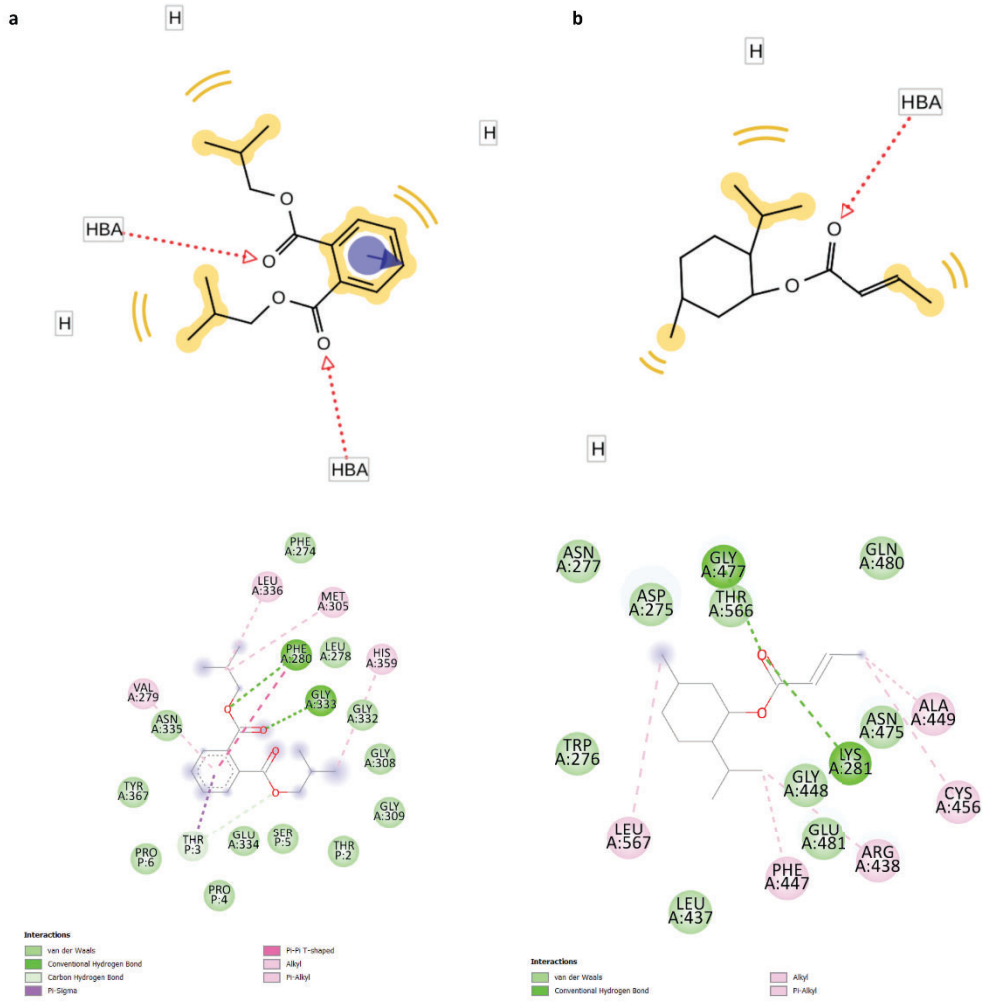


Figure 3. Two-dimensional binding geometry of (a) isobutyl phthalate and (b) crotonic acid, menthyl ester

In silico Toxicity Prediction Results

The bee venom volatile compounds' acute toxicity levels, as analyzed by *in silico* methods, are presented in Table 3.

While components such as junipene and germacrene C exhibit high levels of acute toxicity for both species at minimal concentrations, compounds like tetrahydro furfuryl alcohol and 5-Isopropyl-2,4-imidazolidinedione demonstrate low acute toxic effects even at elevated concentrations. Consequently, it is postulated that the composition of these volatile components exhibits synergistically low or moderate acute toxic effects. Additionally, no mutagenic effects have been identified for any of the volatile components.

ADMET Analysis

SMILES structures were employed for the *in silico* predictions of ADMET properties of certain volatile compounds of bee venom (see Table 4). The radar plots illustrating the general characteristics are presented in Figure 4.

Table 3. I*n silico* toxicity analysis results of some bee venom volatiles

Compounds	Acute toxicity (*Pimephales promelas*) (mmol/L)	Max. tolerated dose (Human) (mmol/kg/day)	Carcinogenicity (Mouse) (mg/L)	Mutagenicity (*Salmonella typhimurium*)
3-Carane	0.212	0.00632	non-carcinogenic	non-mutagenic
Tetrahydrofurfuryl alcohol	8.19	0.217	carcinogenic	non-mutagenic
Menthone	1.2	x	x	non-mutagenic
Perilla alcohol	x	x	non-carcinogenic	non-mutagenic
Junipene	0.00468	0.00803	carcinogenic	non-mutagenic
5-Isopropyl-2,4-imidazolidinedione	10.3	0.0207	x	non-mutagenic
4-Dimethylaminomethylbenzylamine	0.501	0.015	x	non-mutagenic
Germacrene-C	0.0452	x	non-carcinogenic	non-mutagenic
Germacrene-A	0.0458	x	non-carcinogenic	non-mutagenic
Crotonic acid, menthyl ester	0.223	0.0282	non-carcinogenic	non-mutagenic
Diethyl Phthalate	0.143	0.0218	carcinogenic at 0.149	non-mutagenic
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.00705	0.0183	carcinogenic	non-mutagenic
1,2-Benzenedicarboxylic acid, dibutyl ester	0.00359	0.0164	carcinogenic at 0.336	non-mutagenic
2-Dodecen-1-yl (-) succinic anhydride	0.032	0.0203	non-carcinogenic	non-mutagenic

Table 4. SMILES structures of the volatile compounds

Number	SMILES Structure	Compound Name
1	CC1CCC2C(C1)C2(C)C	3-Carane
2	C1CC(OC1)CO	Tetrahydrofurfuryl alcohol
3	CC1CCC(C(=O)C1)C(C)C	Menthone
4	CC(=C)C1CCC(=CC1)CO	Perilla alcohol
5	CC1(CCCC2(C3C1C(C2=C)CC3)C)C	Junipene
6	CC(C)C1C(=O)NC(=O)N1	5-Isopropyl-2,4-imidazolidinedione
7	CN(C)CC1=CC=C(C=C1)CN	4-Dimethylaminomethylbenzylamine
8	CC1=CCCC(=CC=C(CC1)C(C)C)C	Germacrene-C
9	CC1=CCCC(=CCC(CC1)C(=C)C)C	Germacrene-A

Using Diana's approach (Daina et al., 2017), the ADMET properties of certain volatile components have been depicted through radar plots (Figure 4). Most of these components exhibit high lipophilicity and despite a relatively low polarity profile, their structures demonstrate small size and non-flexible

behavior. Given the expected rigid nature of volatile components, such characteristics are within the norm (de Lacy Costello et al., 2014). Furthermore, considering a biopharmaceutical score of 0.55 for all components and their compliance with Lipinski's Rule of five, indicating their drug-like potential, it is plausible to assert that the volatile components of bee venom manifest a favorable ADMET profile.

Figure 4. Radars to assess the general properties and bioavailability of some bee venom volatile compounds.

DISCUSSION

CF is a multifaceted disease characterized by 4 different signaling pathways, for which a definitive treatment has not yet been found. Supportive products related to the disease are often associated with facilitating the dispersion of dense mucus. Mucins are the primary components of mucus produced in the airway epithelium. These are essentially classified as transmembrane or membrane-bound mucins and secreted/gel-forming mucins. As MUC5AC plays a significant role in the pathogenesis of CF, inhibiting the secretion and production of mucin could have a strong impact on the disease (Lillehoj et al., 2013; Samsuzzaman et

al., 2019). On the other hand, Interleukin-13 (IL-13) has been included in this study due to its association with allergic airway inflammation in the context of CF (Nakamura et al., 2017). The target proteins used in the *in silico* analyses were selected within the scope of this study as important markers for revealing their inhibition potential related to the disease. On the other hand, the fact that these ligands selected for computational analyses are bee venom volatiles carries the potential to introduce a new approach to apitherapy.

The analysis of volatile components in bee venom plays a significant role in determining the systematic mechanism of apitherapy applications and elucidating the processes involved. Within the setup established in the study, it is known that the samples obtained from the hive environment contain not only bee venom but also the volatile components of stimulated bees. The intricate social behavior of honeybees involves collective nest protection, executed through an effective system of chemical communication mediated by volatile compounds. Worker bees release some of the volatiles from the Koschevnikov gland during alarm behavior (Noël et al., 2023). The exploration of these volatiles has a longstanding history, initially revealing 3-methylbutyl acetate (isoamyl acetate) as the primary component inducing aggressive behavior (sting pheromone) in bees (Boch et al., 1962).

Subsequent investigations into the chemical composition of bee venoms revealed different components (Simone-Finstrom et al., 2023). In an analysis conducted on hexane extracts of Koschevnikov glands, 22 organic compounds within the C6–C36 range were identified (Camargos et al., 2020). While this study does not directly delve into the analysis of volatile components in bee venom, the shared constituents suggest the synergistic presence of compounds from bee venom, bee pheromones, and other volatile elements in the surrounding environment. Isidorov et al. utilized the same SPME/GC-MS methodology to investigate volatiles in both dry and fresh bee venom (Isidorov et al., 2023). Notably, there are compositional differences in the profiles of volatile components when compared to our results. This is believed to be attributed to factors such as geographical location, bee species, and the harvesting season. Another potential contributing factor is the presence of bee pheromones and bee-derived contaminations. In a study analyzing the volatiles of beehive air, profiles similar to those in our analysis were identified, consisting of aldehyde, acid, and hydrocarbon structures (Abd El-Wahed et al., 2021). Since the samples of bee venom were collected alongside the entire beehive air, it is possible to assert that the content of the hive's air, from which the samples were collected, is parallel to the samples.

On the other hand, when focusing on the *in silico* biotherapeutic effects of these components it has been determined that the volatile components of bee venom exhibit low binding affinities against CFTR targets, and no noteworthy inhibitor activity is demonstrated. It is hypothesized that the thermal behaviors of the protein binding region or the weak profile of volatile components for inhibitor behavior may account for this (Wang et al., 2018; Vega et al., 2016). However, it is possible to state that there is an *in silico* activity against respiratory problems for some of the bee venom volatiles synergistically. The notion that the sample collection environment does not only contain volatile components of bee venom parallels with the content analysis of studies on bee pheromones found in the literature (Li et al., 2014). The compound 1-octanol, reported as an alarm pheromone for *Apis mellifera* species (Wang and Tan, 2019), has been identified in the volatile chemical components of a DVB-PDMS type SPME fiber. This is directly associated with the electrification applied during bee venom collection, inducing bees into an alarmed state and, consequently, leading to venom secretion. The semiochemicals released by bees during their usual activities, compounds such as Geraniol and 1-nonadecene were also found in our study. Similarly, the descriptive fiber for both compounds was DVB-PDMS (Schmitt et al., 2007).

Additionally, crotonic acid, menthyl ester demonstrates high biotherapeutic activity among volatile components, as this observation is consistent with various *in vitro* literature studies (Yassin et al., 2020). When focusing on the potential toxicities of volatile components, no substances exhibiting toxic properties were found, and it is known that the toxic effect of bee venom mainly stems from its enzyme structures and melittin. However, this is specific to bees, and the volatile profiles of venoms from other organisms may exhibit toxicity. For example, phenol-2,4-bis (1,1 dimethyl ethyl) an ant venom volatile compound, display high acute toxicity (Nikbakhtzadeh et al., 2009), emphasizing the significance of approaching bee venom as an allergen despite the low toxicity of its primary volatile components. The recommendation

for the medical use of carcinogenic gases is not a new concept, besides nitric oxide, a well-known carcinogenic gas, is employed in various medical applications, including the treatment of pulmonary hypertension in newborns (Barnes et al., 2020). Fundamental considerations in the utilization of gases and volatile components revolve around concentration and dosage studies. When compared to Table 2, it is observed that the volatile compounds characterized as carcinogenic constitute a relatively small proportion (total 2-3%). Given the consistent exposure of individuals, particularly beekeepers, to similar profiles of compounds during the collection of bee venom, a crucial point emerges: the volatile components of bee venom do not exhibit carcinogenic effects synergistically, as inferred from their chemical composition. This observation is particularly pertinent when considering the continuous inhalation of these compounds during bee venom collection activities (Matysiak et al., 2016).

The utilization of volatile compounds associated with bees in respiratory processes is integratively acknowledged and currently implemented as a complementary method in clinical practices across various regions globally (Abd El-Wahed et al., 2021; Topal et al., 2021). The processes involve inhaling a pre-determined dosage of air by physicians, through an inhaler device emanating from a beehive. Beehives encompass a diverse array of volatile components, ranging from honey to propolis and bee pheromones, demonstrating a synergistic effect collectively. In line with the findings of the present study, the suggested inhalation of bee venom volatiles similarly involves administration through an inhaler extracted from the hive apparatus during the bee venom collection process. The distinction lies in the ambient presence solely of bee pheromones and bee venom volatiles. While the research adopts a cystic fibrosis perspective, it harbors the potential for general relief and positive effects in respiratory disorders of a similar category.

In conclusion, our investigation into the volatile compounds of bee venom unveils a spectrum of bioactive components that exhibit promising potential in cystic fibrosis symptoms. Through SPME-GC/MS analysis, we identified 67 distinct volatile compounds, laying the foundation for a nuanced understanding of the volatile chemical profile of bee venom. The findings presented herein provide a basis for future research initiatives aimed at translating these discoveries into viable clinical applications. This study is setting the stage for deeper exploration and the potential of bee venom volatiles in shaping the future of respiratory diseases management.

Acknowledgment: The authors would like to thank Harun ARSLAN from Ankara Yıldırım Beyazıt University GETAT Center Research Apiary for his help in the care and supply of bees, and Turan ÇIRAK, technical staff of the GETAT Center, for his help in the special hive design.

Author contribution: Nilüfer VURAL: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing – original draft; Writing – review & editing. Sibel KAYMAK: Conceptualization; Formal analysis; Software; Validation; Visualization; original draft; Writing – review & editing. Oğuz YÜCE: Conceptualization; Methodology; Funding acquisition; Supervision

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement: No experimentation on human or animal subjects was involved in this study. At the stage of bee venom sampling from honeybees, ethical permission is not required for insects.

Data availability: Data will be made available on request.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: This research was supported by the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agriculture Research and Policies (TAGEM) with Project number: TAGEM/21/AR-GE/25.

REFERENCES

Abd El-Wahed AA, Farag MA, Eraqi WA, Mersal GAM, Zhao C, Khalifa SAM et al. Unravelling the beehive air volatiles profile as analysed via solid-phase microextraction (SPME) and

chemometrics. Journal of King Saud University–Science, 2021;33:101449.

Allen L, Carr SB, Davies G, Downey D, Egan M, Forton JT et al. Future therapies for cystic fibrosis. A.J.N.C., 2023;14:693.

Barnes M, Brisbois EJ. Clinical use of inhaled nitric oxide: Local and systemic applications, J.F.R.B., 2020;152:422-431.

Boch R, Shearer D, Stone BJN. Identification of iso-amyl acetate as an active component in the sting pheromone of the honey bee. Nature, 1962;195:1018-1020.

Burley SK, Berman HM, Kleywegt GJ, Markley JL, Nakamura H., Velankar S. Protein Data Bank (PDB): the single global macromolecular structure archive. J.P.C.M., 2017;627-641.

Camargos AF, Cossolin JFS, Martínez LC, Gonçalves WG, dos Santos MH, Zanuncio JC et al. Morphology and chemical composition of the Koschewnikow gland of the honey bee *Apis mellifera* (Hymenoptera: Apidae) workers engaged in different tasks. Journal of Apicultural Research, 2020;59:1037-1048.

Castellani C, Assael BM. Cystic fibrosis: a clinical view. Cellular and molecular life sciences, 2017;74:129-140.

Choi HS, Kang SY, Roh DH, Choi SR, Ryu Y, Lee JH. Bee venom stimulation of a lung meridian acupoint reduces inflammation in carrageenan-induced pleurisy: an alternative therapeutic approach for respiratory inflammation. .J.O.V.S.,2018;19:708-715.

Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. J.N.R.G., 2015;16:45-56.

Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. J.S.r., 2017;7:42717.

de Graaf DC, Brochetto-Braga MR, de Abreu RMM, Blank S, Bridts CH., De Clerck LS et al. Standard methods for *Apis mellifera* venom research. Journal of Apicultural Research, 2021;60:1-31.

de Lacy-Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T et al. A review of the volatiles from the healthy human body. J.O.B.R., 2014;8:014001.

Dogru D, Çakır E, Şişmanlar T, Çobanoğlu N, Pekcan S, Çinel G et al. Cystic fibrosis in Turkey: First data from the national registry. J.P.P., 2020;55: 541-548.

Fraser-Pitt D, O'Neil D. Cystic fibrosis–a multiorgan protein misfolding disease. Future science OA, 2015;1(2): DOI: 10.4155/fso.15.57

Fratellone PM, Tsimis F, Fratellone G. Apitherapy products for medicinal use.The Journal of Alternative and Complementary Medicine, 2016;22(12):1020-1022.

Graeber SY, Mall MA. The future of cystic fibrosis treatment: from disease mechanisms to novel therapeutic approaches. J.T.L., 2023;402:1185-1198.

Habryka C, Kruczek M, Drygaś B. Bee products used in apitherapy. J.W.S.N., 2016:254-258.

Hasan A, Qazi JI, Tabssum F, Hussain A. Increased bee venom production in *Apis mellifera* workers on the provision of probiotics and organic acids. ISBAB, 2023;48.

Isidorov V, Zalewski A, Zambrowski G, Swiecicka I. Chemical Composition and Antimicrobial Properties of Honey Bee Venom. Molecules, 2023;28:4135

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A et al. PubChem substance and compound databases J.N.a.r., 2016;44:1202-1213.

Li J, Wang Z, Tan K, Qu Y, Nieh JC. Effects of natural and synthetic alarm pheromone and individual pheromone components on foraging behavior of the giant Asian honey bee, *Apis dorsata*. The Journal of experimental biology, 2014;217:3512-3518.

Lillehoj EP, Kato K, Lu W, Kim KC. Cellular and molecular biology of airway mucins. J.I.R.O.C., 2013;(303):139-202.

Matysiak J, Breborowicz A, Kycler Z, Derezinski P, Kokot ZJ. Immune and clinical response to honeybee venom in beekeepers. A.O.A., 2016;23.

Maunz A, Gütlein M, Rautenberg M, Vorgrimmler D, Gebele D, Helma C. lazar: a modular

predictive toxicology framework. Frontiers in pharmacology, 2013;4.

Naehrig S, Chao CM, Naehrlich L. Cystic fibrosis: diagnosis and treatment. Deutsches Ärzteblatt International, 2017;114:33-34.

Nakamura Y, Sugano A, Ohta M, Takaoka Y. Docking analysis and the possibility of prediction efficacy for an anti-IL-13 biopharmaceutical treatment with tralokinumab and lebrikizumab for bronchial asthma. J.P.O., 2017;12.

Nikbakhtzadeh MR, Tirgari S, Fakoorziba MR, Alipour H. Two volatiles from the venom gland of the Samsum ant, Pachycondyla sennaarensis. Toxicon, 2009;54:80-82.

Noël A, Dumas C, Rottier E, Beslay D, Costagliola G, Ginies C et al. Detailed chemical analysis of honey bee (*Apis mellifera*) worker brood volatile profile from egg to emergence. J.P.O., 2023;18.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC et al. UCSF Chimera—a visualization system for exploratory research and analysis. J.O.C.C., 2004;25: 1605-1612.

Samsuzzaman M, Uddin MS, Shah MA, Mathew B. Natural inhibitors on airway mucin: Molecular insight into the therapeutic potential targeting MUC5AC expression and production. J.L.s., 2019;231:116485.

Schmitt T, Herzner G, Weckerle B, Schreier P, Strohm E. Volatiles of foraging honeybees *Apis mellifera* (Hymenoptera: Apidae) and their potential role as semiochemicals. J.A., 2007;38:164-170.

Simone-Finstrom M, Angove M, Brooks P, Gerdts J. Identification and Discrimination of Volatiles Associated With Chalkbrood Infection in European Honey Bees (*Apis mellifera*), Eastern Australia. J.R.S., 2023;24.

Stela M, Cichon N, Spławska A, Szyposzynska M, Bijak M. 2024. Therapeutic Potential and Mechanisms of Bee Venom Therapy: A Comprehensive Review of Apitoxin Applications and Safety Enhancement Strategies. Pharmaceuticals, 2024;17(9).

Studio, Discovery studio. D.J.A., 2008.

Topal E, Adamchuk L, Negri I, Kösoğlu M, Papa G, Dârjan MS et al. Traces of Honeybees, Api-Tourism and Beekeeping: From Past to Present. J.S., 2021;13.

Ullah A, Aldakheel FM, Anjum SI, Raza G, Khan SA, Gajger IT. Pharmacological properties and therapeutic potential of honey bee venom. S.P.J. 2023; 31(1):96-109.

Vega S, Abian O, Velazquez-Campoy A. On the link between conformational changes, ligand binding and heat capacity. BBA-General Subjects, 2016;5:868-878.

Wang, C, Aleksandrov AA, Yang Z, Forouhar F, Proctor EA, Kota P et al. Ligand binding to a remote site thermodynamically corrects the F508del mutation in the human cystic fibrosis transmembrane conductance regulator. The Journal of biological chemistry, 2018;293:17685-17704.

Wang, Z, Tan K. Honey Bee Alarm Pheromone Mediates Communication in Plant-Pollinator-Predator Interactions. Insects, 2019;10.

Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier JM, Fajloun Z. Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. Molecules, 2019;24.

Weis WA, Ripari N, Conte FL, da Silva-Honorio M, Sartori AA, Matucci RH et al. An overview about apitherapy and its clinical applications. J.P.P., 2022;2:100239.

Wolber G, Langer T. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J.o.c.i.m., 2005;45:160-169.

Yassin MT, Mostafa AA, Al-Askar AA. Anticandidal and anti-carcinogenic activities of *Mentha longifolia* (Wild Mint) extracts in vitro. J.O.K.S.U.S., 2020;32: 2046-2052.