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# Phytochemical profiling of *Artemisia absinthium* essential oils from different regions of Türkiye and preliminary evaluation of their antibacterial and honeybee locomotor effects

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## Abstract

This study aimed to determine the phytochemical composition of essential oils obtained from *Artemisia absinthium* collected in Sivas (AAS), Erzincan (AAE) and Hakkari (AAH) and to evaluate their effects on honeybee locomotor activity. The chemical composition of the essential oils was analyzed by GC–MS, revealing notable regional differences. Chamazulene was identified as the predominant compound in all samples, with the highest concentration in AAH (64.6%), while AAE was characterized by a high β-thujone content (18.3%). Locomotor activity assays showed no significant differences between control and treated groups over a 24-hour period (H (3) = 4.270, p=0.234; F (3,121)=0.867, p=0.460), indicating no detectable acute locomotor alterations under the tested conditions. The antibacterial activity of the essential oils was evaluated using the disc diffusion method against both Gram-positive and Gram-negative bacteria. Antibacterial effects varied depending on the oils' geographical origin and chemical composition. AAE and AAS essential oils showed comparatively larger inhibition zones, particularly against *Staphylococcus aureus* and *Bacillus cereus*, whereas AAH showed selective activity against *Pseudomonas aeruginosa*. No inhibition of Listeria monocytogenes was observed with any of the tested oils. Overall, the oils' antibacterial activity was moderate and selective compared with the positive control, imipenem. These findings suggest that regional variation significantly influences the chemical composition and biological activity of *A. absinthium* essential oils. Furthermore, the tested oils did not produce detectable acute locomotor alterations in honeybees under the conditions tested. These findings provide preliminary insight into the relationship between regional phytochemical variability and the biological properties of *A. absinthium* essential oils.

**Keywords:** Antibacterial activity, *Apis mellifera*, *Artemisia absinthium*, Essential oil, Locomotor activity

## INTRODUCTION

Beekeeping plays a crucial role in Türkiye's agricultural production system and ecological balance, contributing significantly to both economic sustainability and biodiversity conservation. Türkiye's diverse climate zones and rich flora provide favorable conditions for beekeeping activities, positioning the country among the world's leading honey producers (Aydın 2025, Ünal 2022). In addition to honey production, products such as beeswax, royal jelly, and propolis enhance the economic value of beekeeping, while honeybees play a critical role in pollinating agricultural crops, contributing to food security and ecosystem sustainability. Indeed, the bioactive properties and chemical parameters of honeys produced in different regions of Türkiye have been shown to vary significantly based on local floral diversity and environmental conditions (Portakal et al. 2023, Portakal and Çiltepe 2024).

However, beekeeping today faces increasing threats due to environmental stressors such as pests, pathogens, pesticide exposure, and climate change (Allabergenova et al. 2021, Sabo et al. 2024, Nicholson et al. 2024). Synthetic chemicals, widely used in disease and pest control, pose significant problems, including the development of resistance,

environmental pollution, and the risk of residues in bee products. This situation has increased interest in environmentally friendly and sustainable alternative approaches to protecting bee health (Omran et al. 2026).

Among natural products, species of the genus Artemisia (Asteraceae) stand out for their high essential oil content and broad biological activity potential. *A. absinthium* exhibits antimicrobial, antiparasitic, anti-inflammatory, and antioxidant properties due to its terpenoids and other secondary metabolites (Ramakrishna and Ravishankar 2011, Abad et al. 2012, Nigam 2019). However, it has been reported that the chemical composition of *A. absinthium* essential oils varies significantly depending on geographical and ecological conditions, and that there may be marked differences in the proportions of the main components (Orav et al. 2006). These chemical differences are thought to directly affect the biological activity of the oils.

Recent studies indicate that preparations derived from *A. absinthium* may offer potential benefits in controlling *Nosema ceranae* infection in honeybees (Özüiçli et al. 2023). However, research on *A. absinthium* and related Artemisia species has largely focused on antimicrobial, antifungal, and antiparasitic effects, whereas the contact toxicity and behavioural effects of these essential oils on honeybees, particularly in terms of locomotor activity, have been investigated to a limited extent (Asghari et al. 2012, Szopa et al. 2020). Although plant-derived products are increasingly suggested as natural alternatives for disease and pest control in beekeeping, systematic evaluations of the effects of these substances on bee physiology and behaviour are still insufficient (Erdoğan and Cengiz 2020, Neumann and Straub 2023, Sabo et al. 2024).

Locomotor activity is considered a sensitive behavioural endpoint in honeybee toxicology because alterations in movement patterns may reflect early neurophysiological disturbances caused by bioactive compounds. Therefore, locomotor assays are commonly used as preliminary screening tools to evaluate the potential sublethal effects of plant-derived products on pollinators (*Apis mellifera*) (Caren et al. 2025, Giannoni-Guzmán et al. 2014). Furthermore, although it is known that the chemical composition of *A. absinthium* essential oils varies significantly depending on geographical origin, the potential effects of these chemical differences on bee behaviour and safety have not yet been sufficiently elucidated (Bailen et al. 2013, El Ouardi et al. 2024). Therefore, it was hypothesized that geographical variation in the chemical composition of *A. absinthium* essential oils may be associated with differences in their antibacterial activity and short-term effects on honeybee locomotor behaviour.

This study aimed to contribute to understanding the phytochemical variability and the preliminary biological effects of A. absinthium essential oils obtained from different regions of Türkiye.

## MATERIALS AND METHODS

### Plant Material

The plant materials used in this study belong to the species *A. absinthium* and were collected from previously determined locations in the provinces of Sivas, Erzincan, and Hakkari. The identification of the plant species was carried out by Prof. Dr. Ali KANDEMİR for Sivas and Erzincan, and by Prof. Dr. Murat KÜRŞAT for the plants in Hakkari. The plant specimens were identified and registered in the Bitlis Eren University Herbarium with the code BEUH911 for AAH; in the Binali Yıldırım University Herbarium with the code EBYU-0000037 for AAE; and in the Binali Yıldırım University Herbarium with the code EBYU-0000038 for AAS. Following collection from their natural environment, the plant materials were dried in cool, moisture-free environments, out of direct sunlight. The dried plants were subjected to a process of pulverisation, resulting in a powdered form, and subsequently stored in glass jars for subsequent use.

### Extraction of Essential Oils from *A. absinthium* Plants

To obtain essential oils (EOs), the above-ground flowering parts of the plants were dried in the shade. Plant samples (1 kg) were ground in a laboratory-type grinder, and essential oils were obtained by water distillation for 3-4 hours using a Neo-Clevenger apparatus. The obtained essential oils were collected from the collection chamber and stored in dark glass bottles (+4°C). The remaining aqueous portions after distillation were stored in dark glass containers and used for further chemical processing (Tüfekçi et al. 2024).

### Chemical Composition Analysis of Essential Oils by GC-MS Instrumentation

Qualitative and quantitative analyses of essential oils were performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert mass spectrometer (USA). Separation of essential oil components was achieved using an HP-5MS capillary column (30 m × 250 µm inner diameter × 0.25 µm film thickness). Samples were diluted with chloroform at a 1:10 ratio prior to injection, and 1 µL of the stock solution was injected at 280°C with a 30:1 split ratio for each analysis. Helium was used as the carrier gas, with a constant flow rate of 1 mL/min. The oven temperature program was implemented as follows: The initial temperature was held at 50°C for 10 minutes; the temperature was then increased to 72°C at 2°C/min and held steady for 2 minutes. Following this, it was raised to 82°C at 1°C/min and held for 10 minutes, then increased to 120°C at 2°C/min and held for 10 minutes. Subsequently, the temperature was increased to 200°C at 5°C/min then to 250°C at 10°C/min, and held steady for 10 minutes at each stage, resulting

in a total analysis time of 105 minutes. The mass spectrometer parameters were set as follows: ion source temperature 230°C, quadropole temperature 150°C, and MS interface temperature 280°C. The spectrometer was activated after a 3-minute solvent delay, and analyses were performed in full-scan mode over the 50–650 m/z range with a scan time of 0.2 seconds. Compound identification was performed by comparing mass spectra with the NIST, Wiley05, and NIST02 databases. Eclipse indices calculated for all compounds according to the homologous n-alkane series (C8–C30) using the Van den Dool and Kratz equation were validated against the literature (Tüfekçi et al. 2025).

### Antibacterial Activity Assay

Antibacterial activity of the essential oils was evaluated using the disc diffusion method according to previously published protocols (Akşit and Gergin 2025, Gözcü et al. 2026). Briefly, bacterial suspensions were adjusted to the 0.5 McFarland standard (approximately 1.5 × $10^8$ CFU/mL) and uniformly spread onto Mueller–Hinton agar plates. Sterile paper discs (6 mm diameter) were impregnated with 5 µL of each essential oil and placed onto the inoculated agar surface. Plates were incubated at 37°C for 24 h, after which inhibition zone diameters were measured in millimeters. Imipenem discs were used as the positive control, and solvent-treated discs as the negative control. For each bacterial strain and essential oil, the assay was performed as three technical replicates (three independent agar plates inoculated from the same freshly prepared bacterial suspension), using essential oil obtained from a single distillation batch per region. Accordingly, the reported variability (mean ± SD, n = 3) reflects assay/measurement variability rather than between-batch biological variability.

### Locomotor Activity Experiment

The locomotor activity experiment was carried out separately with forager bees. Forager bees were collected from three hives by placing a plastic wire mesh screen at the entrance and collecting the foragers that accumulated on the screen into small containers. Bees in the containers were then released into a 30x30x30 cm flying cage and individually collected from this cage into 15 ml Falcon tubes. Thus, samples collected from multiple hives were pooled and randomly assigned to experimental groups. Bees were then anesthetized by immersing the tubes in an ice bath. Essential oils (EO) were prepared in acetone at a concentration of 50 ppm (50 µg/mL; 1 mg EO/20 mL acetone). Subsequently, 2 µL of the prepared solution was topically applied to the thorax region of each anesthesized bee, corresponding to an approximate exposure dose of 0.1 µg EO per bee. In the control group, only acetone was applied. The bees were again individually put into 15 ml Falcon tubes. Fondant sugar was placed on the lids of Falcon tubes as food, and the candy was covered with gauze to prevent bees from sticking to it. The tubes were then placed into activity monitor modules. The individual locomotor activity monitor (LAM) manufactured by Trikinetics was used in this experiment. This device is a version of the activity monitor previously developed for *Drosophila* and it is adapted for larger insects such as honeybees and wasps. It consists of modules, each containing 32 holes. The width of these holes is enough to fit a 15 ml Falcon tube.

As the honeybees placed in the Falcon tubes move through the tube, a signal is sent to the computer connected to the module whenever they pass through the module-nest area, which contains 4 infrared sensors. Thus, the locomotor activity of each bee sample can be determined from the number of signals received at a specified time. In addition, an environmental monitor connected to the device measures environmental temperature and humidity at specified time intervals and transmits the readings to the computer (Giannoni-Guzman et al. 2014). It was possible to examine 96 bees simultaneously with three activity modules. The control group includes 32 bees, and the experimental group includes 64 bees. Activity modules were placed in the incubator at 33 °C and 60% relative humidity. The experiment lasted 24 hours in a constantly dark environment. At the end of the experiment, dead and alive bees were recorded. Data distribution was evaluated using the Shapiro–Wilk normality test. Where necessary, logarithmic and square-root transformations were applied to improve normality prior to parametric statistical analyses.

## RESULTS

### Chemical Composition Analysis of Essential Oils by GC-MS Instrumentation

The chemical profiles of *A. absinthium* essential oils obtained from AAS, AAE and AAH locations were determined by GC-MS analysis, and the results are presented in Table 1. A total of 106 different compounds were identified as a result of the analyses. It was determined that the main components of the essential oils consist of monoterpenes, sesquiterpenes, and their oxygenated derivatives, while diterpenes are represented in low proportions.

**Table 1**. GC-MS analysis results of EOs of *A. absinthium* species collected from different regions

| No | RT | Compounds name | Formula | $RI_a$ | $RI_b$ | % Area | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | AAS | AAE | AAH |
| **1** | 6.55 | α-Thujene | $C_{10}H_{16}$ | 918 | 925 | - | 0.04 | 0.06 |
| **2** | 6.82 | α-Pinene | $C_{10}H_{16}$ | 926 | 928 | - | 0.17 | 0.14 |
| **3** | 7.45 | α-Fenchene | $C_{10}H_{16}$ | 947 | 955 | - | 0.08 | - |
| **4** | 9.24 | Sabinene | $C_{10}H_{16}$ | 965 | 973 | 0.74 | 3.31 | 2.44 |
| **5** | 9.29 | β-Pinene | $C_{10}H_{16}$ | 972 | 976 | - | - | 1.21 |
| **6** | 10.80 | Artemiseole | $C_{10}H_{16}O$ | 982 | 978 | 0.90 | - | - |
| **8** | 11.56 | β-Myrcene | $C_{10}H_{16}$ | 985 | 991 | 0.88 | 1.92 | 0.62 |
| **9** | 12.36 | α-Phellandrene | $C_{10}H_{16}$ | 995 | 1004 | 1.13 | 4.35 | 1.65 |
| **10** | 13.20 | δ-3-Carene | $C_{10}H_{16}$ | 1006 | 1007 | 0.16 | - | - |
| **11** | 13.04 | α-Terpinene | $C_{10}H_{16}$ | 1015 | 1008 | 0.10 | 0.15 | 0.30 |
| **12** | 13.25 | *p*-Cymene | $C_{10}H_{14}$ | 1025 | 1022 | 1.76 | 1.00 | 1.56 |
| **13** | 13.43 | β-Phellandrene | $C_{10}H_{16}$ | 1030 | 1026 | - | 0.13 | 0.28 |
| **14** | 14.44 | Eucalyptol | $C_{10}H_{18}O$ | 1035 | 1034 | 0.99 | 1.13 | 1.48 |
| **15** | 14.87 | trans-β-Ocimene | $C_{10}H_{16}$ | 1037 | 1037 | - | 0.45 | 0.06 |
| **16** | 15.21 | D-Limonene | $C_{10}H_{16}$ | 1038 | 1040 | 3.94 | - | - |
| **17** | 15.71 | cis-β-Ocimene | $C_{10}H_{16}$ | 1043 | 1047 | - | 0.08 | - |
| **18** | 16.34 | γ-Terpinene | $C_{10}H_{16}$ | 1056 | 1057 | 0.36 | 0.23 | 0.52 |
| **19** | 17.95 | *trans*-4-Thujanol | $C_{10}H_{18}O$ | 1075 | 1069 | 0.16 | - | - |
| **20** | 18.91 | Terpinolene | $C_{10}H_{16}$ | 1081 | 1088 | 0.10 | 0.05 | 0.12 |
| **21** | 19.24 | (*E*)-Linalool oxide | $C_{10}H_{18}O_2$ | 1092 | 1085 | - | 7.77 | - |
| **22** | 19.28 | 6-Camphenone | $C_{10}H_{14}O$ | 1095 | 1095 | 0.42 | - | - |
| **23** | 19.60 | **β-Linalool** | $C_{10}H_{18}O$ | 1096 | 1101 | 8.21 | - | 0.53 |
| **24** | 20.25 | Nonanal | $C_9H_{18}O$ | 1099 | 1102 | 0.23 | - | 0.11 |
| **25** | 20.83 | Hotrienol | $C_{10}H_{16}O$ | 1106 | 1109 | - | 0.25 | - |
| **26** | 20.89 | α-Thujone | $C_{10}H_{16}O$ | 1110 | 1113 | - | 3.98 | 0.10 |
| **27** | 21.95 | **β-Thujone** | $C_{10}H_{16}O$ | 1119 | 1115 | - | 18.3 | - |
| **28** | 22.07 | (*Z*)-*p*-2-Menthen-1-ol | $C_{10}H_{18}O$ | 1119 | 1121 | 0.55 | 0.14 | - |
| **29** | 22.36 | cis-Sabinol | $C_{10}H_{16}O$ | 1143 | 1141 | - | 0.37 | - |
| **30** | 22.51 | (E)-*p*-2-Menthen-1-ol | $C_{10}H_{18}O$ | 1146 | 1141 | 0.18 | - | - |
| **31** | 22.85 | trans-Sabinol | $C_{10}H_{16}O$ | 1150 | 1145 | 0.48 | - | - |
| **32** | 24.33 | cis-Verbenol | $C_{10}H_{16}O$ | 1152 | 1146 | 1.06 | 0.18 | - |
| **33** | 25.04 | 3-Thujanone | $C_{10}H_{16}O$ | 1156 | 1148 | - | 1.28 | - |
| **34** | 25.35 | Lavandulol | $C_{10}H_{18}O$ | 1170 | 1165 | 0.54 | 0.28 | - |
| **35** | 26.69 | Terpinen-4-ol | $C_{10}H_{18}O$ | 1183 | 1185 | 3.70 | 0.80 | 1.33 |
| **36** | 26.77 | α-Terpineol | $C_{10}H_{18}O$ | 1197 | 1191 | 0.75 | 0.24 | 0.26 |
| **37** | 26.92 | 2-Pinen-10-ol | $C_{10}H_{16}O$ | 1198 | 1194 | - | 0.23 | - |
| **38** | 27.03 | cis-Piperitol | $C_{10}H_{18}O$ | 1203 | 1196 | 0.35 | - | - |
| **39** | 27.67 | Grandisol | $C_{10}H_{18}O$ | 1205 | 1200 | 0.44 | - | - |
| **40** | 28.40 | trans-Piperitol | $C_{10}H_{18}O$ | 1211 | 1208 | 0.30 | - | - |
| **41** | 29.32 | trans-Chrysanthenyl acetate | $C_{12}H_{18}O_2$ | 1227 | 1233 | - | 0.13 | - |
| **42** | 31.48 | Ascaridole | $C_{10}H_{16}O_2$ | 1228 | 1235 | 0.38 | - | - |
| **43** | 33.35 | cis-3-Hexenyl isovalerate | $C_{11}H_{20}O_2$ | 1236 | 1238 | - | 0.10 | - |
| **44** | 33.96 | Pulegone | $C_{10}H_{16}O$ | 1243 | 1240 | - | - | 1.43 |
| **45** | 34.88 | 5-Caranol | $C_{10}H_{18}O$ | 1246 | 1244 | 0.41 | - | - |
| **46** | 36.34 | 2,6-dimethyl-1,7-octadiene-2,6-diol | $C_{10}H_{18}O_2$ | 1280 | 1279 | - | - | 0.22 |
| **47** | 37.68 | Perillaldehyde | $C_{10}H_{14}O$ | 1288 | 1286 | - | 0.06 | - |
| **48** | 37.97 | Bornyl acetate | $C_{12}H_{20}O_2$ | 1291 | 1290 | 1.22 | - | - |
| **49** | 38.29 | Lavandulyl acetate | $C_{12}H_{20}O_2$ | 1297 | 1291 | 1.37 | 0.30 | - |
| **50** | 38.31 | cis-2,3-Pinanediol | $C_{10}H_{18}O_2$ | 1311 | 1319 | 0.49 | - | - |
| **51** | 39.17 | Myrtenyl acetate | $C_{12}H_{18}O_2$ | 1318 | 1321 | | 2.58 | - |
| **52** | 40.26 | Cyclohexanone, 2-(3-oxobutyl)- | $C_{10}H_{16}O_2$ | 1349 | 1355 | 0.27 | - | - |
| **53** | 41.79 | Eugenol | $C_{10}H_{12}O_2$ | 1353 | 1361 | | 0.05 | - |
| **54** | 42.72 | Nerol acetate | $C_{12}H_{20}O_2$ | 1359 | 1365 | 0.86 | 0.18 | - |
| **55** | 42.93 | 1H-Indene, 2,3,3a,4-tetrahydro-3,3a,6-trimethyl-1-(1-methylethyl)- | $C_{15}H_{24}$ | 1363 | 1372 | - | 1.36 | 0.17 |
| **56** | 43.41 | Linalyl isobutyrate | $C_{14}H_{24}O_2$ | 1366 | 1374 | 0.48 | - | 0.19 |
| **57** | 44.76 | β-Elemene | $C_{15}H_{24}$ | 1392 | 1395 | 0.14 | 0.43 | - |
| **58** | 45.02 | cis-Jasmone | $C_{11}H_{16}O$ | 1398 | 1397 | 0.55 | - | - |
| **59** | 46.38 | Cyperene | $C_{15}H_{24}$ | 1405 | 1398 | 0.45 | 0.08 | - |
| **60** | 47.13 | 1-Adamantyl methyl ketone | $C_{12}H_{18}O$ | 1409 | 1416 | 1.75 | 0.56 | - |
| **61** | 47.71 | Caryophyllene | $C_{15}H_{24}$ | 1436 | 1429 | 2.04 | 1.19 | 1.10 |
| **62** | 48.72 | γ-Elemene | $C_{15}H_{24}$ | 1444 | 1437 | | 0.07 | - |
| **63** | 49.32 | Linalyl butyrate | $C_{14}H_{24}O_2$ | 1439 | 1447 | - | - | 0.17 |
| **64** | 49.36 | Humulene | $C_{15}H_{24}$ | 1458 | 1454 | 0.41 | - | 0.11 |
| **65** | 49.97 | 2,2,3,3-Tetramethyl-1-indanone | $C_{13}H_{16}O$ | 1458 | 1456 | 0.40 | - | - |
| **66** | 50.10 | Neryl propionate | $C_{13}H_{22}O_2$ | 1461 | 1463 | - | 0.07 | - |
| **67** | 50.86 | γ-Himachalene | $C_{15}H_{24}$ | 1461 | 1467 | 0.29 | - | - |
| **68** | 51.49 | β-Acoradiene | $C_{15}H_{24}$ | 1474 | 1473 | 1.64 | 0.88 | 1.97 |
| **69** | 52.21 | Geranyl propionate | $C_{13}H_{22}O_2$ | 1475 | 1479 | 0.25 | - | - |
| **70** | 52.45 | Germacrene D | $C_{15}H_{24}$ | 1476 | 1481 | - | 0.41 | - |
| **71** | 52.63 | 2-Hydroxy-4-isopropylnaphthalene | $C_{13}H_{14}O$ | 1476 | 1484 | 0.92 | 1.09 | - |
| **72** | 52.90 | β-Eudesmene | $C_{15}H_{24}$ | 1479 | 1486 | 1.28 | 0.31 | 0.91 |
| **73** | 53.16 | Elixene | $C_{15}H_{24}$ | 1498 | 1492 | 0.41 | 0.37 | - |
| **74** | 53.29 | Linalyl isovalerate | $C_{15}H_{26}O_2$ | 1493 | 1497 | 1.73 | 0.25 | 0.30 |
| **75** | 54.29 | β-Himachalene | $C_{15}H_{24}$ | 1501 | 1500 | 0.56 | 0.20 | - |
| **76** | 55.07 | 6-Epishyobunone | $C_{15}H_{24}O$ | 1504 | 1504 | 0.26 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **77** | 55.47 | Geranyl isobutyrate | $C_{14}H_{24}O$ | 1518 | 1514 | 1.33 | 0.37 | - |
| **78** | 56.20 | Geraniol isobutyrate | $C_{14}H_{24}O$ | 1520 | 1515 | 0.65 | 0.44 | 0.26 |
| **79** | 57.65 | Benzene, (2-ethyl-4-methyl-1,3-pentadienyl)-, (E)- | $C_{14}H_{18}$ | 1524 | 1515 | 1.08 | 1.31 | 2.91 |
| **80** | 58.02 | γ-Selinene | $C_{15}H_{24}$ | 1525 | 1532 | 0.23 | - | - |
| **81** | 59.43 | Spathulenol | $C_{15}H_{24}O$ | 1544 | 1548 | 1.35 | 0.15 | 0.40 |
| **82** | 60.90 | Anisole, p-1-cyclohexen-1-yl- | $C_{13}H_{16}O$ | 1551 | 1560 | 0.32 | - | - |
| **83** | 61.98 | Limonen-6-ol, pivalate | $C_{15}H_{24}O_2$ | 1556 | 1560 | 0.39 | - | - |
| **84** | 62.40 | Linalyl 3-methyl butanoate | $C_{15}H_{26}O_2$ | 1582 | 1582 | - | 0.32 | 0.43 |
| **85** | 63.55 | Caryophyllene oxide | $C_{15}H_{24}O$ | 1595 | 1591 | 0.61 | 0.12 | 0.53 |
| **86** | 64.81 | 7-epi-cis-sesquisabinene hydrate | $C_{15}H_{26}O$ | 1577 | 1575 | 0.38 | - | - |
| **87** | 65.63 | Butanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)- | $C_{15}H_{26}O_2$ | 1593 | 1596 | 1.00 | - | - |
| **88** | 66.64 | Isoaromadendrene epoxide | $C_{15}H_{24}O$ | 1622 | 1620 | 0.83 | 0.31 | - |
| **89** | 67.58 | Geranyl pentanoate | $C_{15}H_{26}O_2$ | 1667 | 1659 | - | - | 0.29 |
| **90** | 68.29 | trans-(Z)-α-Bergamotol | $C_{15}H_{24}O$ | 1686 | 1688 | - | - | 0.31 |
| **91** | 69.76 | Juniper camphor | $C_{15}H_{26}O$ | 1687 | 1690 | 1.89 | 0.78 | - |
| **92** | 70.06 | Cedr-8-en-13-ol | $C_{15}H_{24}O$ | 1694 | 1691 | 1.25 | 0.24 | - |
| **93** | 70.93 | β-Santalol | $C_{15}H_{24}O$ | 1721 | 1714 | - | - | **2.69** |
| **94** | 71.16 | Neryl (S)-2-methylbutanoate | $C_{15}H_{26}O_2$ | 1713 | 1717 | 0.95 | 0.28 | 0.39 |
| **95** | 71.84 | (E)-Nuciferol | $C_{15}H_{22}O$ | 1729 | 1733 | | - | 0.38 |
| **96** | 72.59 | **Chamazulene** | $C_{14}H_{16}$ | 1737 | 1734 | 28.2 | 33.5 | 64.6 |
| **97** | 73.95 | 4,4'-Dimethylbiphenyl | $C_{14}H_{14}$ | 1752 | 1756 | 0.70 | - | 1.03 |
| **98** | 75.48 | Azulen-2-ol, 1,4-dimethyl-7-(1-methylethyl)- | $C_{15}H_{18}O$ | 1631 | 1628 | 0.69 | - | - |
| **99** | 76.88 | cis-Lanceol | $C_{15}H_{24}O$ | 1766 | 1761 | - | - | 0.54 |
| **100** | 80.86 | 4',4-Dimethylangelicin | $C_{13}H_{10}O_3$ | 1790 | 1793 | 1.06 | - | - |
| **101** | 85.98 | Geranyl-α-terpinene | $C_{20}H_{32}$ | 1884 | 1883 | 0.96 | 0.49 | 0.50 |
| **102** | 86.34 | Geranyl-*p*-cymene | $C_{18}H_{26}$ | 1893 | 1898 | 1.11 | - | 0.45 |
| **103** | 89.23 | α-Curcumene | $C_{15}H_{22}$ | 1962 | 1953 | 1.15 | - | - |
| **104** | 90.17 | α-Springene | $C_{20}H_{32}$ | 1978 | 1969 | 0.54 | 0.36 | - |
| **105** | 92.86 | 3,7,11,15-Tetramethyl-2-hexadecenol | $C_{20}H_{40}O$ | 1988 | 1992 | - | - | 0.20 |
| **106** | 95.39 | Squalane | $C_{30}H_{62}$ | 2003 | 2004 | - | - | 0.30 |
| | | **Total** | | | | **93.6** | **96.2** | **95.5** |
| | | MH-Monoterpene hydrocarbons | | | | 7.41 | 10.9 | 7.40 |
| | | MO-Oxygenated Monoterpenes | | | | 20.6 | 35.2 | 5.35 |
| | | SH-Sesquiterpene Hydrocarbons | | | | 7.45 | 5.3 | 4.26 |
| | | SO-Oxygenated Sesquiterpenes | | | | 10.6 | 2.45 | 6.26 |
| | | D-Diterpenes | | | | 1.5 | 0.85 | 0.7 |
| | | Others | | | | 46.04 | 41.5 | 71.53 |

$RI_a$ retention index determined on HP-5MS column using the homologous series of n-hydrocarbons;

$RI_b$ retention index determined with HP-5MS column in the literatüre

KI: Kovats indices with those of Wiley spectral library collection and NIST (National 104 Institute of Standards and Technology) library database

In all samples, the most dominant compound was identified as chamazulene, detected at 28.2% in AAS, 33.5% in AAE, and 64.6% in AAH. Regarding oxygenated monoterpenes, the AAE sample showed a concentration of 35.2%, higher than the AAS (20.6%) and AAH (5.35%) samples. Monoterpene hydrocarbons were most prevalent in the AAE sample at 10.9%. The AAS sample was the most prominent in terms of sesquiterpene hydrocarbons (7.45%) and showed the highest concentration of oxygenated sesquiterpenes (10.6%). β-Thujone was detected in high concentrations only in the AAE sample 18.3%, while β-linalool was found in both the AAS 8.21% and AAH 0.53% samples.

### Antibacterial Activity Assay

The antibacterial activity of *A. absinthium* essential oils obtained from three different locations was evaluated using the disc diffusion assay, and the results are presented in Table 2. Among the tested oils, AAE and AAS EOs showed comparatively larger inhibition zones, particularly against Gram-positive bacteria, showing inhibition zones of 13.0 mm against *Staphylococcus aureus* and 12.0 mm against *Bacillus cereus*. The AAH essential oil showed inhibition against *Pseudomonas aeruginosa* (10.0 mm), whereas no inhibition was observed for the other oils against this strain. Moderate inhibitory effects against *Clostridium perfringens* and *Salmonella enteritica* were observed across all essential oils. Notably, *Listeria monocytogenes* showed no inhibition with any of the tested essential oils, whereas the positive control, imipenem, exhibited clear antibacterial activity against all bacterial strains.

**Table 2.** Antibacterial activity of *A. absinthium* essential oils

| Bacterial strains | AAH | AAE | AAS | Imipenem |
|---|---|---|---|---|
| ***Staphylococcus aureus*** | ni | 13.0 ± 0.5 mm | 13.0 ± 0.5 mm | 24 ± 1.0 mm |
| ***Clostridium perfringens*** | 9.0± 0.2 mm | 11.5 ± 0.5 mm | 10.0± 0.5 mm | 22± 2.3 mm |
| ***Enterococcus faecium*** | ni | 10.0± 0.7 mm | ni | 28± 2.1 mm |
| ***Listeria monocytogenes*** | ni | ni | ni | 18± 1.6 mm |
| ***Bacillus cereus*** | ni | 12.0± 0.5 mm | 10.0± 0.6 mm | 23± 1.3 mm |
| ***Pseudomonas aeruginosa*** | 10.0± 0.4 mm | ni | ni | 32± 1.7 mm |
| ***Escherichia coli*** | ni | 11.5± 0.4 mm | ni | 26± 1.8 mm |
| ***Salmonella enteritica*** | 8.0± 0.2 mm | 10.0± 0.3 mm | 11.0± 0.8 mm | 33± 2.2 mm |

ni: no inhibition, *: Positive control (Imipenem), **: inhibition zone diameters in mm (mean ± sd, n = 3)

### Locomotor Activity

The 24-hour locomotor activity assay was divided into two 12-hours time periods and analyzed separately. This was done to determine whether the effects of essential oil treatment on locomotor activity diminish over time or whether the treatments have delayed effects. Sample sizes were 32, 29, 32, and 32 for the control, essential oil-1, essential oil-2, and essential oil-3 groups, respectively. In the comparison of the first 12-hour locomotor activities, log and square root transformation could not normalise the data of all groups (Table 3); therefore, a non-parametric Kruskal-Wallis test was applied. No significant difference ($H(3) = 4.270$; $p = 0.234$) was observed between the groups (Figure 1). No significant difference between groups ($F(3,121) = 0.867$, $p = 0.460$) was also observed in the one-way ANOVA of the data in the second 12-hour time period (Figure 2).

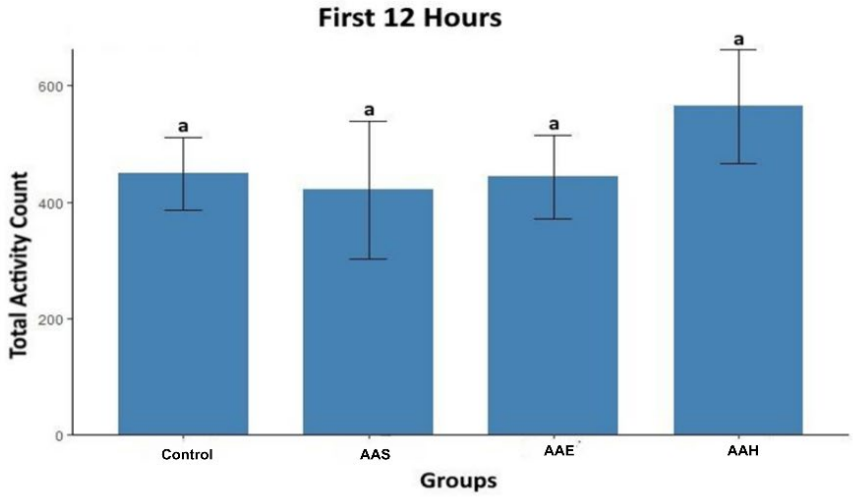


**Figure 1:** Locomotor activities (mean ± standard error) of experimental groups in the first 12-hour time period.

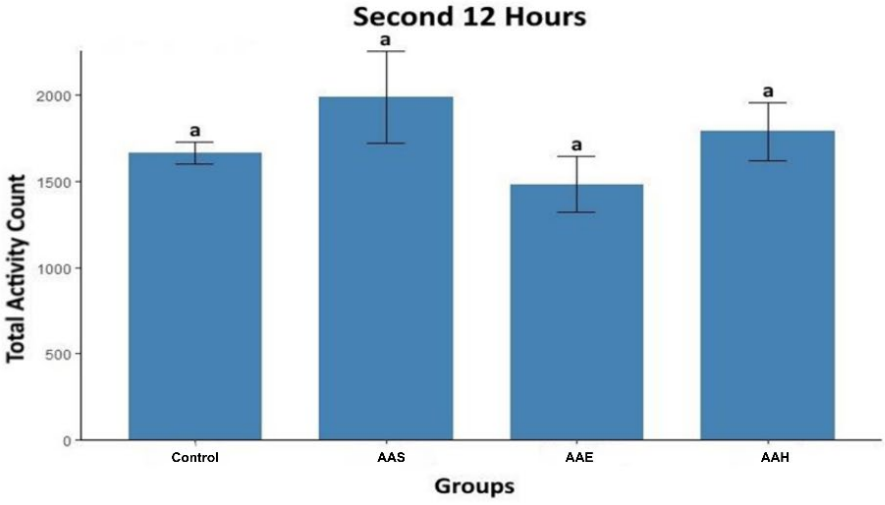


**Figure 2:** Locomotor activities (mean ± standard error) of experimental groups in the second 12-hour time period.

**Table 3:** Shapiro-Wilk normality test results for raw, log-transformed and square root-transformed data of experimental groups.

| Groups | First 12 Hours | | | | | | Second 12 Hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raw | | Log | | Sqr. | | Raw | | Log | |
| | W | p | W | p | W | p | W | p | W | p |
| Control | 0.920 | 0.020 | 0.958 | 0.269 | 0.985 | 0.925 | 0.873 | 0.002 | 0.961 | 0.284 |
| AAS | 0.577 | <0.001 | 0.966 | 0.469 | 0.829 | <0.001 | 0.865 | <0.001 | 0.988 | 0.978 |
| AAE | 0.815 | <0.001 | 0.839 | <0.001 | 0.967 | 0.420 | 0.933 | 0.048 | 0.979 | 0.757 |
| AAH | 0.793 | <0.001 | 0.979 | 0.786 | 0.954 | 0.193 | 0.960 | 0.277 | 0.973 | 0.601 |

## DISCUSSION

In this study, the chemical compositions of essential oils obtained from *A. absinthium* plants collected from three ecological regions of Türkiye (Sivas, Erzincan, and Hakkari) were determined, and the potential contact toxicity of these oils on the locomotor activity of honeybees was evaluated *in vitro*. GC-MS analyses indicated that the essential oils were rich in terpene classes and exhibited marked quantitative and partial qualitative differences among samples. Chamazulene was identified as the dominant compound in all samples, with particularly high levels (64.6%) observed in the AAH essential oil. Previous studies have shown that the chemical composition of *A. absinthium* essential oils may vary considerably under different environmental conditions, thereby influencing their biological properties (Kaya and Solak 2025).

Chamazulene-rich essential oils have previously been associated with antioxidant, anti-inflammatory, and moderate antimicrobial activities, which may contribute to reducing oxidative and physiological stress in biological systems (Batiha et al. 2020, Sharifi-Rad et al. 2022). Although direct evidence regarding the effects of chamazulene on honeybee behaviour or bee parasites remains limited, terpene-rich essential oils have increasingly attracted attention as potential supportive agents in apicultural health management. Variations in the chemical composition of *A. absinthium* essential oils among different geographical regions may be associated with ecological and environmental factors such as climate, altitude, UV radiation, soil characteristics, and precipitation patterns, all of which are known to influence secondary metabolite biosynthesis in aromatic plants (Das and Prakash 2023, Dougué Kentsop et al. 2024, Varlı et al. 2020).

In addition to ecological variability, extraction methodology may also influence essential oil composition and relative constituent abundance. Hydrodistillation using a Clevenger apparatus may affect the recovery of volatile and heat-sensitive compounds, thereby contributing to variations in essential oil profiles (Orav et al. 2006). Terpene-based constituents represent major contributors to the biological activity of essential oils, with monoterpenes and oxygenated derivatives frequently associated with antimicrobial and physiological effects (Batiha et al. 2020).

In parallel, increasing attention has been directed toward evaluating the safety of plant-derived products for honeybees, particularly considering the potential for dose- and composition-dependent responses (Caren et al. 2025). β-thujone was detected exclusively in the AAE sample at a relatively high concentration (18.3%), while only trace amounts were present in the other samples. Previous investigations indicate that β-thujone content in *A. absinthium* essential oils may vary substantially with chemotype, phenological stage, and environmental conditions (Das and Prakash 2023, Msaada et al. 2015, Vieira et al. 2017). β-thujone has been described as a cholinesterase-inhibiting (ChE) compound with potential neuroactive properties, which may influence insect physiology and behaviour (Sharifi-Rad et al. 2022, Zámboriné et al. 2020). Accordingly, the elevated level observed in the AAE sample suggests that its biological effects on honeybees may vary depending on exposure dose and duration (Pașca et al. 2021). β-linalool was present at a comparatively high proportion in the AAS essential oil (8.21%) and at a lower level in the AAH sample (0.53%), while it was not detected in the AAE oil. Variations in essential oil composition are widely recognized as important determinants of biological activity (Dhifi et al. 2016).

In addition to its antimicrobial potential, linalool has been reported to modulate behavioural responses in honeybees, including effects related to reduced aggression or altered activity patterns (Nouvian et al. 2015). The relatively high levels of D-limonene in the AAS sample and (E)-linalool oxide in the AAE sample further suggest that the oils may differ in their biological response profiles. Regarding terpene classes, the AAE essential oil exhibited a high proportion of oxygenated monoterpenes (35.2%). Environmental conditions are known to influence essential oil biosynthesis and secondary metabolite accumulation, potentially leading to pronounced chemotypic variation (Das and Prakash 2023). Oxygenated monoterpenes are frequently associated with enhanced biological activity and may contribute substantially to the observed antimicrobial effects. In contrast, the AAS oil showed a more balanced distribution of oxygenated sesquiterpenes and sesquiterpene hydrocarbons, whereas the AAH oil displayed a distinct profile dominated largely by chamazulene.

Consistent with these chemical differences, the essential oils exhibited variable antibacterial activity. The AAE oil showed larger inhibition zones in the present assay, which may be related to its higher content of oxygenated monoterpenes such as β-thujone, camphor, and 1,8-cineole. Terpene-rich essential oils are commonly reported to exert antibacterial effects through membrane-associated mechanisms, including altered permeability and disruption of cellular homeostasis, particularly in Gram-positive bacteria (Ilyasov et al. 2024, Kamatou and Viljoen 2008, Msaada et al. 2015). In contrast, *Listeria monocytogenes* displayed complete resistance to all tested essential oils, suggesting intrinsic tolerance mechanisms that limit terpene diffusion and intracellular accumulation (Sharifi-Rad et al. 2022). Although chamazulene-rich profiles are often linked to antioxidant and anti-inflammatory properties, they may not necessarily result in pronounced inhibition zones in diffusion-based antimicrobial assays, where minor oxygenated monoterpenes can play critical roles (Batiha et al. 2020, Capuzzo et al. 2014).

The selective inhibitory effect of the AAH essential oil against Pseudomonas aeruginosa may be due to synergistic interactions between chamazulene and minor constituents, such as camphor and 1,8-cineole. Overall, the moderate antibacterial activity observed in this study supports the notion that the bioactivity of A. absinthium essential oils is likely governed by the combined action of multiple constituents rather than a single dominant compound. Previous research has similarly highlighted the broad biological potential of *A. absinthium* essential oils as terpene-rich bioactive mixtures (Rizvi et al. 2023). Moreover, plant-derived essential oils are increasingly regarded as promising natural agents due to their broad-spectrum activity and relatively low environmental burden (Schulz and Wink 2025).

In the 24-h locomotor activity assay, no significant differences were observed between the control and essential oil-treated groups during either observation period. This finding is notable given the presence of bioactive constituents, such as β-thujone, in the AAE oil and suggests that, under the tested conditions, no statistically significant locomotor alterations were observed. Such effects may be influenced by limited dermal uptake, rapid metabolic processing, or interactions among oil components. However, these findings should be interpreted cautiously considering the single-dose exposure design and relatively short observation period. Collectively, these findings suggest that locomotor activity monitoring may be useful as a preliminary behavioural screening approach for evaluating short-term responses of honeybees to plant-derived products. Several limitations should be acknowledged. Although antibacterial assays were repeated, the study remains preliminary in terms of behavioural toxicology because only acute short-term locomotor activity was evaluated. Additional dose-response, chronic exposure, colony-level, and mechanistic studies are required to comprehensively assess the ecological safety of *A. absinthium* essential oils for honeybees

## Conclusion

This study demonstrated that the chemical composition of *A. absinthium* essential oils exhibits marked location-dependent variation, resulting in distinct chemotypic profiles across different regions of Türkiye. Among the analyzed samples, the AAH essential oil was characterized by an exceptionally high chamazulene content, whereas the AAE sample was notable for its elevated levels of oxygenated monoterpenes, including β-thujone, camphor, and 1,8-cineole. These compositional differences were reflected in the antibacterial activity profiles, with the AAE essential oil exhibiting stronger inhibitory effects, particularly against Gram-positive bacteria, while chamazulene-dominant oils showed moderate, selective antibacterial activity.

Importantly, none of the tested essential oils produced statistically significant alterations in honeybee locomotor activity during the 24-hour observation period under the tested experimental conditions. Nevertheless, these findings should be regarded as preliminary due to the limited experimental scale and single-dose exposure design. Further studies involving dose-response analyses and long-term exposure assessments are needed to better understand the biological effects and practical applicability of *A. absinthium* essential oils in beekeeping systems.

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