Feasibility of Detecting Different Genotypes of *Mentha* plant by E-nose Technique

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Abstract

In botanical terms, the classification of plants reveals a multitude of species derived from different sources. The first step for quality control of herbal medicines is to identify their different species and genotypes. The present study investigated the classification of ten different mint genotypes using Gas Chromatography-mass Spectrometry (GC-MS) and an electronic nose (e-nose) system utilizing Metal Oxide Semiconductor (MOS) sensors. Leaf samples were harvested from various mint genotypes, and subsequently, the system sensors' responses to each of these samples were recorded. The classification of plants was performed using biplot diagrams based on GC and GC-MS data, with clustering facilitated by the Ward method. The responses of all enose sensors were further analysed through various approaches, including Principal Components Analysis (PCA), Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), and Artificial Neural Network (ANN). The results from the qualitative analysis of essential oils via GC-MS demonstrate that more than 99% of the identified compounds belong to four chemical groups: hydrocarbon and oxygenated monoterpenes, as well as hydrocarbon and oxygenated sesquiterpenes. Also, based on biplot analysis, different mint populations could be generally divided into 8 groups. The results of principal component analysis showed that the first two main components can cover a total of 97% of the data variance. The classification accuracy achieved through e-nose data for LDA, QDA, and ANN was 98.9%, 99.9%, and 96%, respectively. Proper classification of mint genotypes by e-nose system could be used as a sensitive, reliable, and low-cost alternative to traditional methods.

Keywords: Artificial Intelligent methods, Classification, E-nose, Essential oil

Table of abbreviations	
Definition	Abbreviation
Medicinal and Aromatic Plants	MAPs
Metal Oxide Semiconductor	MOS
Principal Components Analysis	PCA
Linear Discriminant Analysis	LDA
Quadratic Discriminant Analysis	QDA
Artificial Neural Network	ANN
Area Under the Curve	AUC
Retention time of hydrocarbon with smaller alkanes	$t_{r(n)}$
Retention time of hydrocarbon with larger alkanes	$t_{r(N)}$
Retention time of unknown composition	$t_{r \text{ (unknown)}}$
Carbon number of smaller alkanes	N
Kovats Index	KI
The lowest sensor response before the measurement phase (baseline)	$X_{s}(0)$
The sensor response at time t	$X_{s}(t)$
The normalized sensor response at time t	$Y_{s}(t)$

Introduction

In recent years, there has been an increasing demand for the production of medicinal plants due to the consumers' interest in natural products, as they are thought to be safer and more cost-effective (Tangpao *et al.*, 2022). This has led to the development of pharmaceutical, cosmetic, and food industries based on natural products, which, in turn, has

increased the demand for natural raw materials such as medicinal plants (Nguyen, Duong, & Mentreddy, 2019). The global trade in medicinal and aromatic plants, along with their products, has seen remarkable growth both in quantity and quality, indicating a positive outlook (Asl Roosta, Moghaddasi, & Hosseini, 2017). The Lamiaceae family with 200 genera and 3,200 species is one of the largest and most diverse plant families, rich in medicinal plants (Okur et al., 2021a). Different species of mint plant such as Mentha piperita, Mentha spicata, and Mentha pulegium are valuable medicinal and aromatic species of this family, all belonging to the *Mentha* genus. According to the latest published statistics in 2015, the global trade value for mint essential oil exports and imports reached an impressive \$185 million, while essential oils from other species of mint totaled \$322 million. Ranked just behind citrus, mint essential oil boasts a remarkable annual production of 6,000 to 8,000 tonnes, establishing itself as one of the top essential oils worldwide (Banal, Rañola, 2014: Santiago, & Sevilla, Lubbe Verpoorte, 2011). The aerial parts of the mint contain a high amount of active ingredients such as essential oils and various phenolic and flavonoid compounds, recognized for their valuable biological properties. The essential oil and extract of this plant are used in the pharmaceutical, food, and cosmetic industries due to its antimicrobial and high antioxidant properties and special taste (Kiani, Minaei, & Ghasemi-Varnamhasti, 2018). In general, botanically, plants have different species from different sources. The first step for quality control of herbal medicines is to identify their different species and genotypes. In the traditional quality evaluation system, odor serves as a vital quality indicator, as most medicinal plants produce scents that can be associated with their species or legitimacy. Smell is one of the most important sensory properties of food. Smell measurement is an advanced method that is especially effective in obtaining parameters affecting food quality because the smell emitted from food is extremely sensitive the changing

constituents. At present, a sensory method or panel test is used for qualitative evaluation and identification of aromatic substances. Although this method is relatively fast, it has many limitations in standard measurement stability and repeatability (Guohua et al., 2015). Accurate laboratory methods are also used, such as gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), or high-performance liquid chromatography (HPLC) (Kiani et al., 2018; Li, Yu, Xu, & Gao, 2017). Despite having high accuracy, these meticulous methods have a high cost and require knowledgeable people to operate these tools, painstaking sample preparation, and a long time for analysis (Gebicki & Szulczynski, 2018). This led to using non-destructive and less expensive methods, one of which is the emethod. This system includes combination of factors such as understanding of the human olfactory system and rapid advances in sensor technology and pattern recognition systems for smell detection (Zaki Dizaji, Adibzadeh, & Aghili Nategh, 2020). Therefore, olfactory volatile compounds can be identified as a fingerprint. Many studies have been conducted on the application of the e-nose (olfactory machine) technique in the food industry and quality control of some medicinal and aromatic plants such as berry ripening (Aghili Nategh, Dalvand, & Anvar, 2020), sugar cane syrup sucrose detection (Zaki Dizaji et al., 2020), detection and classification of fungal infection in garlic (Makarichian, Chayjan, Ahmadi, & Zafari, 2022), discrimination of flavoured unflavoured olive oils (Rodrigues, Silva, Veloso, Pereira, & Peres, 2021), acrylamide detection in olives (Martín-Tornero et al., 2021) and Lamiaceae (Okur et al., 2021a), qualitative classification of 9 genotypes of (Gorji-Chakespari, Rosa essential oils Nikbakht, Sefidkon, Ghasemi-Varnamkhasti, & Valero, 2017), and isolation of different cultivars and species of Chinese Cymbidium (Zhang et al., 2014), mint (Kiani et al., 2018; Okur et al., 2021b), and basil (Tangpao et al., 2022). To date, no preliminary assessments have been performed to ascertain the genotype of the mint. The present study aimed to investigate the performance of an e-nose system in combination with GC-MS and chemometric instruments to identify genotypes and different species of mint.

Materials and Methods

Preparation of Mentha samples

Different populations of *Mentha* plant (10 genotypes) were harvested from the collection of this plant at the farm of the Department of Horticultural Sciences, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran (Table 1). Plants were harvested from about 5

cm above the ground level at the early reproductive stage. 100 g of aerial parts of each sample of *Mentha* populations, including leaves and inflorescences, were used for essential oil extraction to perform GC test. Oil extraction of plant was performed by water distillation method and Clevenger apparatus for 3 hours. To remove excess moisture from the extracted essential oils, sodium sulphate was added to them after collecting the essential oils in the dark and sealed vials and the oil samples were kept at 4 °C until the analysis of their chemical compounds.

Table 1- List of mint genotypes with botanical name and origin

Number	Genotype code	Botanical name	Origin
1	E1	Mentha spicata L.	Ilam, Iran
2	E4	Mentha spicata L.	Ilam, Iran
3	H1S	Mentha spicata L.	Budapest, Hungary
4	H1P	Mentha imes piperata	Budapest, Hungary
5	Н3	Mentha spicata L.	Budapest, Hungary
6	Н6	Mentha spicata L.	Budapest, Hungary
7	H7	Mentha $ imes$ piperata	Budapest, Hungary
8	H10	Mentha citrata	Budapest, Hungary
9	H16	Mentha spicata L.	Budapest, Hungary
10	T19	Mentha rotondifolia L.	Tehran, Iran

Identification of essential oil components (GC and GC-MS) $\label{eq:GC-MS} % \begin{center} \$

Quantitative and qualitative analysis of the chemical compounds of the essential oil was performed using gas chromatography (SHIMADZU, Model GC-17A) and gas chromatography-mass spectrometry (Agilent, Model B5977) under the following conditions. The gas chromatography (GC) apparatus utilized was equipped with a BP-5 column, measuring 30 m in length, 0.32 mm in diameter, and featuring a stationary phase layer thickness of 0.25 µm. The oven temperature was kept at 60°C for 1 minute and then increased at a rate of 5 °C per minute to 250 °C. This peak temperature was then maintained for an additional 2 minutes. The injector and flame ionization detector (FID) temperatures were 280 and 300°C. respectively, and helium gas with a flow rate of 1.1 mL min⁻¹ was used as the carrier gas.

The gas chromatography-mass

spectrometry (GC-MS) system was fitted with an HP-5 ms column featuring a length of 30 m, a diameter of 0.25 mm, and a stationary phase layer thickness of 0.25 µm. The temperature program of the column included increasing the temperature from 65 to 250°C at a rate of 5°C per minute, which eventually remained at this temperature for 2 minutes. The temperature of the injection chamber and the transmission line to the MS part of the apparatus were 265 and 275°C, respectively, and helium gas was used as the carrier gas at a rate of 1.1 mL min⁻¹. The scan time was 0.6 seconds, and the ionization energy was 70 electron volts. The essential oils were injected into a gas chromatograph-mass spectrometry apparatus, and the mass spectra of the essential oil compounds were obtained. The spectra were identified by utilizing the mass database, assessing inhibition time, and critically analysing the mass spectra of each essential oil component. This process included comparing their failure patterns with standard

alongside reference to reputable spectra sources (Adams, 2007). The quantitative percentage of each compound was determined based on the area under its curve in the GC chromatogram and by computer programming (using GC Solution software). The percentage of chemical compounds constituting each essential oil sample and the Kovats index of each compound were calculated, and spectra were identified by calculating the Kovats Index (KI) with injecting normal hydrocarbons (C4- C28) under the same conditions with injecting essential oils. The Kovats index of essential oil compounds was calculated using the Equation (1):

$$KI = 100 \times [n + ((N - n)) \times \frac{t_{r(\text{unknown})} - t_{r(n)}}{t_{r(N)} - t_{r(n)}}]$$
(1)

where KI = Kovats index, n = carbon number of the smaller alkane, N = carbon number of the larger alkane, $t_{r (unknown)}$ = the retention time of unknown composition, $t_{r (N)}$ = the retention time of hydrocarbon with the larger alkane, and $t_{r (n)}$ = the retention time of hydrocarbon with smaller alkanes.

Preparing samples for e-nose test

20 grams fresh aerial parts of each sample (plant) was poured into the measuring chamber of the e-nose device. Then, this chamber was connected to the e-nose system and the data collection steps were performed from the device. After the sensors started working, data collection of chemical compounds of essential oil from each population of mint was performed by e-nose apparatus.

E-nose device

To conduct the experiments, an e-nose system made at the Shahid Chamran University of Ahvaz was used (Zaki Dizaji et

al., 2020). This system includes a sampling chamber, a system equipment box, and a computer (Figure 1). System equipment consists of a sensor chamber, sample housing, two CL10R0 carbon filters, two micro-pumps (model R-385 with a flow rate of 25 cm³ s⁻¹), three solenoid valves, data collection system, two power supplies 5 and 12 volts, and an inlet air filter. Sensors are tasked with the conversion of chemical changes into electrical signals, highlighting the significance of selecting the correct sensor array (Rafaela, Murilo, Luiza, & Daniel, 2022). The sensor consists of eight metal semiconductor sensors, including MQ2, MQ3, MQ5, MQ8, MQ9, MQ135, MQ137, and MO138 (Hanwai Electronics Co., China). Metal oxide semiconductor sensors are used their high chemical stability, sensitivity, easy manufacturing, and suitability for a wide range of food and agricultural products. The sensors' responses were captured by a production system linked to a computer running LabVIEW 2015 software. The e-nose system was scheduled for three phases: baseline correction (100 seconds), sample smell injection on the sensors (90 seconds), and cleaning of the sensor chamber and sample with clean air (100 seconds). During these phases, the voltage changes in response from the sensors are meticulously recorded over time. In general, the voltage response of the sensors in these 290 seconds is collected by the produced system. The time required for each step is obtained via trial and error. The number of e-nose test repetitions for each sample is 15. The sample chamber consists of a closed chamber with a volume of about 500 cc, maintaining the sample's temperature at room conditions, about 30°C, and a humidity of about 25%.

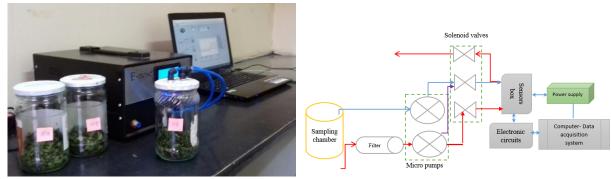


Fig. 1. (Left) The e-nose system, which comprises a sampling chamber, a system equipment box, and a computer, and (right) a schematic block diagram illustrating the components of the e-nose device

Data analysis

To analyse the differences between GC and GC-MS data and to explore the internal relationships among traits using principal component analysis, a two-dimensional biplot diagram was generated through clustering with the Ward method, Euclidean square distance criterion, and SPSS and Genstate software.

Preprocessing is the first step in analysing the response of the array of e-nose sensors. This involves removing irrelevant information to make the information more efficient for the next steps of the analysis. The first preprocessing stage is dedicated to aligning the sensors' responses with a baseline (stable response) to correct for deviations and significantly enhance the contrast of their outputs. In this study, the fractional method was used to correct the baseline (Equation 2). In this method, the baseline is subtracted from the sensor response and then divided by the baseline. The response obtained is not only dimensionless but also normalized and can be used for small or large signals (Zaki Dizaji et al., 2020).

$$Y_s(t) = [X_s(t) - X_s(0)]/X_s(0)$$
 (2)

In this regard, X_s (0) is the lowest sensor response before the measurement phase (baseline), X_s (t) is the sensor response at time t, and Y_s (t) is the normalized sensor response. After these steps, the pre-processed data are analysed in different ways, and finally the sample is evaluated. In general, pattern recognition is done by two methods: statistical methods or artificial neural methods. These methods are based on qualitative expression or classification of data. This research involved

the analysis of data utilizing various statistical and intelligent techniques, including PCA, LDA, QDA, and ANN. In order to analyse the performance of these methods, the parameters of the classification performance such as accuracy, precision, sensitivity, specificity, and area under the curve (AUC) were used based on the values of the confusion matrix (Kaushal, Nayi, Rahadian, & Chen, 2022; Mahmodi, Mostafaei, & Mirzaee-Ghaleh, 2019).

The main component analysis follows the idea of reducing the data dimension. In fact, a number of interconnected characteristics are expressed in the form of several compact and independent indices that are the main components of the main multiple characteristics. The linear discriminant analysis is one of the most widely used methods for classifying observations different classes, especially when it has more than two classes. The quadratic discriminant analysis is a statistical method used to find the quadratic composition of properties that best separate two or more groups of objects. A multi-layer perceptron (MLP) algorithm was also used. The classification involved a network that features an input layer, a hidden layer, and an output layer. The hidden layer contains several neurons that represent a nonlinear network system. Considering that this research utilizes a neural network for realtime classification, it is better to be close to the desired error rate with the same number of epochs or fewer repetitions. Therefore, a hidden layer was considered for the network to increase the speed of training. The hyperbolic tangent activation function was used for the hidden layer. The post-diffusion algorithm was used to train the network, and through a trial-and-error approach, it was concluded that the optimal number of neurons for the hidden layer is 8. Finally, the optimal model was selected. Based on the data from eight sensors at the network input, and with an output layer composed of 10 nodes, the samples are classified using a structure of 8-8-10. Data processing was performed in Microsoft Excel 2019, MATLAB 2015, and Unscrambler 10.3 (CAMO) software.

Results and Discussion

GC and GC-MS results

According to the results of qualitative analysis of essential oil by GC-MS apparatus, 72 different compounds were identified in the essential oils of different populations of Mentha plant (Table 2). More than 99% of the identified compounds were in four chemical groups, including hydrocarbon and oxygenated monoterpenes, as well as hydrocarbon and oxygenated sesquiterpenes (Hawrył et al., 2015). Oxygenated monoterpenes made up about 75% of the essential oils from various populations of mint, followed by hydrocarbon monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes in that order. In other words, aroma of Mentha plant covers the subgroups of terpenes, esters, alcohols, and ketones. These results align closely with the majority of results presented in other studies (Kiani et al., 2018; Okur et al., 2021a).

According to the results of biplot and cluster analysis, different populations of mint are generally divided into 8 groups (Table 3). This separate division showed that the desired traits were appropriate criteria for creating diversity in the studied populations (Figure 2). The first group includes H7 and H1P populations of mint with high levels of menthone (60.05%)and Isomenthone (10.93%) compounds. The second group includes the population of H3, rich in Menthol compounds (26.58%) and Menthyl acetate (3.16%). The third group includes E1 and H10 populations with high levels of Linalool

compounds (39.93%), α-Cubebene (9.71%), Caryophyllene (E) (5.73%), and Geranyl acetate (2.39%). The fourth group includes E4 populations rich in Limonene (19.27%), α-Terpineol (7.19%), Pulegone (2.81%), *cis*-Sabinene hydrate (1.66%), and α -Humulene (1.23%). The fifth group includes the population of H16 with a high level of Carvone compounds (70.06%). The sixth group has the population of H6 rich in Carvone compounds (62.27%) and 1,8-Cinnamol (14.97%). The seventh group includes the population of T19 containing Pipritenone compounds (5.21%) and α-Pinene (1.12%). Lastly, the eighth group includes the population of H1S with high levels of cis-Carvyl acetate compounds (47.41%), Myrcene (6.97), Germacrene D (3.52%), β -Pinene (1.80%), Sabinene (1.32%), and β -Elemene (1.15%).

According to the results of principal components analysis, the biplot diagram, and clustering of different populations of mint based on essential oil components, biosynthetic pathway of Menthone isomenthone monoterpenes seems to be active in H7 and H1P populations, and genes responsible for encoding enzymes and the production pathway of Menthol and menthyl acetate were most highly expressed in the H3 population. The results demonstrated that the path of synthesis of essential oil compounds in E1 and H10 populations could result in the production of Linalool and Geranyl acetate monoterpenes α-Cubebene and and Caryophyllene (E) sesquiterpenes. Also, the high levels of Limonene, α-Terpinyl, Pulegone, and *cis*-sabinene hydrate monoterpenes in the E4 population indicated that the biosynthetic pathway enzymes of these compounds are more active in the above population. The study of the essential oil components of H16, H6, and T16 populations showed the predominance of the biosynthesis Carvone of 1.8-cineole pathway and compounds in the H16 and H6 populations, and Pipritenone and α-Pinene in the T16 population. Pinene, Sabinene, Germacrene D, and β-Elemene sesquiterpenes have been the most active biosynthetic pathways of essential

oil compounds in the H1S population.

Table 2- The essential oil chemical composition of different accessions of mint by GC-MS

	Table 2- 11													
Row	Component	RT 5.10	KI _c	KI _r	E4	E1	H1S	H1P	H3	H6	H7	H10	H16	T19
1	α-Pinene	5.10	931	932	0.71	0.31	1.02	0.60	0.52	0.41	0.53	0.24	0.55	1.12
2	Camphene	5.38	946	946	0.13	0.06	1 22	0.40	0.52	0.22	0.42	0.60	0.52	0.14
3 4	Sabinene β-Pinene	5.81 5.94	969 976	969 974	0.64 1.05	0.81 0.67	1.32 1.80	0.49 0.90	0.52 0.79	0.33 0.71	0.43 0.83	0.60 0.57	0.52 0.67	1.11 1.78
5	Myrcene	6.17	988	988	0.89	11.99	6.97	0.70	0.79	0.71	0.69	2.10	3.17	1.78
6	α-Phellandrene	6.45	1002	1002	-	0.12	0.13	-	0.08	0.49	0.09	2.10	0.10	0.09
7	α-Terpinene	6.78	1015	1014	0.07	0.05	0.13	_	0.09	0.15	_	0.06	-	-
8	Limonene	6.98	1023	1024	19.27	0.82	10.71	1.67	5.24	-	2.74	1.02	11.72	12.60
9	1,8-Cineole	7.14	1030	1026	4.62	6.19	8.50	4.23	3.88	14.97	3.66	5.33	1.55	7.50
10	β-Ocimene (Z)	7.19	1032	1032	0.43	0.76	0.42	0.40	0.38	0.56	0.35	0.90	0.11	0.25
11	β-Ocimene (E)	7.50	1045	1044	0.22	1.01	0.09	0.09	0.12	0.25	0.09	0.91	0.06	0.11
12	γ-Terpinene	7.79	1056	1054	0.17	0.30	0.15	-	0.18	-	-	0.22	0.17	-
13	cis-Sabinene hydrate	7.95	1063	1065	1.66	0.16	0.51	0.06	1.22	0.30	-	0.25	1.34	0.28
14	Terpineol	8.55	1087	1086	0.08	0.39	0.16	-	0.07	-	-	0.37	0.10	0.07
15	Linalool	8.74	1095	1095	-	39.93	0.60	0.16	0.34	-	0.18	39.76	0.17	0.16
16	trans-Sabinene hydrate	8.80	1097	1098	0.23	-	0.06	-	0.08	0.16	-	0.11	0.09	0.17
17	cis-Thujene	8.91	1102	1101	0.13	0.63	0.06	0.05	-	0.12	-	0.30	-	0.06
18	cis-p-menth-2-en-1-ol	9.36	1117	1118	0.11	0.46	0.08	-	0.07	0.17	-	0.38	0.07	-
19	trans-p-mentha-2,8-dien-1-ol	9.41	1119	1119	0.05	-	0.13	-	-	0.12	-	-	-	-
20	trans-Limonene oxide	9.93	1136	1137	0.07	-	0.13	0.11	0.10	0.06	0.13	-	0.07	0.05
21	Comphor	10.02	1139	1141	-	-	-	0.13	0.06	-	0.12	-	0.09	-
22	cis-β-Terpineol	10.14	1143	1140	-	-	0.14	- 52.70	0.12	0.17	-	0.07	0.06	-
23	Menthone	10.50	1155	1148	- 0.00	0.40	0.50	53.78	39.29	0.17	60.05	0.07	0.06	- 0.00
24 25	Isomenthone Borneol	10.67	1161 1165	1158 1165	0.90	0.49 0.05	0.58	10.93	7.17	0.09 0.17	10.44	0.16	0.29	0.98
25 26	Menthol	10.78 10.86	1165	1165	-	0.05	-	- 14.44	26.58	0.17	8.52	0.07	-	-
27	cis-Linalool oxide (Pyranoid)	10.80	1170	1170	0.85	0.03	0.59	-	-	0.12	0.62	0.07	0.45	0.13
28	Terpinene-8-ol	11.12	1176	1174	-	-	0.21	0.18	0.18	-	0.02	-	-	0.13
29	Isomenthol	11.28	1181	1179	0.30	_	0.49	0.18	0.19	_	0.16	_	0.32	0.43
30	α-Terpineol	11.38	1185	1186	7.19	6.73	0.28	-	-	0.17	-	6.90	-	1.28
31	Dihydrocarveol	11.54	1190	1192	-	-	0.14	0.08	0.10	2.02	0.06	0.94	0.06	0.09
32	trans-Dihydrocarvone	11.84	1200	1200	0.14	0.13	0.20	-	_	0.44	-	_	0.25	0.12
33	trans-Carveol	12.29	1217	1215	1.30	1.01	0.06	-	0.07	1.97	-	0.88	0.96	0.62
34	cis-Carveol	12.56	1228	1226	0.08	_	0.16	0.09	_	_	0.07	_	_	-
35	Pulegone	12.68	1232	1233	2.81	_	0.11	1.26	0.71	0.61	2.47	0.06	0.13	0.13
36	Carvone	12.78	1236	1239	40.63	16.75	0.48	0.81	2.57	62.27	0.87	13.45	70.06	50.98
37	Pipperiton	13.10	1248	1249	-	-	-	-	-	0.36	-	-	0.12	0.14
38	cis-Carvone oxide	13.42	1260	1259	-	-	0.58	-	-	-	-	-	0.06	0.08
39	Geranial	13.55	1265	1264	-	0.07	0.55	-	-	-	-	-	-	-
40	trans-Carvone oxide	13.76	1273	1273	0.27	-	-	0.15	0.35	0.13	-	-	0.10	3.50
41	Isobornyl acetate	14.07	1285	1283	0.08	0.15	0.08	0.09	-	-	0.09	-	0.07	-
42	Menthyl acetate	14.25	1291	1294	-	-	0.37	2.94	3.16	0.11	1.54	-	-	-
43	Carvacrol	14.33	1294	1298	0.13	-	0.24	-	-	0.16	-	-	-	-
44	trans-Carvyl acetate	15.58	1339	1339	1.22	-	0.31	-	0.05	0.40	-	-	0.13	
45	Piperitenone	15.63	1341	1340	0.28		-	-	-	0.08	-	-	-	5.21
46	α-Cubebene	15.83	1348	1345	-	3.30	-	-	-	-	-	9.71	-	-
47	Neryl acetate	16.07	1357	1359	-	-	0.05	-	-	-	-	-	-	0.13
48	cis-Carvyl acetate	16.36	1367	1365	0.18	1.20	47.41	-	-	0.11	-	1.05	0.07	3.73
49	Geranyl acetate	16.75	1381	1379	1.26	2.39	- 0.00	0.12	- 0.22	1 14	0.15	2.07	- 0.50	0.12
50 51	β-Borbonene	16.89 17.05	1386 1392	1387 1389	0.55	0.05 0.35	0.08 1.15	0.13 0.42	0.23 0.17	1.14 0.78	0.15 0.42	0.08 0.85	0.59 0.70	0.13 0.16
52	β-Elemene	17.03	1410	1411	0.33	-	0.06	0.42	0.17	0.78	0.42	-	-	0.16
53	cis-α-Bergamotene Caryophyllene (E)	18.82	1410	1417	3.46	5.73	4.99	1.83	0.11	3.76	1.82	2.82	1.11	1.36
54	trans-α-Bergamotene	18.20	1434	1432	3.40	5.75	0.06	1.03	-	5.70	1.02	2.02	1.11	1.50
55	Aromadendrene	18.51	1445	1439	0.22	_	-	0.09	0.05	0.21	0.08	_	0.11	0.13
56	α-Humulene	18.76	1455	1452	1.23	0.55	0.82	0.29	0.54	0.88	0.31	0.50	0.47	0.67
57	Germacrene-D	19.51	1483	1484	1.67	1.81	3.52	1.89	1.75	1.76	1.76	2.48	0.95	0.51
58	Bicyclogermacrene	19.91	1498	1500	0.34	0.52	1.24	0.33	0.61	0.30	0.33	0.23	0.49	0.05
59	γ-Cadinene	20.35	1515	1513	0.16	0.11	_	-	-	0.14	-	0.15	-	-
60	Δ-Cadinene	20.58	1523	1522	0.49	0.05	0.08	0.05	0.06	0.23	-	0.05	0.08	0.12
61	α-Cadinene	20.95	1538	1537	0.14	-	-	-	-	0.07	-	-	-	-
62	Elemol	21.21	1548	1548	0.08	0.72	-	-	-	-	-	2.14	-	-
63	Germacrene-B	21.45	1557	1559	0.10	0.05	0.15	-	-	0.06	-	0.07	-	0.17
64	Spathulenol	21.95	1576	1577	0.22	0.09	0.29	-	0.15	0.11	-	0.12	0.09	0.05
65	Caryophyllene oxide	22.16	1584	1582	0.39	0.30	0.32	0.11	-	0.23	0.13	0.22	0.06	0.14
66	Viridiflorol	22.35	1592	1592	-	0.82	-	0.11	0.42	-	0.09	0.06	-	-
67	10-epi-γ-Eudesmol	23.07	1621	1622	0.44	0.07	-	-	-	0.22	-	0.14	0.06	0.20
68	γ-Eudesmol	23.39	1634	1630	0.15	0.16	0.07	-	-	-	-	0.37	-	-
69	epi-α-Morolol	23.57	1642	1640	0.36	0.38	0.07	-	0.07	0.08	-	0.18	-	0.05
70	α-Morolol	23.66	1646	1644	-	-	-	-	-	0.44	-	0.15	-	-
71	α-Eudesmol	23.82	1652	1652	- 0.45	0.05	- 0.15	-	-	- 0.22	-	0.16	- 0.15	- 0.06
72	α-Cadinol	23.92	1656	1652	0.45	0.14	0.15	0.06	0.10	0.22		0.31	0.15	0.26
	Monoterpene hydrocarbons	-	-	-	23.66	7.30	22.84	4.85	8.50	3.29	5.66	6.99	17.24	18.76
	Oxygenated monoterpenes	-	-	-	64.52	76.57	63.09	89.66	86.30	85.46	89.05	72.07	76.51	75.90

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Sesquiterpene hydrocarbons	-	-	-	8.41	12.52	12.15	5.03	4.06	9.38	4.88	16.95	4.50	3.37
Oxygenated sesquiterpenes	-	-	-	2.09	2.74	0.90	0.28	0.73	1.31	0.29	3.85	0.36	0.70
Total				98 67	99.13	98 98	99.82	99.59	99 43	99.87	99.86	98 61	98 73

RT: Retention time; KI_c: Calculated kovats index; KI_r: Reference kovats index

Table 3- Results of biplot analysis and cluster analysis of different populations of mint by GC-MS

Group	Construe	Compo	ound					
Group	Genotype	High	Zero or low					
1	H7 and H1P	Menthone (60.05%), Isomenthone (10.93%)	cis-Sabinene hydrate, α-Terpineol, trans- Carvone, Piperitenone, α- Cubebene, Carvyl acetate, Geranyl acetate, α-Humulene					
2	Н3	Menthol (26.58%), Menthyl acetate (3.16%)	Piperitenone, α-Cubebene, Carvyl acetate, Geranyl acetate, β-Pinene					
3	E1 and H10	Linalool (39.93%), α- Cubebene (9.71%), Caryophyllene E (5.73%), Geranyl acetate (2.39%)	<i>cis</i> -Sabinene hydrate, β-Pinene, Menthone, Pulegone, Menthyl acetate, Piperitenone					
4	E4	Limonene (19.27%), α-Terpineol (7.19%), Pulegone (2.81%), <i>cis</i> -Sabinene hydrate (1.66%), α-Humulene (1.23%)	Linalool, Menthone, Menthyl acetate, α -Pinene					
5	H16	Carvone (70.06%),	Menthone, α-Terpineol, Menthyl acetate, Pipritenone, α-Cubebene, Geranyl acetate					
6	Н6	Carvone (62.27%), 1,8-cineole (14.97%)	Limonene, α- Cubebene, Geranyl acetate					
7	T19	Piperitenone (5.21%), α-Pinene (1.12%)	β-Elemene, Germacrene D, Menthone, Menthyl, Geranyl acetate					
8	H1S	<i>cis</i> -Carvyl acetate (47.41%), Myrcene (6.97%), Geranyl acetate (3.52%), β-Pinene (1.80%), Sabinene (1.32%), β-Elemene (1.15%)	Menthone, Menthol, Piperitenone, α- Cubebene					

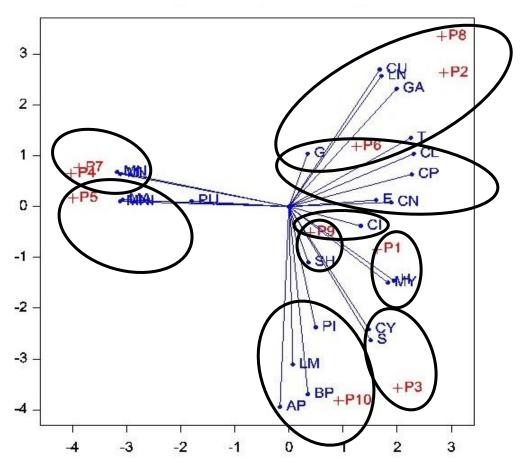
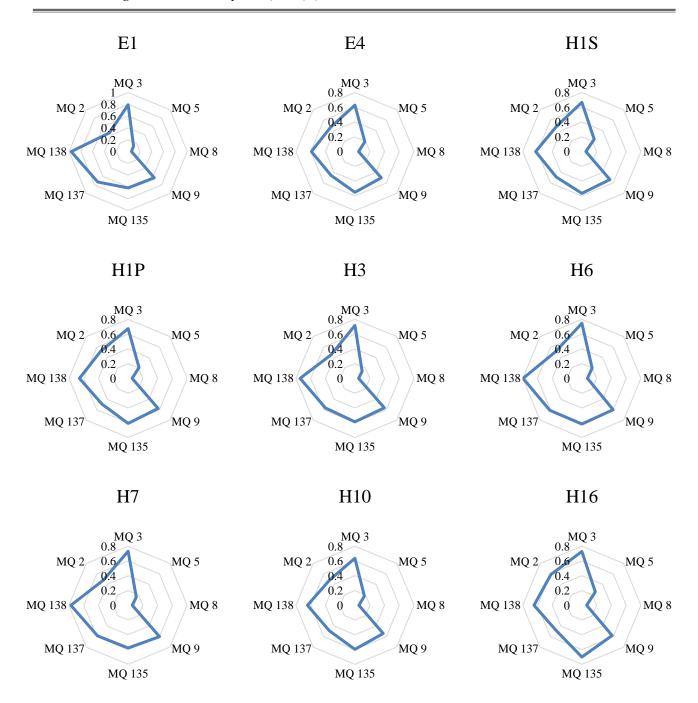


Fig. 2. Graphic display of biplot of different populations of mint using two main components derived from the dominant components of essential oil; P1, P2, P3, P4, P5, P6, P7, P8, P9, and P10 = E4, E1, H1S, H1P, H3, H6, H7, H10, H16, and T19; AP = α-Pinene, S = Sabinene, BP = β-Pinene, MY = Myrcene, LM = Limonene, CI =1,8-cineole, SH = *cis*-Sabinene hydrate, LN = Linalool, MN = Menthone, MNI = Isomenthone, ML = Menthol, T = α-Terpineol, CL = *trans*-Carvone, PU = Pulegone, CN = Carvone, MA = Menthyl acetate, PI = Piperitenone, CU = α- Cubebene, CY = *cis*-Carvyl acetate, GA = Geranyl acetate, E = β-Elemene, CP = caryophyllene E, H = α-Humulene, and G = Germacrene D

E-nose results

The study of the signal form of the sensors of the e-nose system shows that the response set of sensors varies for different types of mint genotypes, indicating that the organic matter of each genotype is distinct. The response pattern of sensors to the essential oil of E1, H3, H6, and H7 genotypes showed that the volatiles from these genotypes had the highest

response in the MQ138 sensor, while the highest numerical response for the H16, E4, HIS, and H1P genotypes was in the MQ3 sensor (Figure 3). Both sensors demonstrate impressive accuracy in detecting alcohol compounds, which, as evidenced by GC results, are among the most significant compounds found in mint genotypes.



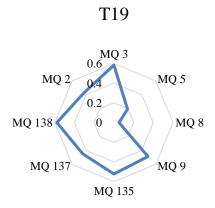
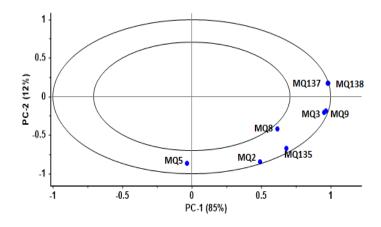


Fig. 3. The mean response of e-nose sensors to ten genotypes

PCA results

The two main components of PCA covered 97% of the variance in the data. According to Figure 4, the amount of variance in the first and second major components was 85% and 12%, respectively. Genotypes E1, H16, T19, and H6 are clearly identifiable, which is rather consistent with the results of the GC (Table 3). There is a lot of overlap among H1P, E4, H1S, and H10 genotypes. The loading diagram was used to investigate the participation of sensors in the detection of the degree of processing. These sensors are displayed in the loading values diagram with the of specific coefficients. The high coefficient value for a sensor in the loading diagram highlights its important role in detecting different types of mint genotypes. Moreover, by removing the least crucial sensors from the process of

identifying mint genotypes and streamlining the data analysis, the overall expenses associated with constructing a sensor array can be significantly lowered (Heidarbeigi et al., 2015). Although all sensors were effective in detecting mint genotypes, the MQ5 sensor showed the least involvement (Figure 4), so it is possible to remove it from the olfactory system when detecting the mint genotypes. In one study, the use of e-nose for identification the ripeness stage of berries investigated. The PCA technique was applied for data analysis. Results from the PCA indicated a significant categorization of the volatile profiles of berries at the ripe, nearly ripe, intermediate, nearly unripe, and unripe stages (Aghili Nategh et al., 2020).



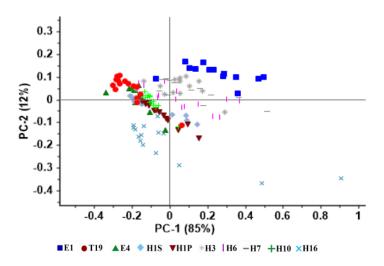


Fig. 4. (top) Loading and (bottom) score plots of PCA to detect the mint genotypes

LDA results

Figure 5 illustrates the diagram for the LDA linear resolution analysis, showcasing the first two principal components (LD1-LD2) derived from e-nose signals for mint genotype Accordingly, the detection. LDA determine the genotypes of the mint plant well. In particular, genotypes E1, E4, and T19 are completely differentiated. There was a noticeable overlap not only between H1P and T19 genotypes, but also between H1S and H16, as well as between H6 and H10 genotypes. According to the confusion matrix (Table 4), the analysis accuracy was 95.33%. Table 5 gives the performance parameters of the classifier according to the

mentioned confusion matrix, including classification accuracy, precision, sensitivity, specificity, and AUC for genotypes of mint. The average per class accuracy, precision, sensitivity, specificity, and AUC were 98.9%, 95.7%. 95.3%, 99.3%, and 97.6%. respectively. Lin et al. isolated different species of Apiaceae flower via e-nose data in combination with the LDA method (Lin et al., 2013). In a study, researchers used ANN, LDA, and SVM to classify contaminated and healthy mushrooms over a 28-day storage with LDA showing performance (Makarichian et al., 2022).

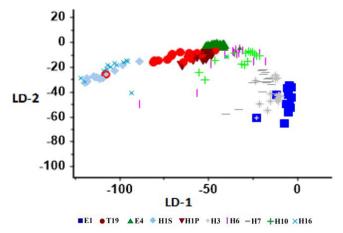


Fig. 5. Score plot of LDA analysis for detecting the mint genotypes

Гable 4-	Conf	fusior	n mat	rices	obtaiı	ned fr	om I	DΑ,	QDA	, and	ANN					
		1	2	3	4	5	6	7	8	9	10					
	1	13	0	0	0	0	0	0	0	0	0					
	2	0	15	0	0	0	1	0	0	0	0					
	3	0	0	15	0	0	0	0	0	0	1					
	4	0	0	0	15	0	0	0	0	0	0					
	5	2	0	0	0	13	0	0	0	0	0					
LDA	6	0	0	0	0	0	14	0	0	0	0					
	7	0	0	0	0	2	0	15	0	0	0					
	8	0	0	0	0	0	0	0	15	1	0					
	9	0	0	0	0	0	0	0	0	14	0					
	10	0	0	0	0	0	0	0	0	0	14					
		Correct classification: 95.33%														
	1	15	0	0	0	0	0	0	0	0	0					
	2	0	15	0	0	0	0	0	0	0	0					
	3	0	0	15	0	0	0	0	0	0	0					
	4	0	0	0	15	0	0	0	0	0	0					
	5	0	0	0	0	13	0	0	0	0	0					
QDA	6	0	0	0	0	0	15	0	0	0	0					
	7	0	0	0	0	2	0	15	0	0	0					
	8	0	0	0	0	0	0	0	15	0	0					
	9	0	0	0	0	0	0	0	0	15	0					
	10	0	0	0	0	0	0	0	0	0	15					
	Correct classification: 98.33%															
	1	13	0	0	0	٠	0	0	0	0	0					
	2	0	15	0	0	0	0	0	0	0	0					
	3	0	0	13	0	0	0	0	0	0	0					
	4	0	0	0	15	0	0	0	0	0	0					
	5	1	0	0	0	14	0	0	0	0	0					
ANN	6	0	0	0	0	0	15	0	0	1	0					
	7	1	0	0	0	1	0	15	0	0	0					
	8	0	0	0	0	0	0	0	15	0	0					
	9	0	0	0	0	0	0	0	0	14	0					
	10	0	0	2	0	0	0	0	0	0	15					
			(ct cla			: 96%								

Table 5- Performance measurements of LDA, QDA, and ANN

Classifi er Method			LDA					QDA		ANN					
Class	Precisi on	Sensitivi ty	Specifici ty	Accura cy	AU C	Precisi on	Sensitivi ty	Specifici ty	Accura cy	AU C	Precisi on	Sensitivi ty	Specifici ty	Accura cy	AU C
E1	1.00	0.87	1.00	0.99	0.93	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	0.99	0.93
E4	0.94	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1	1.00	0.99	0.99	1.00
H1S	0.94	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	0.99	0.93
H1P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
H3	0.87	0.87	0.98	0.97	0.93	1.00	0.87	1.00	0.99	0.93	0.93	0.93	0.99	0.99	0.96
H6	1.00	0.93	1.00	0.99	0.97	1.00	1.00	1.00	1.00	1.00	0.94	1.00	0.99	0.99	1.00
H7	0.88	1.00	0.98	0.99	0.99	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.98	0.99	0.99
H10	0.94	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
H16	1.00	0.93	1.00	0.99	0.97	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	0.99	0.97
T19	1.00	0.93	1.00	0.99	0.97	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.98	0.99	0.99
Average	0.957	0.953	0.993	0.989	0.97	1	0.987	1	0.999	0.99	0.963	0.96	0.993	0.992	0.97

QDA results

This method is extensively used in statistics, recognition pattern, and machine learning to find a combination of unique traits.

Table 4 shows the confusion matrix for the Quadratic Discriminant Analysis (QDA) employed in the nonlinear resolution analysis of e-nose signals for the detection of mint genotypes. According to the confusion matrix, the analysis accuracy was 98.33%. The sample is only misdiagnosed in the H1P genotype 2. The average per class accuracy, precision, sensitivity, specificity, and AUC were 99.9%, 100%, 98.7%, 100%, and 99.3%, respectively (Table 5). In previous research, the quadratic analysis successfully used to classify dieselbiodiesel blends (Mahmodi *et al.*, 2019).

ANN results

To minimize neural network training time, only one hidden layer was considered. The best network was found with a topology of 8-11-10, and a network with 11 neurons in the hidden layer. Table 4 shows the confusion matrix. Achieving a 96% classification accuracy across 10 genotypes can likely be explained by the significant variety and abundance of aromatic compounds present in these genotypes. The average class accuracy, precision, sensitivity, specificity, and AUC were 99.2%, 96.3%, 96%, 99.3%, and 97.7%, respectively (Table 5). Aghili Nategh et al. demonstrated that (2020)the optimal architecture (10-11-5) effectively classifies samples into five distinct categories in ANN analysis, achieving an impressive accuracy of 100% for blackberries and 88.3% for white berries.

Conclusion

The e-nose system is essential for the rapid, non-destructive determination of quality indicators without the need for manual measurements in the medicinal and aromatic plants industry. The results of qualitative analysis of essential oil by GC-MS showed that there were 72 unique compounds in the

essential oil of different populations of mint. More than 99% of the identified compounds were in four chemical groups, including hydrocarbon and oxygenated monoterpenes and sesquiterpenes. The average values of yield parameters (AUC, Accuracy, Precision, Specificity, and Sensitivity) and classification analysis show that QDA was the best method for the classification of different genotypes of mint, while principal component analysis, linear discriminant analysis, and artificial neural network were less accurate than quadratic discriminant analysis.

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Declaration of interest statement

The authors declare no conflict of interest.

Authors Contribution

- H. Zaki Dizaji: Supervision, Methodology, Conceptualization, Validation, Data acquisition, Text mining, Review and editing services
- M. Mahmoodi Surestani: Methodology, Data pre and post processing, Review and editing services
- N. Aghili Nategh: Statistical analysis, Technical advice, Review and editing services
- A. Boveiri Dehsheikh: Data acquisition, Statistical analysis, Software cervices

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امکانسنجی تشخیص ژنوتیپهای مختلف گیاه نعنا با تکنیک E-Nose

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چکیده

مطالعه حاضر به بررسی طبقهبندی ده ژنوتیپ نعناع توسط یک سیستم بینی الکترونیکی مبتنی بر حسگرهای نیمههادی اکسید فلزی (MOS) می پر دازد. نمونههای برگ از ژنوتیپهای مختلف نعناع بر داشت شد، سپس پاسخ سنسورهای سیستم به هر نمونه ثبت شد. برای طبقهبندی گیاهان، پاسخ تمام حسگرهای بینی الکترونیکی با روشهای تجزیه و تحلیل اجزای اصلی (PCA)، تجزیه و تحلیل تشخیص خطی (LDA)، تجزیه و تحلیل متمایز درجه دوم (QDA) و شبکه عصبی مصنوعی (ANN) مورد بررسی قرار گرفت. بر اساس نتایج تجزیه و تحلیل کیفی اسانسها توسط دستگاه GC-MS، بیش از ۹۹ درصد ترکیبات شناسایی شده در چهار گروه شیمیایی شامل هیدروکربنها و مونوترپنها و سسکوئی ترپنهای اکسیژن دار قرار داشتند. همچنین بر اساس تجزیه و تحلیل بای پلات، جمعیتهای مختلف نعناع را میتوان بهطور کلی به ۸ گروه تقسیم کرد. نتایج تحلیل مؤلفههای اصلی با استفاده از دادههای به دست آمده از سیستم نشان داد که دو مؤلفه اصلی اول می توانند در مجموع ۹۷ درصد از واریانس دادهها را پوشش دهند. دقت طبقهبندی با استفاده از دادههای بینی الکترونیکی برای تجزیه و تحلیل وضوح خطی، تجزیه و تحلیل وضوح درجه دوم و شبکه عصبی مصنوعی بهترتیب ۹۸/۹، ۹۸/۹ و ۹۶٪ بهدست آمد. طبقهبندی مناسب ژنوتیپهای نعناع توسط سیستم بینی الکترونیکی میتواند بـهعنوان جـایگزینی حسـاس، قابل اعتماد و کمهزینه برای روشهای سنتی مورد استفاده قرار گیرد.

واژههای کلیدی: اسانس، بویایی ماشینی، روشهای هوشمند، طبقهبندی

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