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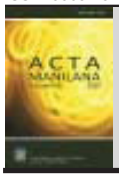

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Differentiation of mint species and varieties through headspace analysis based on polymer-modified piezoelectric quartz crystal sensors

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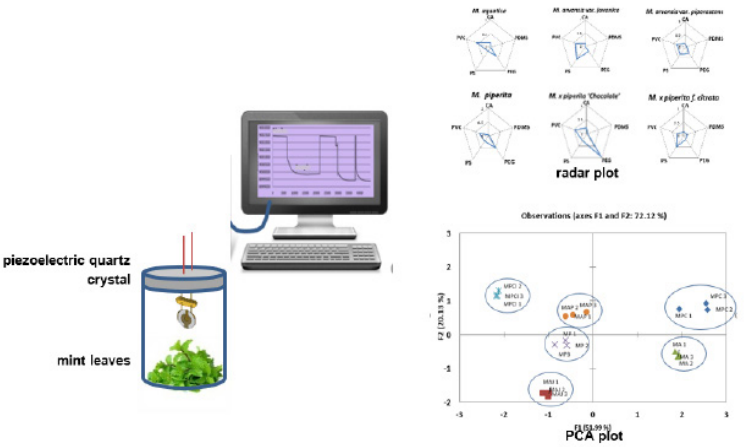
Graphical Abstract



Abstract

Mint plants have been traditionally used in flavors, fragrances, and medicines throughout the world. Polymer-modified piezoelectric quartz crystal sensor through headspace analysis was used to discriminate the six varieties of mint plant, namely: Chocolate mint (*Mentha* x *piperita* 'Chocolate'), Java mint (*Mentha arvensis* var. *javanica*), Water mint (*Mentha aquatica*), Peppermint (*Mentha* x *piperita*), Lemon mint (*Mentha* x *piperita f. citrata*) and Japanese peppermint (*Mentha arvensis* var. *piperascens*). Five quartz crystals were coated separately with the following polymers: polyethylene glycol (PEG), polystyrene (PS), polyvinyl chloride (PVC), cyanoacrylate (CA) and polydimethylsiloxane (PDMS) on both sides of the quartz crystal. The sample of mint leaves was placed in a sample chamber and were studied using one polymer-coated sensor at a time. The headspace vapors from the incubated sample interact with the polymer-coated sensor causing a frequency shift. The data collected for each plant for the five sensors were analyzed using pattern recognition methods.

Keywords: polymer-modified piezoelectric quartz crystal; mint plant species and varietal differentiation; headspace measurement; pattern recognition

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Introduction

The mint plants have attracted considerable interest because of the increasing economic prospects of the essential oil derived from these plants. These plants belong to the genus *Mentha* which includes herbs with strongly aromatic leaves [1]. The well-known species in this genus are peppermint (*Mentha* x *piperata*) and spearmint (*Mentha spicata*). The leaves contain essential oils, which are composed of volatile organic compounds with a characteristic odor. The essential oils from the different *Mentha* species and varieties are characterized by cooling and astringent properties [1]. These oils are highly prized as fragrances and flavors in the culinary, food and beverage, personal care and cosmetics, pharmaceutical and aromatherapy industries.

The mint essential oils have a dynamic global market estimated to increase to USD346.6 million in 2027 [2]. The expanding demand for these essential oils has engendered a concern for quality control of the herbal material. The authenticity of the herbal species must be confirmed to safeguard the sensory profile and efficacy of the essential oil product. Adulterated or misdeclared, or misidentified plant raw materials must be detected to guarantee product quality and sustain market confidence.

Several quality control methods have been developed for the analysis of the mint herb starting materials. A macroscopic evaluation method has been applied, which involves visual analysis of the anatomical features of the plant [3]. However, this method applies only to fresh plant materials and is unreliable when dried or crushed leaves samples are used [4] in the pharmaceutical and cosmetic industry. DNA-based methods, such as DNA-barcoding, have been employed for the accurate identification of *Mentha* species [5, 6], but these methods are lengthy and require expensive materials and equipment [4].

Chemical methods based on instrumentation have been employed to differentiate mint plants through their essential oils. Gas chromatography/mass spectrometry coupled with chemometric methods yielded fingerprints which were useful in identifying mint species [7]. Ultraviolet/visible spectrometry and Fourier transform infrared spectrometry provided spectral data, which were processed through chemometric methods into fingerprints that differentiated mint species [4]. These two methods required the isolation of the essential oils from the plant samples. Raman spectroscopic measurements carried out on the mint plant trichomes containing the essential oil were able to discriminate not only mint species but also subspecies and varieties [8].

Discrimination of mint species has also been achieved through chemical analysis of the vapor phase above the essential oil or the mint plant sample. Known as headspace analysis, this technique is seen as a sampling method in the determination of volatile substances from a liquid, such as the essential oil, or a solid system, such as the plant tissue containing the essential oil [9]. Headspace analysis has been carried out using gas chromatography to identify the compounds present in the headspace of mint essential oils [10]. Headspace analysis has also been done using modified quartz crystal microbalance devices to differentiate mint species [11,12].

This work aimed to discriminate mint species and varieties through static headspace measurement using a set of polymer-modified piezoelectric quartz crystals (PQC). Solid-phase extraction of compounds in the vapor phase is performed by the polymer coating of the PQC, leading to an increase in surface mass, and consequently, a decrease in the oscillation frequency of the PQC. A dynamic headspace sampling has been earlier applied for the differentiation of mint plants through polymer-coated PQCs. Metal oxide nanoparticles and metal-organic frameworks have also been used to modify the surface of PQC sensors for the identification of mint scents [13]. The responses of the sensor array to the various mint leaves samples were processed through multivariate pattern recognition techniques for the differentiation of species and varieties of sample plant of the genus *Mentha*.

Materials and methods

Materials. Polymers of different polarities were used as sensing phase in this study. The polymers, together with their polarities are listed in Table 1. The solvents used to dissolve the polymers were toluene (Tol), tetrahydrofuran (THF) and trichloromethane (TCM), which were obtained from Sigma-Aldrich Chemicals (Singapore) and used as received, without further purification.The piezoelectric quartz crystal (Beijing Chenjing Electronics Co., China) consisted of a 1-μm thick AT-cut 9 MHz quartz crystal wafer (12.5 mm diameter) sandwiched by two polished gold disc electrodes (6 mm).

Six plants of genus *Mentha* were included in this study (Table 2). These plants were obtained from the Bureau of Plant Industry (BPI, Manila, Philippines) and the Manila Seedling Bank (MSB, Quezon City, Philippines), where they were propagated and authenticated. Certificates were provided to vouch for the identity of the plants. The samples included a *Mentha* species (*M. aquatica*), a naturally occurring hybrid (*M. piperata*) and two sets of *Mentha* varieties. Photographs of the plants are presented in Figure 1.

Table 1. Polymers used as coatings in the study.

Polymer	Polarity
polycyanoacrylate (CA)	moderately polar
poly(dimethyl)siloxane (PDMS)	slightly polar
polyethylene glycol 6000 (PEG)	polar
polystyrene (PS)	nonpolar
polyvinyl chloride (PVC)	moderately polar

Table 2. *Mentha* species used in the study.

Scientific name	Common name	Source
Mentha aquatica	Water mint	MSB
Mentha arvensis var. *javanica*	Java mint	MSB
Mentha arvensis var. *piperascens*	Japanese mint	BPI
Mentha x *piperita*	Peppermint	BPI
Mentha x *piperita* 'Chocolate'	Chocolate mint	MSB
Mentha x *piperita f. citrata*	Lemon mint	BPI

Figure 1. The *Mentha* plants used in the study.

Sensor fabrication. The piezoelectric quartz crystal (PQC) was first cleaned by immersion in chloroform for five minutes and then dried in an oven at 104°C for one hour. The surface of the PQC was modified by coating on both sides with the polymeric sensing phase through a drop casting technique. The coating material was prepared by dissolving 0.02 g of polymer in 10 mL solvent: PVC and CA in THF; PS and PDMS in Tol; PEG in TCM. A 5 µL drop of the polymer solution was applied on the gold electrode on each side of the PQC, and air-dried for five minutes.

Instrumentation. The instrumentation for measuring the oscillation frequency of the PQC consisted of a sensing chamber, an oscillator circuit, a data acquisition system and a laptop computer. Figure 1 shows a diagram of this system. The sensing chamber was a 30-mL vial which housed the fabricated sensor and contained the leaf sample. The PQC electrodes were connected to a Pierce-IC-based oscillator (SN7400N) which drove the quartz crystal to oscillate in the gas environment. A data acquisition system (National Instruments NI-DAQ SCB-68) measured the oscillation frequency of the PQC, and the data were stored and displayed in a computer system.

Measurement Procedure. The leaves samples (0.60 g) were shredded and transferred into a 30-mL sample vial. To ensure the removal of any adsorbed volatile matter, the vial was heated in the oven for ten minutes and then flushed with nitrogen gas. The leaves sample was incubated in the vial at ambient temperature to allow the vaporization of the volatile compounds in the leaves and its equilibration with the solid phase. An empty sensing chamber was used to obtain the baseline response. The empty vial was then replaced with a vial containing the sample. The oscillation frequency of the crystal was monitored until a steady-state value was reached.

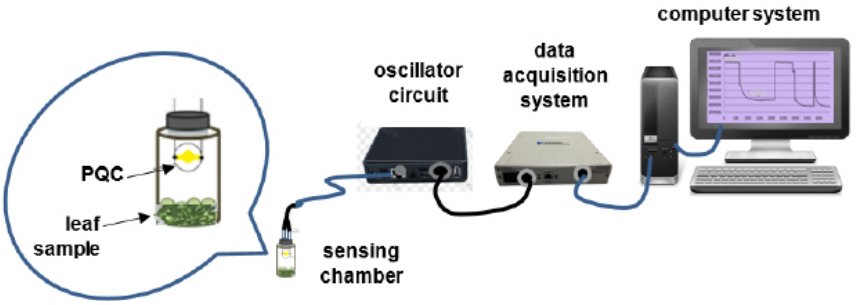

Figure 2. Diagram of the instrumentation for oscillation frequency measurement.

Between replicate measurements, the sample container was dried in the oven at 104°C for one hour to remove the remaining volatiles in the sample container. The sensor was purged with nitrogen gas for cleaning and recovery of the baseline response.

Data analysis. Graphical representations such as bar graphs and radar graphs were done using Microsoft Excel to visualize the pattern of the sensor responses. Multivariate analysis such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) was performed using the XLSTAT software package (Addinsoft, U.S.A.).

Results and Discussion

Sensor response. The sensors responded immediately upon exposure to the plant headspace, the frequency decreasing and then attaining a steady-state value. A blank (or empty) cell yielded the baseline reading. When the blank cell was replaced by the sample cell, a change in the frequency of the crystal occurred. The sensor response is due to an increase in the surface mass on the PQC caused by the vapor components extracted by the polymer coating. The steady state value indicates an equilibrium between the components in the polymer and the vapor phase. A typical sensor response graph is shown in Figure 3. The time to reach the steady varied with the polymer and with the plant sample, ranging from 3.8 to 16.6 minutes.

The sensor responses were reversible and repeatable. The baseline response was recovered when the sample container was replaced with an empty container and purged with a stream of nitrogen gas. The sensor responses exhibited relative standard deviation of less than 10% for three successive readings. This behavior can be attributed to the reversible physical forces involved in the adsorption-desorption process between the polymer coating and the vapor phase.

Plant response profile. Each polymer-modified PQC responded differently to the six plant samples. All the sensors, except the PDMS-modified PQC, exhibited a frequency shift of above 200 Hz. It should be noted that PDMS is a silicon-based polymer, unlike the other polymers used. For each plant sample, a unique pattern was observed for the responses of the set of sensors, as revealed in the bar graph in Figure 4. This response profile could provide a fingerprint for each of the plant samples.The uniqueness of the response profile of each plant is made more evident if the data are normalized and represented as a radar plot (Figure 5).

Different shapes are obtained for each plant which can be easily discerned as a signature pattern that can discriminate one plant from the others. These plots differentiated each plant sample not only on the species level but down to the variety level.

The response profile of a plant sample is related to the chemical compounds present in the vapor phase. It is dependent on how the volatile components of the herbal sample partition itself between the polymer coating of the sensor and the vapor phase. In general, polar compounds will be sorbed preferentially by a polar polymer. The amount of a compound retained in the polymer phase varies with the polarity of the polymer. In a PQC sensor, the amount of the sorbed compounds determines the magnitude of the sensor response. Thus, for an array of sensors involving polymer coatings of different polarities, each sensor will exhibit a characteristic response to the volatile compounds from a plant sample, and the array will generate a unique response profile for each plant.

Principal component analysis. Principal component analysis reduced the dimensionality of the multivariate data set, which consisted of the responses of the sensor array to the plant samples. Two principal components were generated and visualized through a biplot (Figure 6). From the plot, it can be easily recognized that data points from a plant species or variety clustered close to each other, and the clusters were well separated from each other. PCA highlighted and expressed the variation of the response profile with the species or variety of the *Mentha* plant samples quantitatively.

The PCA biplot demonstrates the capability of the sensor array of polymer-modified PQC to discriminate the plant species in the genus *Mentha*, even down to the variety level. The differentiation of plant species and varieties was much better than those observed using UV-VIS and FTIR spectral data [8] and an electronic nose based on quartz crystal microbalances modified with metal oxide nanoparticles, metal organic framework and conjugated polymers [12]. The variety of interactions, such as hydrogen bonding and hydrophobic interactions, between the functional groups in the polymer coating and the volatile compounds, contributed to the unique fingerprint obtained for each species and variety.

Hierarchial cluster analysis. Hierarchical cluster analysis of the multivariate data set of the response profile yielded the dendrogram shown in Figure 7. The diagram is based on the Euclidean distance computed between data points and clusters of data points in the multivariate data set. The closeness of points or clusters provides a measure of the similarity. Each group occurring in the dendrogram corresponded to a plant species, hybrid or variety.Close inspection of the dendrogram indicates similarity between *M. piperata* and *M. arvensis* var. *piperascens*.

The essential oil of these two plants contain menthol and menthone as the dominant components [13,14]. A similarity is also indicated between *M. piperata* var. *citrata* and *M. arvensis* var. *javanica*.Both plants exhibited a response profile with the highest response generated by the PS-coated PQC sensor (see Figure 4). The essential oil derived from these plants contains a major component with hydrophobic groups, such as linalyl acetate in *M. piperata* var. *citrata* [15] and pulegone in *M. arvensis* var. *javanica* [14].

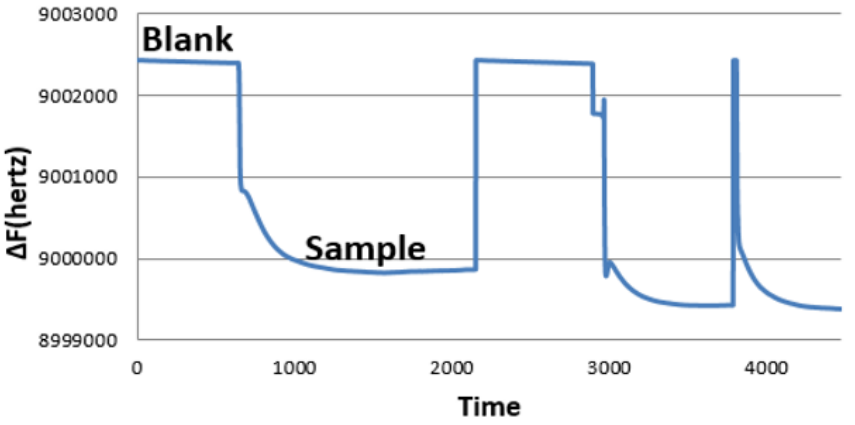

Figure 3. A typical response of PEG-modified PQC to Chocolate mint.

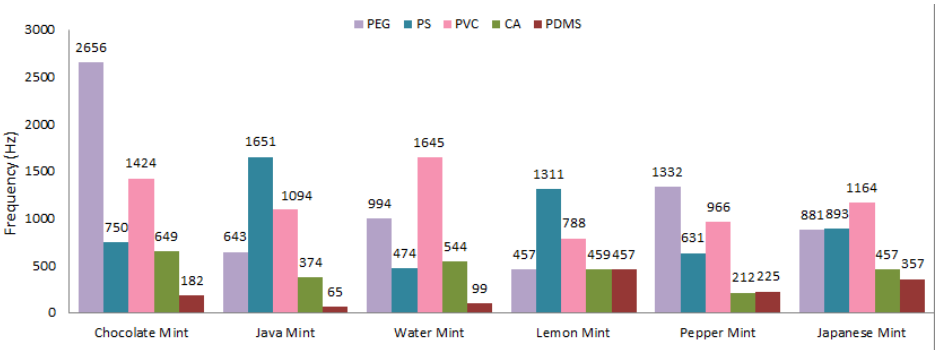

Figure 4. Response profile of the *Mentha* plant samples. Bar graphs were calculated from triplicate data.

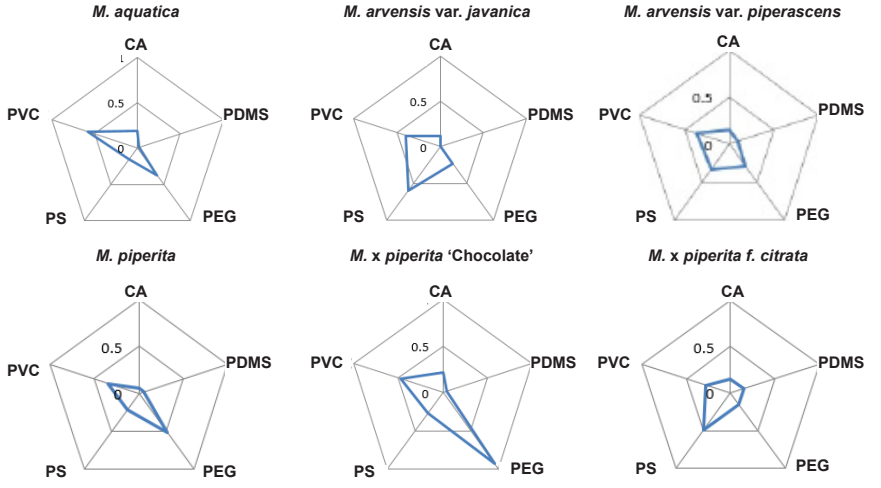

Figure 5. Radar plots of the normalized response of the PQC sensors to the *Mentha* plant samples.

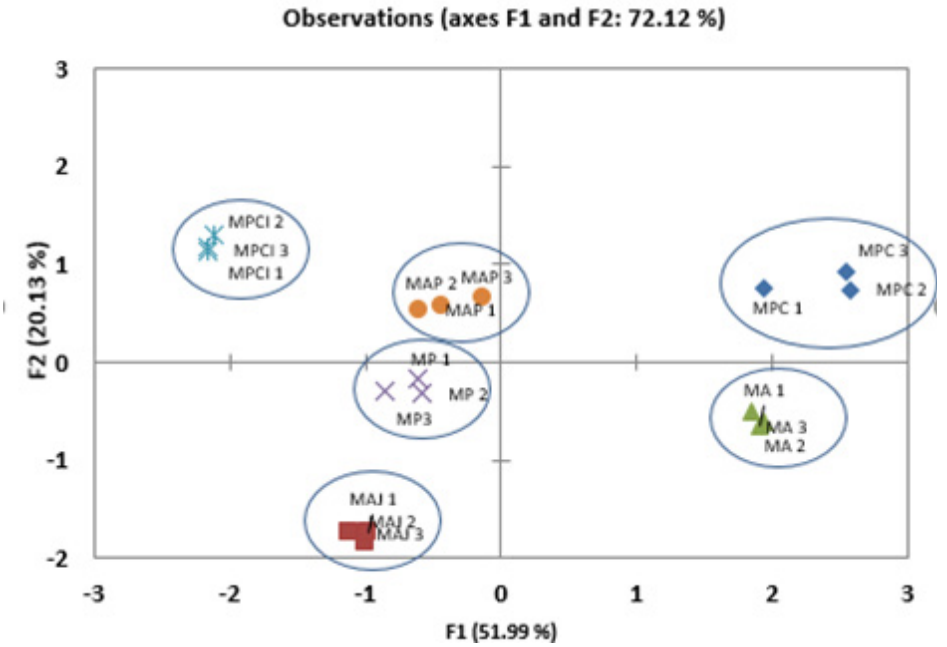

Figure 6. Score plot of the two principal components of the PQC sensor responses obtained from *Mentha* plant samples: *M. aquatica* (MA), *M. arvensis* var. *javanica* (MAJ), *M. arvensis* var. *piperascens* (MAP), *M. piperita* (MP), *M. piperita* var. 'Chocolate' (MPC), *M. piperata* var. *citriata* (MPCI).

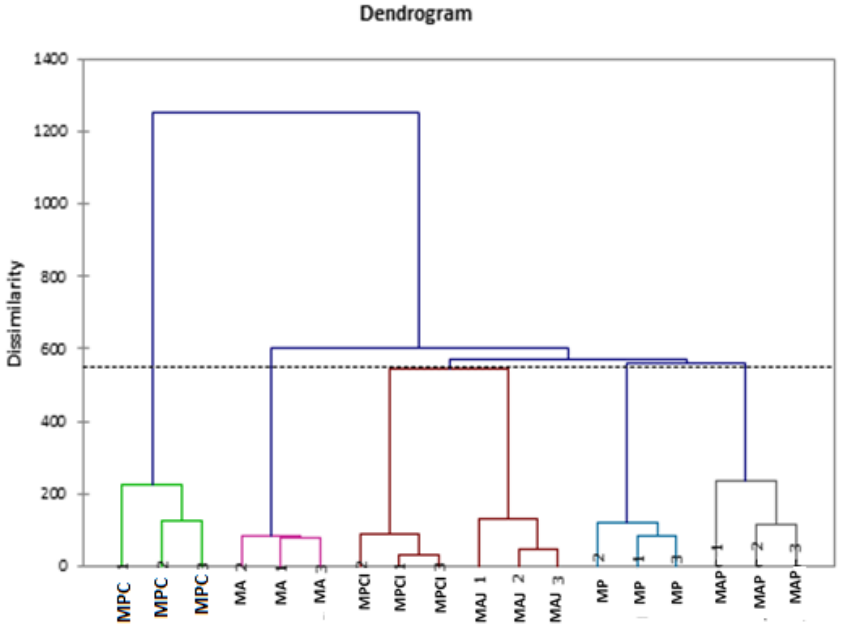

Figure 7. Dendrogram of the PQC sensor responses obtained from *Mentha* plant samples: *M. aquatica* (MA), *M. arvensis* var. *Javanica* (MAJ), *M. arvensis* var. *piperascens* (MAP), *M. piperita* (MP), *M. piperita* var. 'Chocolate' (MPC), *M. piperata* var. *citriata* (MPCI).

Conclusion

Mentha plants of different species and varieties were differentiated by a set of five polymer-modified PQC sensors. The method used in the study was simple since measurements were performed by the sensors on the headspace of the plant samples and did not require the extraction of the essential oil from the plant sample. The measurement of the sensor response required a relatively short time since a static headspace analysis involved a solid-vapor phase distribution equilibrium of the compounds in the headspace. The measurement resulted in an efficient differentiation of the plant samples, since polymers of different polarities were used in the sensing phase, resulting in a unique response profile for each plant sample. Discrimination of the plant samples was observed in the radar plot of the sensor responses, in the biplot generated by principal component analysis, and in the dendrogram resulting from hierarchical cluster analysis. A simple, rapid and efficient method for the differentiation of *Mentha* species can be developed based on the results of this study.

Conflict of Interest

The authors declare no conflict of interests related to the manuscript.

Author Contributions

MRSC, DRBA and FSIII were actively involved in the conduct of the work and the writing of the paper. FSIII conceptualized the work, DRBA planned the instrumentation assembly and MRSC carried out the laboratory work. All authors have read and agreed to the final version of the manuscript.

Institutional Review Board Statement

The study did not involve any human or animal subject.

References

[1] M. da M. Gomes et al., We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %, Intech, 15(1), p. 13, **2013**, [Online]. Available:https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttps://doi.org/10.32725/jab.2004.016.

[2] Mint Essential Oils Market Growth, Demand & Trends, 2020-2027, **2020**. https://www.reportsanddata.com/report-detail/mint-essential-oils-market.

[3] W. H. O. (WHO), Quality control methods for herbal materials,World Heal. Organ. Geneva, Switz., p. pp 187, **2011**, doi: ISBN 978 92 4 150073 9.

[4] K. Kucharska-Ambrożej, A. Martyna, J. Karpińska, A. Kiełtyka-Dadasiewicz, & A. Kubat-Sikorska, Quality control of mint species based on UV-VIS and FTIR spectral data supported by chemometric tools, Food Control, 129 May, **2021**, doi: 10.1016/j.foodcont.2021.108228.

[5] V. V. Thakur, S. Tiwari, N. Tripathi, & S. Sapre, DNA barcoding and phylogenetic analyses of Mentha species using, Ann. Phytomedicine, 5(1), pp. 59–62, **2016.**

[6] F. F. Wang et al., Comparative study for morphological identification, chemical component analysis, and DNA barcode determination of three plants and their varieties in Genus Mentha, Yaoxue Xuebao, 54(11), pp. 2083–2088, **2019**, doi: 10.16438/j.0513-4870.2019-0395.

[7] M. A. Hawrył et al., GC-MS fingerprints of mint essential oils, Open Chem., 13(1), pp. 1326–1332, 2015, doi: 10.1515/chem-**2015**-0148.

[8] P. Rösch, W. Kiefer, & J. Popp, Chemotaxonomy of mints of genus Mentha by applying Raman spectroscopy, Biopolym. - Biospectroscopy Sect., 67(4–5), pp. 358–361, **2002**, doi: 10.1002/bip.10099.

[9] O. V. Rodinkov, A. S. Bugaichenko, & L. N. Moskvin, Static Headspace Analysis and Its Current Status, J. Anal. Chem., 75(1), pp. 1–17, **2020,** doi: 10.1134/S106193482001013X.

[10] H. B. Hyun, K. H. Boo, H. R. Kang, & S. K. Cho, Analysis of mint essential oils from Jeju Island, Korea by gas chromatography-mass spectrometry and headspace-gas chromatography-mass spectrometry, J. Appl. Biol. Chem., 58(2), pp. 175–181, **2015**, doi: 10.3839/jabc.2015.028.

[11] J. B. Igat, D. B. Albano, & F. Sevilla III, Electronic Discrimination of the Mint Plants Based on Quartz Crystal Microbalance Sensors, Acad. Res. Int., 4(2), pp. 1–9, **2013**.

[12] S. Okur et al., Identification of mint scents using a QCM based e-nose, Chemosensors, 9(2), pp. 1–14, **2021**, doi: 10.3390/chemosensors9020031.

[13] M.-L. Tsai, C.-T. Wu, T.-F. Lin, W.-C. Lin, Y.-C. Huang, & Chao-Hsun Yang, Chemical Composition and Biological Properties of Essential Oils of Two Mint Species, Trop. J. Pharm. Res., 12(4), pp. 577–582, **2013**, doi: 10.4314/tjpr.v12i4.20.

[14] T. Myoda et al., Components of essential oil of the Japanese mint 'Hokuto' and its deodorization against human malodors, J. Essent. Oil Res., 30(6), pp. 431–436, 2018, doi: 10.1080/10412905.**2018**.1494045.

[15] P. Kumar, S. Mishra, A. Malik, & S. Satya, Efficacy of Mentha×piperita and Mentha citrata essential oils against housefly, Musca domestica L., Ind. Crops Prod., 39(1), pp. 106–112, **2012,** doi: 10.1016/j.indcrop.2012.02.021.