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Determination of vitamin E content in *Momordica charantia* L.

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SUMMARY: *Momordica charantia* L. (bitter melon) has long been used in traditional medicine for both external applications, such as wound and burn treatment, and internal use in conditions like gastrointestinal disorders and cancer. These uses often involve fresh fruits or their macerations in olive oil or honey. One of its key bioactive components, α-tocopherol (a form of vitamin E), is known for its strong antioxidant activity and plays a therapeutic role in managing oxidative stress-related conditions, including cancer, cardiovascular diseases, and aging. In this study, the α-tocopherol content in fresh bitter melon fruits, as well as their olive oil and honey macerates, was quantified using a validated high-performance liquid chromatography method with diode array detection (HPLC-DAD). The method was validated through linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and recovery assessments. A comparative analysis revealed that the highest α-tocopherol content was found in the fresh fruits (3.346 µg/100 mg), followed by the olive oil macerate (3.04 µg/100 mg). The honey macerate contained α-tocopherol below the quantifiable limit of the method. These findings suggest that both fresh bitter melon and its olive oil macerations may serve as potential sources of vitamin E. The results also provide a valuable basis for further investigations into the therapeutic potential and bioavailability of α-tocopherol in such traditional formulations.

KEY-WORDS: *Honey; HPLC; Momordica charantia; Olive oil; Validation; Vitamin E.*

RESUMEN: ***Determinación del contenido de vitamina E de Momordica charantia L.*** *Momordica charantia* L. (melón amargo) se ha utilizado desde hace mucho tiempo en la medicina tradicional, tanto para aplicaciones externas, como el tratamiento de heridas y quemaduras, como para uso interno en afecciones como trastornos gastrointestinales y cáncer.
Estos usos suelen incluir frutas frescas o sus maceraciones en aceite de oliva o miel. Uno de sus componentes bioactivos clave, el α-tocoferol (una forma de vitamina E), es conocido por su potente actividad antioxidante y desempeña un papel terapéutico en el manejo de afecciones relacionadas con el estrés oxidativo, como el cáncer, las enfermedades cardiovasculares y el envejecimiento. En este estudio, se cuantificó el contenido de α-tocoferol en frutos frescos de melón amargo, así como en sus macerados de aceite de oliva y miel, mediante un método validado de cromatografía líquida de alta resolución con detección por arreglo de diodos (HPLC-DAD). El método se validó mediante evaluaciones de linealidad, límite de detección (LOD), límite de cuantificación (LOQ), precisión, exactitud y recuperación. El análisis comparativo reveló que el mayor contenido de α-tocoferol se encontró en las frutas frescas (3,346 µg/100 mg), seguido del macerado de aceite de oliva (3,04 µg/100 mg). El macerado de miel contenía α-tocoferol por debajo del límite cuantificable del método. Estos hallazgos sugieren que tanto el melón amargo fresco como sus maceraciones en aceite de oliva podrían ser fuentes potenciales de vitamina E. Los resultados también proporcionan una base valiosa para futuras investigaciones sobre el potencial terapéutico y la biodisponibilidad del α-tocoferol en estas formulaciones tradicionales.

PALABRAS CLAVE: *Aceite de oliva; HPLC; Miel; Momordica charantia; Validación; Vitamina E.*

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1. INTRODUCTION

Momordica charantia L., commonly known as bitter melon, is a member of the Cucurbitaceae family. Native to tropical regions such as the Amazon, East Africa, Asia, India, South America, and the Caribbean, it is also referred to as "kudret narı" in Türkiye. This perennial climbing plant bears immature fruits that are white or green, developing a pronounced bitter taste upon ripening. The genus name "*Momordica*" derives from the Latin word for "to bite," alluding to the plant's jagged-edged leaves that appear as if bitten (Das *et al*., 2015).

Bitter melon extracts, primarily derived from its fruits, have been investigated for their potential therapeutic applications in conditions such as diabetes, dyslipidemia, microbial infections, and certain types of cancer. In Ayurvedic medicine, the fruit is utilized as a tonic, stomachic, stimulant, emetic, and laxative. Despite its traditional use in stimulating digestion and alleviating ailments like dyspepsia and constipation, some clinical trials and traditional reports suggest that it may trigger reflux and ulcers (Grover and Yadav, 2004).

Phytochemical analyses reveal that *M. charantia* contains various bioactive compounds, including glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids. Its unripe fruits are particularly rich in vitamins A and C, phosphorus, and iron. Notable constituents encompass sterols, triterpenes, and bioactive proteins such as α- and β-momorcharins; sterols like daucosterol; and triterpenes including goyaglycosides a-h, goyasaponins I-III, cucurbitacins, and their glycosides (e.g., momordicosides E1, F1, F2, F-K). Low molecular weight compounds such as gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin are also prevalent (Semiz and Sen, 2007; Güneş, 2018; Gurbuz *et al*., 2000).

While *M. charantia* is not indigenous to Türkiye, it is extensively utilized in traditional medicine, particularly in the western and southwestern regions. Common applications include the treatment of peptic ulcers and tumors. Ethnomedical reports indicate its use in managing various ulcers, diabetes, and infections. The reported adverse effects of *M. charantia* include hypoglycemic coma and convulsions in children, elevated levels of γ-glutamyl transferase and alkaline phosphatase, and headaches. Due to potential abortifacient effects and teratogenicity, consumption of its seeds during pregnancy is not recommended. Given the documented hypoglycemic effects and seizures in children, it is advised to avoid administering raw fruit or its preparations to pediatric populations and during lactation (Ilhan *et al*., 2015).

In Türkiye, *M. charantia* is frequently employed topically for wound healing and orally for conditions such as peptic ulcers, colds, and fevers, as well as for its laxative, antioxidant, and cholinergic properties. A prevalent traditional remedy involves macerating the fruit in olive oil. Studies have demonstrated that this preparation significantly contributes to wound healing by stimulating fibroblast epithelialization, neovascularization, and proliferation, leading to accelerated recovery (Pişkin *et al*., 2012). The absence of harmful side effects in external applications suggests that the olive oil macerate of bitter melon can be safely utilized in wound treatment.

Vitamin E, first identified by Evans, is a fat-soluble antioxidant essential for various bodily functions (Evans *et al.,* 1936). Its most biologically active form, α-tocopherol, exists in eight isomers, with only four detectable in human plasma; among these, RRR-α-tocopherol is the naturally occurring form in foods. Rich dietary sources include green leafy vegetables, legumes, nuts, seeds, and whole grains. As a potent antioxidant, Vitamin E protects cells from oxidative damage, supports immune function, and may reduce the risk of cardiovascular diseases by preventing LDL (Low Density Lipoprotein) oxidation. Deficiency, though rare, can lead to neurological issues and hemolytic anemia, particularly in individuals with fat malabsorption disorders (Traber, 1999; Baysal, 2008; Pazirandeh and Burns, 2020). While supplementation has been explored for conditions like non-alcoholic fatty liver disease and age-related macular degeneration, excessive intake may pose health risks, including an increased likelihood of hemorrhagic events. Therefore, maintaining adequate, but not excessive, levels of Vitamin E through a balanced diet is recommended for optimal health (Evans and Lawrenson, 2017).

M. charantia holds a prominent position in traditional medicine systems across various cultures, including Turkish, due to its diverse therapeutic properties. However, further randomized studies are necessary to conclusively establish its safety and efficacy.

Despite the extensive documentation of *M. charantia's* ethnomedicinal uses and phytochemical richness, limited studies have focused on how different maceration media affect the retention and stability of lipophilic compounds such as α-tocopherol. In particular, natural carriers like olive oil and honey, which are widely used in traditional preparations, have not been comparatively evaluated in terms of their efficacy in preserving

this essential antioxidant. Therefore, the present study aims to investigate the α-tocopherol content in *M. charantia* preparations macerated in extra virgin olive oil and flower honey. By evaluating these two commonly used carriers under standardized conditions, the study seeks to highlight their role in stabilizing bioactive compounds and contribute to the development of safe, natural, and functional formulations. This work offers an original perspective by combining traditional maceration practices with modern analytical evaluation of vitamin E content, particularly addressing the gap in comparative studies on maceration efficiency in natural matrices.

2. MATERIALS AND METHODS

2.1. Plant materials

Momordica charantia L. samples were collected from the İskele area of the Urla district of Izmir in September 2024 after they had reached maturity (Figure 1). The seeds were removed and discarded, and the remaining 600 grams of fruit were cut into small pieces and divided into three parts of 200 grams each. The first part was kept fresh in the deep freezer. 500 grams of extra virgin olive oil (Taris, Türkiye) was added to one of the other two parts and kept for a month, stirring occasionally. 500 grams of flower honey (Floral honey, Balparmak, Türkiye) were added to the other and left to macerate under the same conditions.

FIGURE 1. The appearance of a fully ripe bitter melon fruit and the appearance of fruits left to ripen, respectively (Civelek *et al.,* 2016)

2.2. Extraction and preparation of samples

40 grams were taken from yellow-orange mature bitter melon (without seeds), honey and olive oil macerations stored in the freezer. 250 ml of n-hexane were added to each well and the mixtures were incubated at room temperature on a mechanical shaker (DLAB Scientific Inc.) for approximately 8 hours. At the end of the period, the extraction solvent was filtered by using Whatman No. 1 filter paper with an approximate pore size of 11 µm and the filtrate was evaporated to dryness in a rotavapor at 50 °C (Chen *et al*., 2019). The samples evaporated to dryness were stored at -5 °C, and protected from light until analysis. These samples were then dissolved in methanol (≥ 99.9%, gradient grade, purchased from Merck KGaA, Darmstadt, Germany) to a concentration of 500 mg/ml.

2.3. High performance liquid chromatography

In the high-performance liquid chromatography (HPLC) method, 50 MPa pressure is applied to pass the liquid solvent containing the sample mixture through the column filled with a solid adsorbent (Inertsil® ODS-3 (25 cm × 4.6 mm × 5 µm). Different components move through the column at different speeds, which allows different components to reach the column outlet at different times. Then, a UV light source (190-950 nm) is used to determine the amount of the components. HPLC is a frequently used device for

analytical separation techniques. It is widely used for the separation of drug substances, proteins, vitamins and pesticides (Bird, 1989).

In our study; we used the HPLC method to determine the amount of α-tocopherol found in bitter melon, and honey and olive oil macerates. The HPLC operating conditions used in the study are given in Table 1. The analysis was carried out using an Agilent 1100 HPLC system equipped with a Diode Array Detector (DAD), which allows the simultaneous monitoring of multiple wavelengths within the 190–950 nm range, providing precise and reliable detection. The chromatographic separation was achieved using a reversed-phase Inertsil® ODS-3 column (25 cm × 4.6 mm, 5 µm particle size), maintained at a constant temperature of 40 °C. The mobile phase consisted of methanol (MeOH), delivered at a flow rate of 1 mL/min, and the injection volume was set to 20 µL. A pressure of approximately 50 MPa was applied to ensure efficient solvent flow through the packed column. Due to the hydrophobic nature of the stationary phase (C18 bonded silica), the method was suitable for the effective retention and separation of non-polar compounds like α-tocopherol. HPLC is a widely recognized analytical technique used in pharmaceutical, nutritional, and environmental studies for the precise quantification and separation of bioactive compounds.

TABLE 1. Conditions of the HPLC system

System	Conditions
HPLC system	Agilent 1100 HPLC-DAD
Detector	Diode Array Detector (DAD) (190-950 nm)
Column	Inertsil® ODS-3 (25cm x 4.6mm x 5µm)
Column temperature	40 °C
Flow rate	1 ml/min
Mobile phase	Methanol (isogratic mode)
Application volume	20 µl
Pressure	50 MPa
Run time	40 min

In line with this information, the amount of vitamin E was determined using the HPLC method. Vitamin E is thought to be one of the active substances responsible for the effect of the olive oil and honey macerates prepared from bitter melon fruits which are widely used among the public in our country. These macerates are used externally in the treatment of wounds and burns, and internally as an aid in the treatment of stomach and intestinal ulcers and cancer.

2.4. Validation of the method

Method validation is used to test the method's compliance with the performance criteria in determining the method parameters. Method validation can be applied for more than one reason: the first application of the method in the laboratory, changes in the currently applied method, the application of a method with known validity in a different laboratory, and the application of the method with different people or devices. Parameters vary according to the applied method and the purpose of application. While the specificity parameter is at the forefront for qualitative analyses, in quantification; linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and realism gain importance (Bayraç and Camizci, 2020). For the linearity parameter, the calibration curve created in line with the response of the method to the prepared analyte concentrations is used. According to the EuroChem guide, in order to create a calibration curve, three replicates of the analyte at least at six different concentrations are required, while ISO standards accept at least two replicates of at least three different concentrations.

In our study, five different concentrations (0.1; 0.2; 0.3; 0.4; 0.5 µg/mL) were used for the calibration curve. The calibration curve was created with the peak area values formed in the chromatograms corresponding to the prepared concentrations.

Method validation also includes the realism parameter. The realism parameter includes the use of a validated method and proficiency test results, along with certified reference laboratory material (Shabir, 2003; Bayraç and Camizci, 2020).

Another parameter, precision, refers to the closeness of the results of the method applied under the same conditions. It has two separate components, repeatability and reproducibility. Repeatability is the experiment where the same method is applied with the same equipment in the same laboratory by the same person with equivalent test substances and the same results are obtained. Reproducibility is the experiment where the same method is applied with different equipment in a different laboratory by different people with equivalent test substances and the same results are obtained. In our study; three concentrations were selected from the calibration curve for the repeatability application, and five repetitions were made on the same day and three repetitions on three different days.

2.5. Statistical analysis

Data were expressed as means ± standard deviation (SD) for five independent experiments in triplicate. Comparisons of means between groups were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P value less than 0.05 was considered significant.

3. RESULTS

3.1. Yield of the extracts

In our study, the percentage yields of the extracts obtained as a result of extracting bitter melon, its olive oil and honey macerations with n-hexane are given in Table 2. According to the results obtained, the fruit extract of *Momordica charantia* showed a yield of 0.023%, the oil maceration yielded 0.533%, and the honey maceration yielded 0.325%. Among these, the highest yield was obtained from the oil maceration, while the lowest yield was observed for the fruit extract.

TABLE 2. Percentage yields of *Momordica charantia* extracts

Extract	Extraction Yield %[a]
Fruit extract of *Momordica charantia*	0.023 ± 0.021
Oil maceration of *Momordica charantia*	0.533 ± 0.092
Honey maceration of *Momordica charantia*	0.325 ± 0.082

[a] Mean ± S.D.

3.2. Results of HPLC analysis

Studies on the determination of α-tocopherol amount in bitter melon, olive oil and honey macerations of bitter melon were carried out by HPLC method and the area values belonging to it were calculated. The HPLC spectra of bitter melon, olive oil, and honey macerations of bitter melon are shown in Figure 2. When the spectra were examined, it was observed that the analysis of the honey maceration of bitter melon fell outside the measurable limits.

A run time of 40 minutes was applied in the HPLC analysis to ensure that the baseline remained stable throughout the chromatographic process. The HPLC spectrum of the standard tocopherol peak given in the 0.1-0.5 µl concentration range was determined to have a retention time of 11.5 minutes and a maximum wavelength of 330 nm. The equation obtained from the calibration curve was y=354.97x – 0.6816 0.9996.

The amount of α-tocopherol calculated using the standard equation and the area values of the α-tocopherol amount for the samples are given in Table 3. A comparative evaluation of the HPLC analysis results revealed that bitter melon had the highest α-tocopherol content, at 0.73 µg/100 mg. The olive oil macerate of bitter melon was calculated to contain 0.39 µg/100 mg α-tocopherol. The α-tocopherol content in the honey macerate of bitter melon was outside measurable limits.

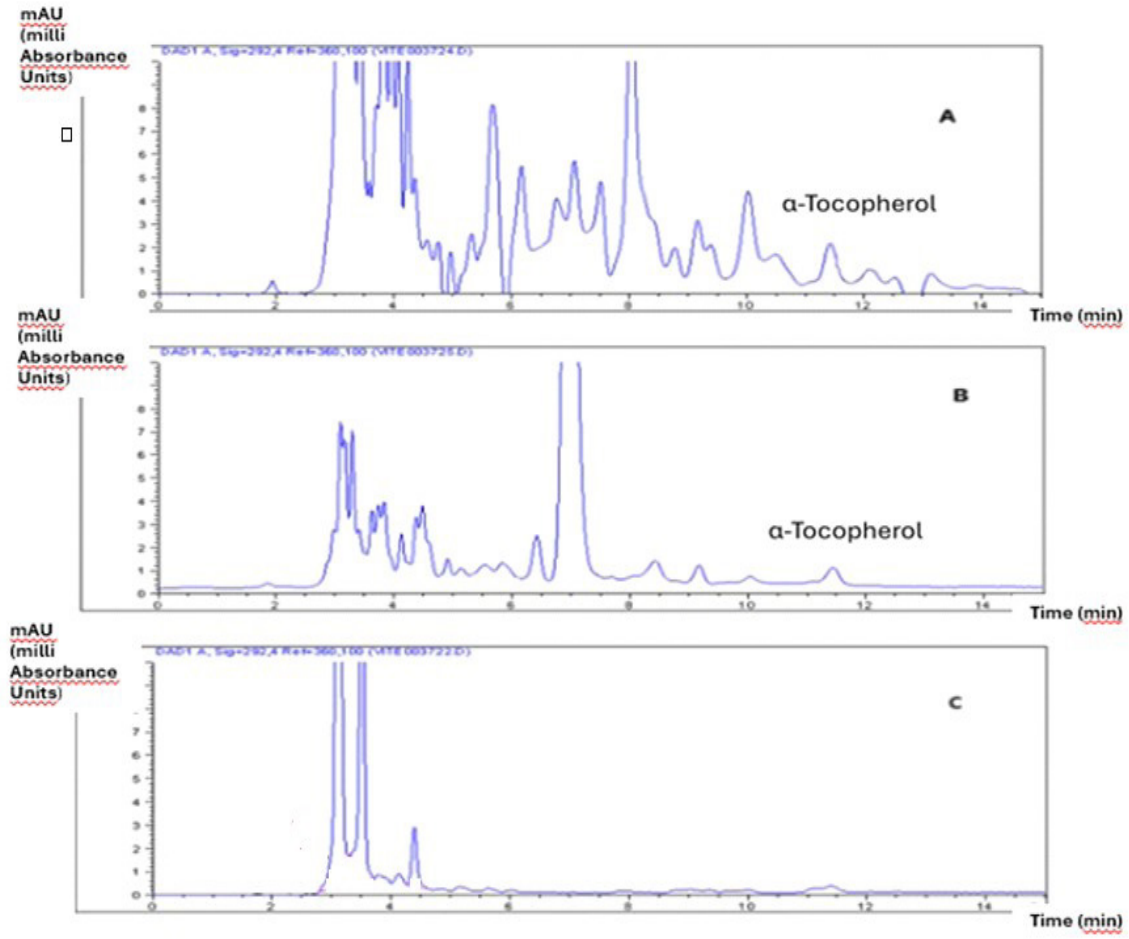

FIGURE 2. HPLC spectrums of extracts (A: *M. charantia* fruit, B: olive oil maceration, C: honey maceration). HPLC chromatograms of α-tocopherol in bitter melon extracts: (A) fruit extract, (B) oil maceration, and (C) honey maceration. Separation was performed using an Inertsil® ODS-3 column (25 cm × 4.6 mm, 5 µm); mobile phase: methanol; flow rate: 1 mL/min; column temperature: 40 °C; detection at 292.7 nm (DAD). The pink area in chromatogram C indicates the integration area for the α-tocopherol peak.

TABLE 3. α-tocopherol contents in *Momordica charantia* extracts

Extracts	Area Values of α-tocopherol Amounts of Samples	α-Tocopherol (µg/100 mg)
Fruit extract of *Momordica charantia*	25.2662[a]	0.73 ± 0.04 [a]
Oil maceration of *Momordica charantia*	13.1495	0.39 ± 0.06

[a] Mean ± S.D.

3.3. Data of validation methods

A linearity equation was obtained by applying the linearity study conducted within the scope of method validation between 0.1-0.5 µl. The determination and detection limits of α-tocopherol, which we used as a standard substance, and the linearity analysis results are given in Table 4.

The results of the repeatability application we conducted within the precision parameter of the method validation are given in Table 5.

TABLE 4. Linearity equation, determination and detection limit results of α-tocopherol

Standard substance	LOD µg/ml [a]	LOQ µg/ml	Linearity	R^2
α-tocopherol	0.039±0.011	0.201±0.013	y=354,97x – 0,6816	0,9996

[a] Mean ± S.D.

TABLE 5. Intraday and interday repeatability study results

Standard substance	Amount added µg/ml	Average recovery	RSD %
Intraday α-tocopherol	0.5	93.8±0.19	4.14
(n=5)	1	94.3±0.03	1.11
Interday α-tocopherol	0.5	95.1±0.04	1.40
(n=3)	1	96.5±0.10	1.33

n: number of analyses, RSD: relative standard deviation

4. DISCUSSION

In the current study, the amount of α-tocopherol in bitter melon, olive oil and honey maceration, which are widely used in traditional medicine both in the world and in our country, externally for wound and burn treatment and internally for stomach and intestinal diseases, were determined comparatively. While the primary aim was to quantify α-tocopherol content in these preparations, no direct biological or clinical evaluations were conducted regarding their health benefits. However, since α-tocopherol is a well-known antioxidant and plays a vital role in protecting cells from oxidative damage, the quantitative findings obtained in this study may serve as a basis for future research into the potential health-promoting properties of these traditional formulations.

When the comparative HPLC analysis results were evaluated, it was seen that the highest α-tocopherol content belonged to bitter melon with 0.73 µg/100 mg. It was calculated that the olive oil macerate of bitter melon contained 0.39 µg/100 mg α-tocopherol. The α-tocopherol content in the honey macerate of bitter melon was below the limit of quantification (LOQ), indicating that its concentration was too low to be reliably measured using the applied HPLC method. In all three repetitions of the honey maceration of bitter melon, the amount of α-tocopherol remained outside the measurable limits. Although α-tocopherol was found in the determination of the amount in bitter melon, it is thought that one of the reasons why it remained outside the measurable limits in the honey maceration is that vitamin E, a fat-soluble vitamin, did not pass into the honey.

In the literature research, a comparative study of the chemical and functional contents of honey produced by Ecuadorian bees and multifloral honey was found (Guerrini *et al*., 2009). According to the results of the study, no tocopherol species other than β-tocopherol was found in honey produced by Ecuadorian bees, while four different tocopherol species were found in trace amounts in multifloral honey. It was thought that the differences between the ingredients in honey could be due to geographical and botanical differences. In line with these results, it was thought that the geographical and botanical conditions in which the honey we purchased for our experiment was produced could cause differences in vitamin E content.

There are differences between the results obtained in the literature research and the results we found regarding the amount of vitamin E found in bitter melon (*Momordica charantia*) (Haefelfinger, 1981; Shah *et al*., 2000; Gustavo and Angeles, 2007). The literature results are higher than our results. For example, Haefelfinger (1981) reported α-tocopherol content of up to 27.1 mg/100 g, Shah *et al.* (2000) found values ranging between 18.3 and 22.5 mg/100 g, and Gustavo and Angeles (2007) reported 25.6 mg/100 g in bitter melon seed oil extracts. In contrast, the amount of α-tocopherol determined in our study was significantly lower (0.39-0.73 mg/100 g). This discrepancy may be due to several factors such as the climatic conditions in which the plant grows, harvest time, storage conditions, and differences in extraction or analytical methods. The bitter melon samples used in our study were collected from Urla, Izmir, Turkiye and were not cultivated under controlled conditions. The fruits were stored at room temperature and extracts were prepared within 10 days of collection. In future studies, using bitter melon grown and stored under controlled and varied

conditions may provide more comparable results. Similarly, literature studies have shown that bitter melon seeds contain high levels of vitamin E. In our study, the seeds of the bitter melon were removed and only its fruits were used because bitter melon seeds are known to have abortive and teratogenic effects.

The findings suggest that fresh bitter melon fruits and their olive oil macerations could serve as valuable sources of vitamin E, which is known for its strong antioxidant properties and potential therapeutic effects in wound healing, cancer, cardiovascular diseases, and age-related conditions (Jiang, 2014; Huang *et al*., 2016). The higher α-tocopherol content in fresh fruits is consistent with previous studies highlighting the rich vitamin E composition of *M. charantia* (Basch *et al*., 2003). Furthermore, the presence of α-tocopherol in olive oil macerate supports the hypothesis that oil-based extractions may help retain fat-soluble vitamins more effectively than aqueous or sugar-based preparations (Kamal-Eldin and Appelqvist, 1996).

Recently, Gayathry and John (2022), in their comprehensive review, emphasized the rich presence of vitamin E and other lipophilic antioxidants in *M. charantia* and highlighted the influence of extraction methods on their yield, particularly noting that non-polar techniques, such as hexane-based extractions, optimized tocopherol recovery (Gayathry and John, 2022). Additionally, some researchers investigated the biochemical properties and antioxidant potential of bitter melon seed oil and highlighted that while seeds contained a richer tocopherol profile, fruit-only preparations —consistent with our study design— naturally exhibited lower vitamin E levels (Balde Samba *et al.,* 2022). A bioavailability study emphasized that oil-based carriers enriched with unsaturated fatty acids and tocopherols enhanced the stability and absorption of lipophilic antioxidants in topical and nutritional applications. These recent insights support our approach and underscore the significance of extraction solvent polarity, chosen plant tissue, and matrix composition in determining α-tocopherol yield and its potential bioefficacy in *M. charantia* preparations (Infante *et al.,* 2023).

These results emphasize the importance of extraction methods in preserving bioactive compounds. The results emphasize the potential of the applied extraction method in preserving bioactive compounds, particularly α-tocopherol. The use of n-hexane as a non-polar solvent in maceration has been widely recognized as effective for isolating lipophilic compounds, including tocopherols, due to its high selectivity and ability to minimize oxidative degradation during extraction (Demirkaya and Kadioglu, 2007; Müller *et al*., 2010). Although only one method was used in the present study, its selection is supported by existing literature as a suitable approach for maintaining the integrity of vitamin E compounds. Future research may benefit from comparative evaluations of different extraction techniques and carriers to further enhance the stability and bioavailability of α-tocopherol in *M. charantia* preparations.

5. CONCLUSIONS

This study provides a comparative evaluation of the α-tocopherol content in *Momordica charantia* preparations macerated in olive oil and honey, as well as in the fresh fruit itself. The findings revealed that fresh M. charantia fruits contained the highest level of α-tocopherol, followed by olive oil macerates, while the honey-based preparation exhibited no measurable levels of this fat-soluble antioxidant. The absence of detectable α-tocopherol in the honey macerate is likely attributed to its aqueous and sugar-rich matrix, which may hinder the solubilization of lipophilic compounds such as vitamin E.

The comparatively lower α-tocopherol content identified in this study, when contrasted with values reported in the literature, may stem from factors such as plant origin, environmental conditions, harvest timing, post-harvest handling, and methodological variations. The removal of seeds—which are known to be rich in tocopherols—further explains the reduced vitamin E content in the tested fruit samples.

Despite using a single extraction method, the application of n-hexane as a non-polar solvent proved effective in isolating α-tocopherol from both the fresh fruit and oil-based macerates, supporting existing literature on its suitability for extracting lipophilic compounds. These results underline the importance of solvent and carrier selection in preserving bioactive compounds in herbal preparations.

In conclusion, fresh M. charantia fruits and their olive oil macerates represent promising natural sources of α-tocopherol, a compound with recognized antioxidant and therapeutic properties. Further studies involving a broader range of extraction methods and controlled cultivation conditions are recommended to optimize

the recovery, stability, and potential health benefits of α-tocopherol in traditional and modern formulations of M. charantia.

RESEARCH DATA POLICY

DATA AVAILABILITY

The data obtained in this study is stored in a fileshare account. The access link to this account is as follows: https://fileshare.ikc.edu.tr/index.php/apps/files/?dir=/&fileid=902334

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DECLARATION OF COMPETING INTEREST

The authors of this article have declared that there are no conflicts of interest.

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AUTHORSHIP CONTRIBUTION STATEMENT

P.Tastan: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. E.I.Yilmaz: Conceptualization, Formal analysis, Investigation, Methodology. T.Gonenc: Investigation, Methodology, Writing – review & editing.

Artificial Intelligence Usage

Authors have not used Artificial Intelligence tools in this article.

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