

Analytical Methods

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4 **Chemical composition of the essential oils from the hulls of *Pistacia vera* L. by**
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6 **using magnetic nanoparticle-assisted microwave (MW) distillation:**
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9 **Comparison with routine MW and conventional hydrodistillation**
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15 Hamid Hashemi-Moghaddam*¹, Majid Mohammhosseini ² and Mohammad Salar ¹
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17
18 ¹ *Department of Chemistry, College of Basic Sciences, Damghan Branch, Islamic Azad*
19
20 *University, Damghan, Iran*
21

22
23 ² *Department of Chemistry, College of Basic Sciences, Shahrood Branch, Islamic Azad*
24
25 *University, Shahrood, Iran*
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31 **Abstract:**

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33 In this paper, classical hydrodistillation (HD) and microwave assisted hydrodistillation
34 (MAHD) were used for extraction of *Pistachio* hull essential oil combined with subsequent gas
35 chromatographic mass spectrometric (GC-MS) determination. The major focus is on synthesis of
36 amine-functionalized magnetite nanoparticles (AFMN) and their effects on essential oils from
37 quantitative and qualitative points of view. In addition, experimental parameters such as power
38 and time were optimized in the proposed and improved MAHD technique. The obtained results
39 show that the quantity of the extracted essence is negligible both in presence and absence of
40 AFMN in the HD method. However, the essential oil yield was significantly increased when
41 using MAHD method particularly in the presence of AFMN, in which more yield (0.8%) and
42 more chemical diversity (25 compounds) were obtained. Generally, the most important
43 compounds in the extracted essential oil in the presence of magnetic nanoparticles included: α -
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3 pinene (31.5%), α -terpinolene (20.2%), myrcene (11.5%), pinocarvone (6.2%) and camphene
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5 (4.8%). On the other hand, the most important compounds in the extracted compounds in the
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7 absence of magnetic nanoparticles were found to be α -pinene (31.8%), α -terpinolene (20.3%),
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9 myrcene (12.2%), pinocarvone (6.2%) and camphene (4.7%). According to the results,
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11 simultaneous use of microwave and nanoparticles significantly increase the quality and quantity
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13 of volatile essential oils of *Pistacia vera*.
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16 17 **Keyword index**

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19 Essential oil, Microwave-assisted hydrodistillation (MAHD), Hydrodistillation (HD), *Pistacia*
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21 *vera* L., Magnetic nanoparticles
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Introduction

The *Pistachio*, *Pistacia vera*, a member of the cashew family, is a small tree originally from Central Asia and the Middle East. *Pistachio* trees can be found in some regions of Iran, Syria, Lebanon, Turkey, Greece, Tunisia, Kyrgyzstan, Tajikistan, Turkmenistan, India, Pakistan, Egypt, Italy, Uzbekistan, Afghanistan, and the United States, particularly in California. The tree produces a seed.^{1, 2} The pistachio is a drupe, which means it has an outer fleshy covering over the hard-shelled nut, like the nutmeg. In the harvest season, the fruit is gathered and subsequently soaked to remove the outer soft red or yellow covering prior to drying the hard shells containing the nut in the sun.

Pistachio fruits are sold on a large scale in market either as seeds or enclosed inside their hard dehiscent shell representing the endocarp. In fact, the latter is covered by a soft envelope constituting the fleshy mesocarp and the epicarp. This envelope or hull releases a pleasant scent reminding that of *Pinus* resin due to its richness in pinenes.³ Empty *Pistachio* shells can be recycled in several ways. Practical uses include fire starters, mulchs for shrubs and plants that require acid soils as well as media for orchids. In addition, shells from salted *Pistachios* can be placed around the base of plants to deter slugs and snails. Some investigations indicate that *Pistachio* shells may be helpful in cleaning up pollution created by mercury emissions.^{4, 5} Moreover, in the literature, sporadic reports are found concerning the antioxidant activity,^{6, 7} photoprotective,⁶ antibacterial,^{8, 9} antimicrobial,^{10, 11} antiviral,⁸ and antifungal^{8, 12} properties of *Pistachio*.

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3 In the present work, we focused on the volatile constituents of *Pistachio* hull in order to
4 valorize it, since a variety of its novel aroma compounds are widely used and exploited in
5 various fields, such as culinary, pharmaceutical, therapeutic, industrial and cosmetic cases.^{5, 13-15}
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11 Essential oils are complex mixtures of volatile substances usually present at low
12 concentrations. Their components can be analyzed and identified by gas chromatography-mass
13 spectrometry (GC-MS) after their isolation from the initial plant matrix.
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19 Hydrodistillation (HD), steam distillation (SD), Soxhlet extraction, and solvent extraction are
20 conventional methods to extract the essential oils from medicinal plants.¹⁶⁻²⁰ However, these
21 procedures often require large amounts of samples and are also time-consuming, not economical
22 and laborious. Furthermore, appreciable loss of some volatile compounds, low extraction
23 efficiency, and toxic solvent residue in the extract may be encountered using these extraction
24 methods. These disadvantages have led to the consideration of new techniques which use less
25 solvent, time, and energy for the extraction of essential oils. Nowadays, modern sample
26 preparation in analytical chemistry is characterized by simplification, miniaturization, high
27 enrichment and minimization of sample amount and solvent.²¹ Of the advanced methods used
28 for oil and or volatile fraction isolation, phytochemists widely use microwave or SPME+-based
29 methods.²¹⁻²⁴
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45 Microwave heating involves internal heating based on conduction and dielectric polarization
46 caused by microwave irradiation.²⁵ It is therefore not only more efficient when compared to
47 traditional heating but also may result in an external temperature much lower than that of the
48 sample with control of the time and output power of the microwave irradiation.
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54 Microwave assisted extraction is suitable for the recovery of a vast array of compounds, and
55 is recognized as a versatile and efficient extraction technique intensively applied for isolation of
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3 the essential oils from different organs of the plants all over the world in recent years.²⁶⁻³⁶
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5 Compared with classical reflux and Soxhlet-based extractions, microwave assisted extraction
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7 generally shows evident advantages involving shorter extraction time, higher extraction yield,
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9 higher selectivity and better quality of the target extracts.³⁷ It is also a relatively cost-effective
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11 approach when compared to accelerated solvent extraction.^{23, 38, 39}
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15 Traditionally, microwave assisted extraction utilizes a distinct volume of an organic solvent
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17 like methanol. Moreover, extraction efficiency of microwave technique may be very poor when
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19 either the target compounds or solvents are non-polar, or when the viscosity of solvent is
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21 extremely high.⁴⁰ In general, microwave assisted extraction is not recommended for the
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23 extraction of the thermally labile compounds.³⁸
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27 In the present work, a simple magnetite nanoparticle-assisted microwave distillation method
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29 was proposed and successfully developed for the effective extraction and analysis of the essential
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31 oil of *Pistachio vera* L. Accordingly, amine functionalized magnetite nanoparticles (AFMN)
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33 practically served as a microwave absorption solid medium. This approach can be considered as
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35 a feasible way in the extraction of the essential oils from a variety of the plant materials. The
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37 types of materials being used in this method must have promising microwave absorption
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39 capacities.^{41, 42} A simple comparison of the chemical profiles by this method and the HD method
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41 confirms the higher capability and effectiveness of AFMN-based method for the separation of
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43 essential oils of *P. vera*.
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Experimental

Materials and methods

Plant material

Pistachio vera trees used in this study belong to the variety of “Shahpasand”. The samples were picked up from Damghan region (North Eastern of Iran), Semnan Province, at Latitude: 36°.1666667 and Longitude: 54°.3438889 on 20 September 2012. Damghan along with Rafsanjan (Kerman Province, South Eastern, Iran) are great poles of *Pistachio* export in the Middle East.

The plant was identified by a local botanist, and a voucher specimen was deposited at the Herbarium (number 44520) of the Botanical Department of Islamic Azad University, Damghan Branch, Iran. Hulls (epicarp without mesocarp) were then taken off from fruits and were dried at shadow to avoid extra damaging and minimizing cross-contamination. In the next step, *Pistachio* shell sample was dried at shade (one week) and was exactly weighed.

Preparation of the amine-functionalized magnetite nanoparticles

At first, 3.0 g of pure ferric chloride hexahydrate was added to 12.0 g sodium acetate followed by addition of 12.7 mL of 1,6-hexanediamine to 90 mL of glycol. In the next step, the reagents were gently mixed to obtain a transparent solution. The mixture was sealed in a PTFE-lined, stainless steel autoclave and was heated at 200 °C for 6 h. The product which settled at the bottom of the autoclave, was washed with hot water and ethanol (three times) under ultrasonic conditions to remove the solvent and unbound 1,6-hexanediamine, effectively. Immediately after, the precipitate was dried at 50 °C to gain the black powder. The XRD image of amine-functionalized magnetite nanoparticles is shown in Figure 1. The mean diameter of synthesized

nanoparticles, as one of the prominent characteristics of diverse nanoparticles, was calculated by the Scherrer equation.⁴³⁻⁴⁷ In X-ray diffraction and crystallography, the Scherrer equation, relates the size of sub-micrometre particles, or crystallites, in a solid to the broadening of a peak in a diffraction pattern^{48, 49}. This equation has the following formula (eq. 1):

$$\tau = \frac{K\lambda}{\beta \cos \theta} \quad (\text{eq. 1})$$

Where the terms τ , K , λ and β account for mean size of the ordered (crystalline) domains, dimensionless shape factor (with a value close to unity), the X-ray wavelength, the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians, respectively. The term FWHM is also sometimes denoted as $\Delta(2\theta)$; θ is the Bragg angle. Accordingly, the average diameter of the synthesized magnetic nanoparticles was found to be 50 nm; the larger surface/volume ratio of the synthesized nanoparticles makes them absorb microwave irradiation better than micro-size magnetic particles which have a higher load capacity.

Hydrodistillation

Conventional hydrodistillation was carried out using a circulatory commercially available Clevenger apparatus. Accordingly, 150-g portions of the dried hulls of *P. vera* were exactly weighed and immersed in water in the ratio of 1:10, followed by collecting the volatile oils at sequential times. For each distillation time, experiments were conducted twice. The maximum distillation period was 5 h.

Microwave assisted hydrodistillation (MAHD)

The microwave oven used for MAHD was provided by Samsung (South Korea) trade mark operating at 2450 MHz. The maximum power of the oven was regulated at 1000 W. The dimensions of the interior cavity of the oven were 29×37×40 cm. Microwave oven was modified

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3 by drilling a hole at the top. Flat bottom flask having a capacity of 1000 mL was placed in the
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5 oven, and directly connected to Clevenger apparatus through the hole.³⁴
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8 For MAHD, fifty-gram portions of the sample were soaked in 500 mL of distilled water at
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10 room temperature (25 °C) for 1 h in order to hydrate its external layers, and the excess water was
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12 drained off. Soaking time, during which the maximum amount of absorption is achievable, was
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14 determined from a preliminary experiment. Afterwards, the moistened sample was placed in a
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16 flat-bottom flask combined to a Clevenger apparatus. During the process, the vapor continuously
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18 passed through the condenser located at the outside of the microwave cavity where it was
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20 condensed. The MAHD process was performed for different times, and was repeated until no
21
22 more essential oil was obtained. For each condition, experiments were replicated twice. The
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24 essential oil was collected in amber brown vials, dehydrated with anhydrous sodium sulfate,
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26 capped under nitrogen and kept at 4 °C until being analyzed. In the magnetic nanoparticle-
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28 assisted microwave (MW) distillation method, 0.2 g-portions of AFMN were added to the flask
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30 and the MAHD approach was performed as describe above.
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36 **Gas chromatography and gas chromatography–mass spectrometry**

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39 Quantitative and qualitative evaluations of the oils were performed by means of GC and GC-
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41 MS instruments. The GC analyses were carried out on a Varian (CP 3800) gas chromatograph
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43 equipped with a split/splitless (10:1) injector (280 °C) and a flame ionization detector (250 °C).
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45 N₂ was used as the carrier gas (0.8 mL/min). The capillary column used was CP-Sil 5 CB (30 m
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47 × 0.25 mm × 0.25 μm film thickness). The oven temperature was held at 60 °C for 3 min, heated
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49 to 220 °C at a rate of 6 °C min⁻¹, and finally held for 3 min. Quantitative data were obtained
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51 through system area percentage.
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3 GC-MS determinations were performed on an HP-6890 GC system coupled with a 5973
4 network mass selective detector, and equipped with an HP-5MS capillary fused silica column (30
5 m × 0.25 mm I.D. × 0.32 μm film thickness). The operating conditions were the same as
6 described above but the carrier gas was He with a flow rate of 0.8 mL/min. Mass spectra were
7 taken at 70 eV. Mass spectra were recorded over the m/z range 20-500 amu. All
8 chromatographic measurements were carried out in triplicate, and the mean of the retention times
9 and percentage compositions of each component were taken into consideration. Duplicate times
10 were discarded if they differed by more than 1 s, and the experiments were repeated again in
11 duplicate.
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27 **Results and Discussion**

28 **Effect of microwave power on extraction yield of the *Pistacia vera* L essential oil**

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31 The impacts of microwave powers were investigated on the extraction yield. Different
32 microwave powers were examined in primary experiments. The times of all experiments were
33 fixed at 120 minutes. At low powers, the extraction yield is too low and the applied energy does
34 not produce adequate oil. On the other hand, the yield of extraction is low at maximum powers
35 most probably due to rapid boiling or decomposing of essential oils. Thus, routine powers of 400
36 W were selected for further studies and the related results are given in Table 1.
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48 **Effect of time on extraction yield of the *Pistacia vera* L essential oil by MAHD**

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50 In another part of this study, the impacts of different extraction times were investigated on
51 yield of the MAHD extraction method. Microwave powers of all experiments were regulated
52 under the optimized power of 400 W. Different times were examined in primary experiments.
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3 The obtained results are given in Table 2. Based upon these results, the optimal time of 120 min,
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5 was selected for further studies.
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10 **Effect of amount of AFMN on extraction yield of the *Pistacia vera* L essential oil by MAHD**

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12 The influences of different amounts of AFMN were investigated on the extraction yield
13 through the MAHD procedure. Microwave powers of all experiments were regulated at 400 W.
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15 Then, different amounts of AFMN (0.05 g, 0.1 g, 0.15 g, 0.2 g, 0.4 g and 0.6 g) were examined
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17 in the preliminary experiments. Based on the obtained results, the extraction yield considerably
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19 increased in the presence of AFMN .However, when using more than 0.2 g of AFMN, no
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21 significant increase in the yield of extraction was noted. Thus, 0.2 g was chosen as the optimal
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23 amount of AFMN in the complementary attempts.
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29 To determine the precision of magnetic nanoparticle-assisted microwave distillation, the
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31 experiment was repeated three times and the respective RSD was calculated. The obtained result
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33 of RSD was about 1.5 which demonstrates good repeatability of the method.
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39 **Identification and quantification of essential oil compounds**

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41 In this work, determination and characterization of the constituents of the oil were made
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43 through:
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46 i) Comparing their mass spectral fragmentation pattern regarding authentic samples and
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48 retention indices (RI) relative to C₉-C₂₁ *n*-alkanes with those given in the literature and some
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50 previously published papers^{22, 50-52}
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53 ii) The data stored in a MS library (Wiley 275)
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iii) Matching the fragmentation pattern with those in National Institute of Standards and Technology Mass Spectra Library (NISTMSL) package with a resemblance percentage above 85%. Relative percentage amounts of the components were directly calculated from peak area using an HP-6890 GC system on the HP-5MS column considering the sum of all eluted peaks as a hundred percent without the use of the correction factor. Identical volumes of each essence were separately injected to GC-MS and the respective results are shown in Table 3, where the constituents are sorted in the order of their elution from an HP-5MS column.

By using the HD method, both in presence and absence of magnetic nanoparticles no essence appeared in the Clevenger system. Figures 2 and 3 show the GC-MS chromatograms of the essential oils of *P. vera* L hull extracted by MAHD method, the former without of magnetic nanoparticles and the latter by the aid of nanoparticles, respectively.

Using the routine MAHD method (Table 1), 15 components could be identified in the oil from the hull of *P. vera* totally accounting for 97.3% of the oil profile: Six monoterpene hydrocarbons (72.1%), three oxygenated monoterpenes (9.4%), two sesquiterpene hydrocarbon (5.6%) and four non-terpene hydrocabons (10.2%) while one component still remained unidentified. Specifically, the major components in the volatile oil of the hulls of *P. vera* were found to be α -pinene (31.8%), α -terpinolene (20.3%), myrcene (12.2%), pinocarvone (6.2%) and camphene (4.7%) (see Figure 2), while (E)-caryophyllene (4.1%), pulegone (2.1%), eucalyptol (1.7%), limonene (1.4%), α -farnesene (1.5%) and carvacrol (1.1%) occurred in lower contents.

Using AFMN-assisted MAHD method, we were able to identify 25 volatile compounds constituting 99.5% of the total oil (Figure 3) composition including α -pinene (31.5%), α -terpinolene (20.2%), myrcene (11.5%), pinocarvone (6.2%) and camphene (4.8%). The other identified compounds were found to be (E)-caryophyllene (3.7%), pulegone (2.2%), eucalyptol

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3 (1.8%), limonene (1.4%), carvacrol (1.1%), germacrene (1.1%), terpinen-4-ol (0.8%), p-cymene
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5 (0.8%), δ -3-carene (0.7%), sabinene (0.5), α -farnesene (0.5%), β -elemene (0.4%), santolina
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7 triene (0.4%), decanal (0.4%), allo-ocimene (0.4%), and allo-aromadendrene (0.3%) as minor
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9 constituents. In terms of general categories, eleven were monoterpene hydrocarbons (74%), four,
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11 oxygenated monoterpenes (10.3%), five, sesquiterpene hydrocarbons (6.0%) and five, non-
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13 terpene hydrocarbons (9.2%).
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16 17 18 **Chemical composition of *Pistacia vera* L. in similar reports**

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20 There have been several reports on the constituents of essential oils from *P. vera* in different
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22 parts of world especially in Iran and Turkey.
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25 Alma et al.¹¹ have reported, based on GC and GC-MS analyses, that the main components of
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27 water-distilled volatile oils from the gum of *P. vera* grown in Turkey in a profile consisting of :
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29 monoterpenes (89.7%), oxygenated monoterpenes (8.1%) and diterpenes (1.2%) were α -pinene
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31 (75.6%), β -pinene (9.5%), trans-verbenol (3.0%), camphene (1.4%), trans-pinocarveol (1.20%),
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33 and limonene (1.0%). In the work of Chahed et al.⁵³ focused on characterization of main lipid
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35 class contents (polar lipids, diacylglycerols, triacylglycerols and free fatty acids), fatty acid and
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37 methyl esters (FAMES) contents in ripe seeds of *P. vera* from four localities of Tunisia and
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39 subsequent analysis by gas chromatography (GC), oleic acid was the major fatty acid in all
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41 samples. In a complementary attempt of this research team, monoterpene hydrocarbons, mainly
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43 represented by α -pinene (15.0-47.4%) and terpinolene (32.2-51.1%) as prominent constituents
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45 during all fruit development, totally representing 90.3% of the oil at full ripeness.³
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51 A similar report is found in the literature in which the limonene is the most abundant
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53 compound in the essential oil followed by α -terpinolene.⁵⁴
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3 In a related study Duru et al.,¹² reported that the water-distilled essential oil of Turkish *P.*
4 *vera* was consisted of mainly of monoterpenes, specifically α -pinene (29.2%), limonene (12.4%),
5 α -terpineol (9.6%) and camphene (8.3%).
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10 Kalalinia et al.⁹ have isolated the essential oil from the resins of *P. vera* and have reported α -
11 pinene, β -pinene, and α -thujene as the most dominant components in a profile involving twenty
12 compounds and accounting for 99.5 % of the total identified components. Similarly, Ramezani et
13 al. have pointed out the dominancy of α -pinene, β -pinene, and α -thujene in the hydrodistilled oil
14 from the gum of *P. vera*.⁵⁵
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22 Ozel et al.⁵⁶ have also analyzed the hulls of *P. vera* fruits by the aid of direct thermal
23 desorption method. Accordingly, the volatile oil was resolved using comprehensive gas
24 chromatography (GC \times GC) coupled with time-of-flight mass spectrometry (TOF-MS). This study
25 revealed the presence of α -pinene and (Z)- α -terpineol as the major components at all
26 temperatures. Finally, another similar report on water-distilled essential oil from fruits and the
27 leaves of *P. vera* growing in Greece revealed the presence of 21 compounds in the oil,
28 predominately including (+)- α -pinene (54.6 %) and terpinolene (31.2 %) in the fruit oil and 33
29 constituents in the leaves oil containing α -pinene (30.0 %), terpinolene (17.6 %) and bornyl
30 acetate (11.3 %) being the most frequent ones.⁵⁷ In short, monoterpene hydrocarbons contribute
31 as the major fractions in the chemical profiles of the essential oils separated from different parts
32 of *P. vera*.
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48 **Conclusions**

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50 Since microwave irradiation was used in the recovery of the important components from plant
51 materials in 1986, there have been numerous reports on microwave assisted extraction of plant
52 secondary metabolites. The growing rate of published reports in the literature, suggested that
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3 microwave assisted extraction has some considerable merits such as shorter extraction time,
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5 higher extraction yield and less solvent consumption compared to conventional extraction
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7 methods.^{23, 38, 39, 41, 42}
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11 Microwave assisted extraction was currently regarded as a robust alternative technique to
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13 traditional extraction ones, especially in the case of the sample preparation for analytical
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15 purpose. Practically speaking, combining of microwave-assisted extraction with other extraction
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17 techniques may overcome the limitations of the single extraction technique and obtain the
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19 satisfactory extraction efficiency. In these hyphenations, unlike the conductive heating,
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21 microwave heats the whole plant samples-solvent mixture simultaneously and rapidly,
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23 accelerating the extraction process of the target molecules from plant matrix.⁵⁸ More
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25 specifically, the use of magnetic nanoparticles, as a microwave absorption solid medium, in the
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27 extraction of the essential oils by applying the microwave beams is a new idea possessing
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29 promising outputs.⁵⁹ Compared with conventional methods, microwave assisted
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31 hydrodistillation accelerated by magnetic nanoparticles is much more efficient and may result in
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33 an external temperature which is considerably lower than the traditional techniques. It is also a
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35 flexible method since it has some adjustable parameters like microwave power and time which
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37 can be optimized in order to approach the best experimental conditions. In the present study, the
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39 optimal conditions were a microwave power of 400 W along with 120 min. for sample
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41 irradiation step. Additionally, in the main part of our study, microwave-assisted extraction was
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43 successfully developed for the separation of essential oils from the hull of *P. vera* in presence
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45 and without synthesized magnetic nanoparticles. Accordingly, we found out that addition of
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47 magnetic nanoparticles (AFMN) can greatly improve the extraction efficiency of the volatile oil
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49 from the hulls of *P. vera*. This approach gave rise to an increase in the total number of extracted
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3 compounds. It seems rationale that the synthesized AFMN can absorb the microwave radiation
4 properly and can enhance the extraction rate and yield of the isolated essential oils more
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6 efficiently. In view of its obvious merits, great effort should put into optimizing microwave
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8 assisted extraction in presence of magnetic nanoparticles, identifying its mechanisms, and more
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10 specifically scaling up excellent laboratory results for industrial utilization. Some
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12 complementary attempts are also undertaken by our research group to improve the capability of
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14 this strategy and to facilitate its use.
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21
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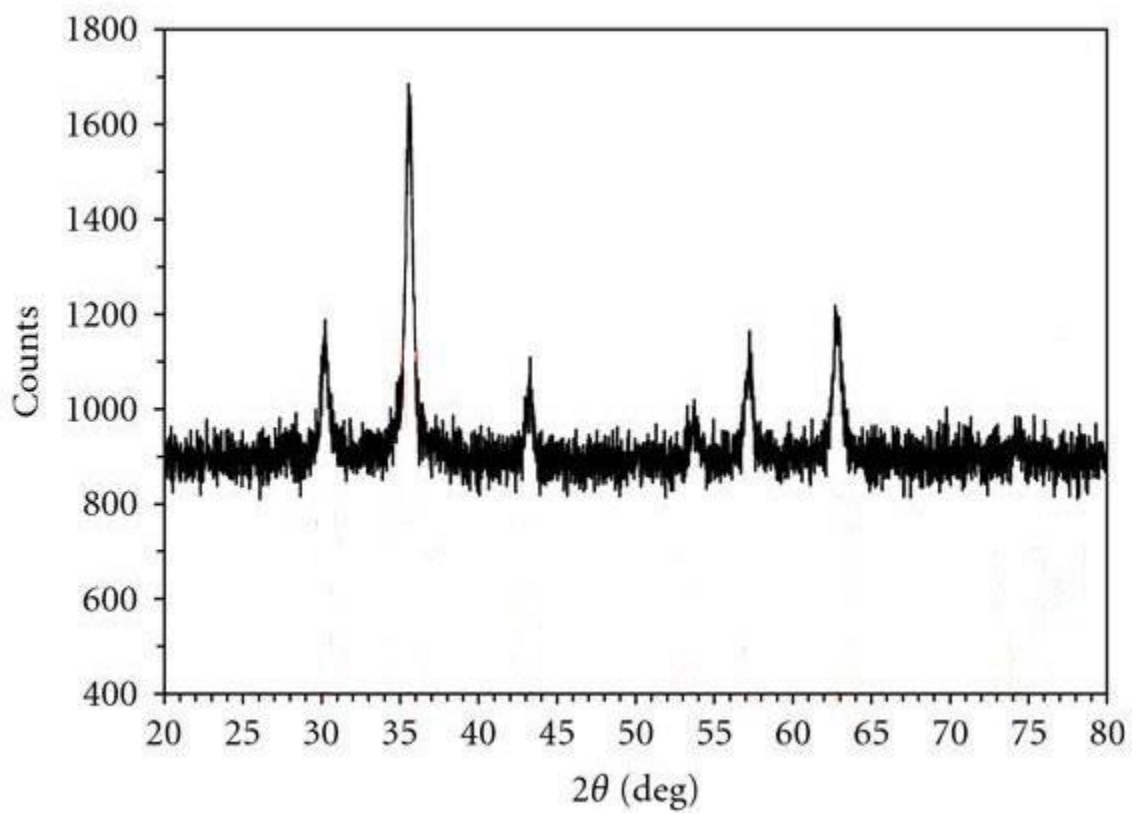


Fig.1 The XRD pattern of amine-functionalized magnetite nanoparticles

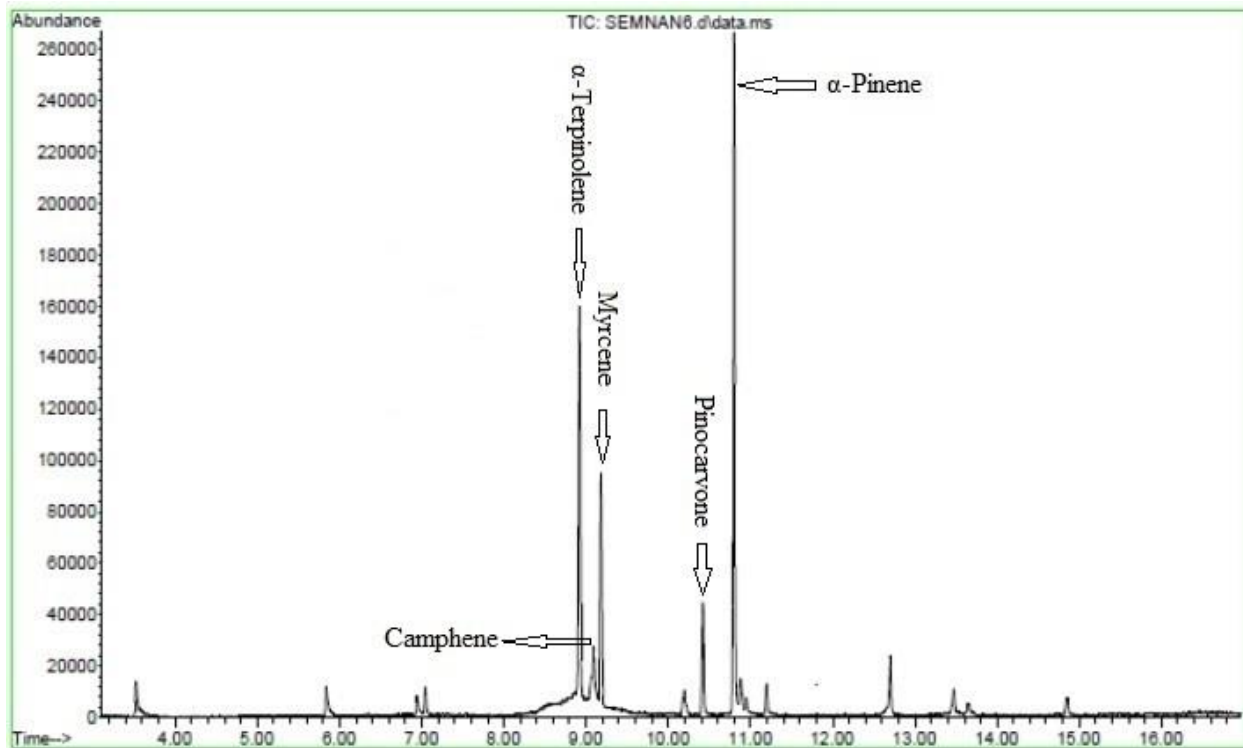


Fig. 2 The GC-MS chromatogram of essential oils from hulls of *Pistachio vera L* extracted using MAHD method, without magnetic nanoparticles.

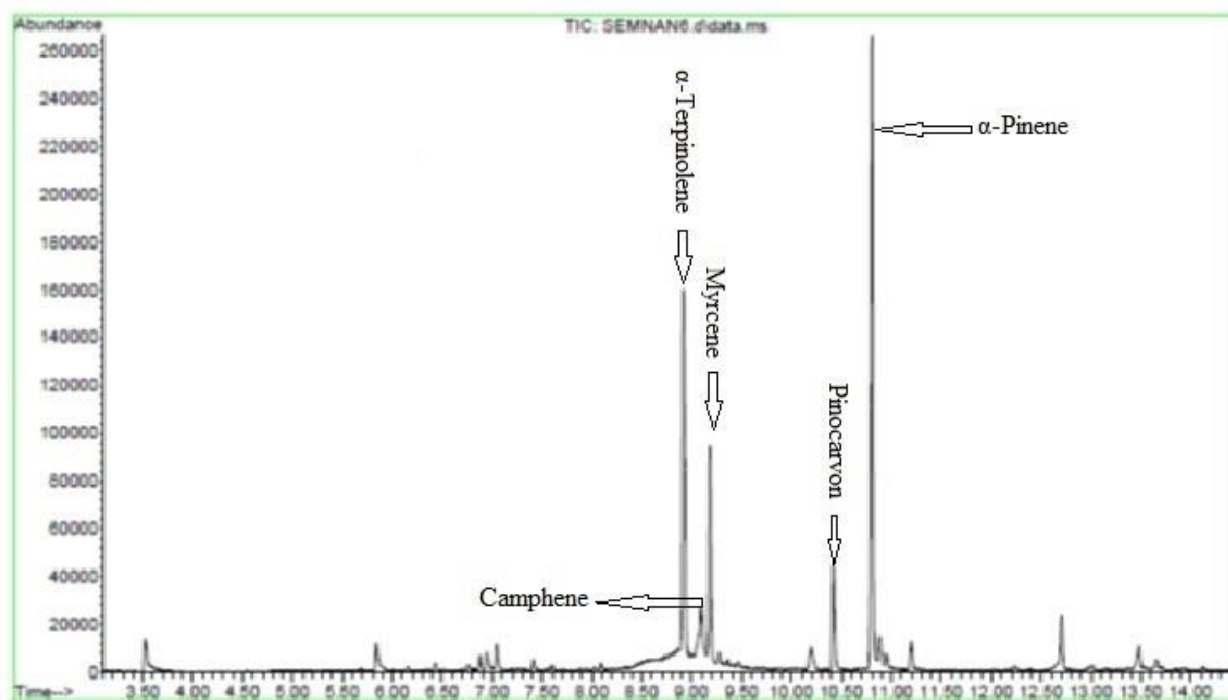


Fig. 3 The GC-MS chromatogram of essential oils from hulls of *Pistachio vera* L extracted by MAHD method, in presence of magnetic nanoparticles.

Table 1 Effect of microwave power on the yield of essential oil of *Pistacia vera* L.^a

Power (W)	200	300	400	500	600
Yield	%0.19	%0.34	%0.62	%0.52	%0.48

^a The time dedicated to isolate the essential oils was 120 min. in all experiments.

Table 2 Effect of sequential times on extraction yield of essential oil of *Pistacia vera* L usingMAHD technique ^a

Time (min.)	100	120	135	165	180
Yield	0.47	0.62	0.60	0.55	0.53

^a The power applied to separate the essential oils was 400 W in all experiments.

Table 3 The chemical composition of essential oils from by the MAHD technique in presence and without magnetic nanoparticles along with their percentages ^a

NO	Constituents	Class	R _t ^g	RI ^h	Magnetic nanoparticles	
					Absence ⁱ	Presence
1	Octane	N.H. ^b	3.52	800	3.0	2.1
2	Santolina triene	M.H. ^c	7.60	906	-	0.4
3	α -Pinene	M.H.	10.80	932	31.8	31.5
4	Camphene	M.H.	9.09	946	4.7	4.8
5	Trans-2-Heptenal	N.H.	5.83	947	2.5	2.1
6	Sabinene	M.H.	6.42	969	-	0.5
7	Myrcene	M.H.	9.18	988	12.2	11.5
8	δ -3-carene	M.H.	7.41	1008	-	0.7
9	p-Cymene	M.H.	6.88	1020	-	0.8
10	Limonene	M.H.	6.94	1024	1.4	1.4
11	Eucalyptol	M.H.	7.04	1033	1.7	1.8
12	α -Terpinolene	M.H.	8.91	1086	20.3	20.2
13	Allo-Ocimene	M.H.	9.46	1128	-	0.4
14	Pinocarvone	O.M. ^d	10.41	1160	6.2	6.2
15	Terpinen-4-ol	O.M.	9.27	1174	-	0.8
16	Decanal	N.H.	8.08	1201	-	0.4
17	(2E)-Decenal	N.H.	11.95	1260	1.9	1.8
18	Pulegone	O.M.	10.19	1233	2.1	2.2
19	Carvacrol	O.M.	10.94	1298	1.1	1.1
20	(2E,4E)-2,4-Decadienal	N.H.	10.88	1315	2.8	2.8
21	β -elemene	S.H. ^e	12.23	1389	-	0.4
22	(E)-Caryophyllene	S.H.	12.70	1417	4.1	3.7
23	Allo-Aromadendrene	S.H.	13.02	1458	-	0.3
24	Germacrene-D	S.H.	13.49	1484	-	1.1
25	α -Farnesene	S.H.	13.65	1505	1.5	0.5
26	N.I. ^f		14.88	-	1.1	-
	Number of total components				16	25
	Total %				98.4	99.5
	Yield (%)				0.6	0.8

^a The compounds have been sorted according to their Kovats retention indices on an HP-5MS capillary column^b N.H.: Non-terpene hydrocarbons^c M.H.: Monoterpene hydrocarbons^d O.M.: Oxygenated monoterpenes^e S.H.: Sesquiterpene hydrocarbons^f N.I.: Not identified^g R_t: Retention time (min.)^h RI (Lit): Retention indices given in the literatureⁱ Optimized microwave-assisted hydrodistillation conditions are: 400 W and 120 min for power and time of extraction, respectively.

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