Original article

Authentication and quality control of some polyherbal oils used in Persian Traditional Medicine (PTM)

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Abstract

Background and objectives: Traditional polyherbal oils are still in use in Persian Traditional Medicine (PTM). Most of these formulations are prepared via traditional procedures such as maceration of herbs in oils or evaporating aqueous herbal extracts in boiling or heating oils as the vehicle. Thus, their quality control, standardization and authentication are real challenges due to the lack of scientific studies. The present study provided data and methods to authenticate some of these oils and has compared applicability of different fingerprinting methods for their authenticity. **Methods**: Thirteen oils were prepared according to the traditional manuscripts. High performance thin layer chromatography (HPTLC), ultraviolet (UV) and infrared (IR) fingerprinting data were analyzed using MATLAB software. For HPTLC fingerprints a special coding system was designed according to the R_f values. The fingerprinting data were subjected to principal components (PCs) analysis. Melting point and thermal behavior of the oils were obtained by differential scanning calorimetry (DSC). Also, the refractive indices, acid and peroxide values were obtained for the oils. **Results**: The designed coding system for HPTLC was successfully able to produce a discriminative unique fingerprint for each sample. Among UV, IR and HPTLC fingerprinting, the last one seemed more reliable than others to authenticate the oils. The acid values (0.22-3.85), peroxide values (2.31-34.35 meq/kg) and refractive indices (1.4622 - 1.4706) were in acceptable ranges for most of these oils. **Conclusion**: Despite lack of knowledge about constituents of traditional polyherbal oils, this study was able to provide some data and fingerprinting methods for their authentication.

Keywords: fingerprint, HPTLC, MATLAB software, standardization, traditional polyherbal oil

Introduction

Traditional medicinal polyherbal oils are often mentioned as the most applicable dosage forms in clinical practice during the medieval era, as well as today's folk medicine [1]. This type of herbal

preparation was mostly applied for topical administration and included different preparation methods. From ancient times, many medical traditional cultures throughout the world have considered herbal oil products for clinical approaches [2].

It is remarkable that in current knowledge, herbal oils are mostly divided into fixed and volatile derivatives and are less likely involving other oil preparations. But with reference to Persian folk medicine, Ayurveda and Persian Traditional Medicine (PTM) literatures, these dosage forms have been introduced as oils obtained from oil bearing plant or animal parts and also, polyherbal oils which have been prepared by fixed or volatile oils as vehicles of non-oily herbal materials or as solvents to extract phytoconstituents of other plants [3]. Direct compression of oily parts, as well as distillation of aromatic plants to obtain the volatile oil could be represented as preparation methods for oil bearing herbal parts. On the other hand, plants components having no oil composition were traditionally macerated into some common oils such as almond, sesame or olive oil and were heated to a certain temperature for a period of time. Moreover, boiling and evaporating of aqueous extracts containing plant compositions in oils was also reported as an old method of oil preparation [4,5].

Preparation of polyherbal oils in line with traditional procedures is still in use in Iran. Many of these preparations are easily produced and dispense by traditional healers and local practitioners [6]. For such occasions, quality of formulation, validity of content and standardization, misidentification and probable adulterations may come noticeable [7]. Regarding the ease of preparation and application of medicinal oils, as well as large requests for herbal preparations especially by elders in Iran [8], concerns about safety, quality control, authenticity and real efficacy of such polyherbal oil preparations are considerable.

A review on herbal medicinal oils used in pharmaceutical manuscripts of Persian Traditional Medicine has already been released to clarify the framework related to this subject [1]. Although many considerable points regarding traditional herbal oils were gained in comparison to the contemporary knowledge, pharmacognostical and phytochemical characteristics of these preparations are still remained unexamined.

In this regard, the present work was carried out to assess and introduce first steps of experimental evaluation toward standard regulation for traditional herbal oils which are currently applied in PTM. This study gives different data and methods to authenticate some of these polyherbal oils and compares applicability of different fingerprinting methods for their authenticity.

Experimental
Oil preparation
Among most common PTM herbal oils which are currently used in local herbal markets and also PTM manuscripts, thirteen different oils were selected and prepared according to the instruction of traditional manuscripts. Preparation method for those selected oils were derived and employed according to the instructions in *Qarabadin-e-Salehi*, a well-known Persian pharmacopeia encompassed more than 200 kinds of dosage forms in alphabetic order [4]. The selection also, regarded the type of oil preparation with the intention to encompass main the traditional preparation methods. Since the extraction of essential oil and concerned analytical procedure is well practiced in current knowledge, oils with this type of preparation were excluded. Table 1 has represented the applied herbs to obtain the respective oils along with preparation methods. According to this table, samples 3-5, 8, 10 and 12-13 were prepared via macerating the related botanical parts in heated vehicle (1:10) for a certain time mentioned in the text. On the other hand, samples, 6, 7 and 11 were prepared via boiling and evaporation method in Sesame oil. In this procedure, related parts are decocted in water (1:10). The yielded extract is subsequently boiled in an oily vehicle to evaporate the water portion (1:1).

Table 1. Traditional oils for experimental study

No	Species used for preparing the oils	Plant family	Part used	Preparation method
1	*Anethum graveolens* L. (dill)	Apiaceae	Leaf	Boiling and evaporation in sesame oil oil
2	*Boswellia* spp. (frankincense)	Burceraceae	Oleo gum resin	Boiling water bath (sesame oil)
3	*Citrus medica* L. (citron)	Rutaceae	Peel	Maceration in heated sesame oil
4	*Cucurbita pepo* L. (pumkin)	Cucurbitaceae	Fruit	Maceration in heated sesame oil
5	*Lippia citriodora* (Lam.) Kunth (lemon verbena)	Verbenaceae	Leaf	Maceration in heated olive oil
6	*Myrtus communis* L. (myrtle)	Myrtaceae	Leaf	Maceration in heated sesame oil
7	*Ocimum basilicum* L. (basil)	Lamiaceae	Leaf	Maceration in heated olive oil
8	*Olea europea* L. (olive)	Oleaceae	Fruit	Direct compression
9	*Peganum harmala* L. (harmel)	Nitrariaceae	Seed	Boiling and evaporation in olive oil
10	*Petroselinum crispum* Mill. (parsley)	Apiaceae	Leaf	Maceration in heated sesame oil
11	*Sesamum indicum* L. (sesame)	Pedaliaceae	Seed	Direct compression
12	*Vitis vinifera* L. (grape vine)	Vitaceae	Fruit	Boiling and evaporation in olive oil
13	*Zataria multiflora* Boiss. (Shirazi thyme)	Lamiaceae	Aerial	Maceration in heated olive oil

To prepare the Frankincense oil, *Boswellia* gum was dispensed in Sesame oil (1:10) in a glass container. The container was then submerged in boiling water until the gum was fully dissolved in the oil [4].

Among these oils, olive and sesame oils with a certain known commercial brand were purchased from a local market. Maceration in heated oil, as well as boiling and evaporation of the aqueous extract in oil were the main methods for preparation [4,5,9]. As an exception, oil from Frankincense oleo gum resin was prepared by immersing a piece of oleo gum resin into a beaker placed in a boiling water bath containing sesame oil. While the sesame oil was warmed up in the water bath, the oleo gum resin was dissolved in the oil [4].

High performance thin layer chromatography (HPTLC) fingerprinting

HPTLC was carried out using a Camag TLC system fitted with WinCats (version 1.2.3) software. To screen the primary and secondary metabolites, the selected polyherbal oils were diluted in dichloromethane (1: 4) and 2 μL of oils were applied on a silica gel plate 60F254 (10×20 cm, Merck, Germany). The TLCs of polyherbal oils were developed in a mobile phase containing chloroform: acetone: water (98: 1.99: 0.01). Chromatographic bands were visualized first with ultraviolet lamps at 254 and 365 nm and then

with different reagents including phosphomolybdic acid (vis.), dragendorff, 5% potassium hydroxide (vis. & UV_{365} nm), orcinol, natural product (ethanolamine diphenylborate)/PEG 400 in methanol ,UV_{365} nm), Liebermanne Burchard (UV_{365} nm or vis.), 3% $FeCl_3$ (vis.), vanillin-sulfuric acid and anisaldehyde-sulfuric acid [10,11]. All chemicals and solvents were prepared as analytical grade from Merck (Germany) or Sigma Aldrich (USA). In order to analyze the polyherbal oils based on their HPTLC fingerprints, the R_f values of the bands in the selected HPTLC strip was calculated. A special coding system was designed in such a way to produce a discriminative unique fingerprint for each sample. Briefly, the HPTLC strips were divided into 100 R_f zones from 1 to 100. The first R_f zone was related to the bands with the R_f range of 0-0.01, the second was related to R_f range 0.01-0.02 and so on. A 100 digit bit string was thereafter generated for each HPTLC string based on the presence or absence of bands within the specified R_f zones. The m-file for conversion of HPTLC raw data into the encoded bit strings was implemented in MATLAB software. The resulted data were then subjected to principal component analysis and 10 PCs were extracted for each sample.

FT- infrared spectroscopy

In this study, IR spectroscopy was used as a

finger print tool for the polyherbal oils. The samples were subjected to a Vertex 70 FT-IR spectrometer (Ettlingen, Germany) applying attenuated transmittance reflectance (ATR) method. The IR spectra were taken for the samples with and without a blank. Since components of the vehicle (sesame or olive oil) were present in all yielded oil samples, it was important to reduce their effect as a noise in further analysis. Accordingly, samples acquisition was carried out after baseline correction with the vehicle oil. In other word, the vehicle oil was used as blank and its baseline was corrected for data acquisition. The transmittance values were taken in 600-3400 cm^{-1}.

Ultraviolet absorbance profiling
Using a spectrophotometer, PG Instruments - T80, the UV spectra of prepared polyherbal oils were obtained. For this process, both sesame and olive oils were diluted to one-fourth with dichloromethane and were used as blank. Subsequently the spectrum of each oil samples was recorded against the respective blank. The absorbance data of samples were plotted as excel files and the graphs were designed using GraphPad Prism (Version 6.01, San Diego, USA) software.

Statistical comparative analysis of fingerprints
MATLAB software was used to analyze the HPTLC, IR and UV spectra data statistically. The preprocessing procedure was done by means of standard normal variety (SNV) to suppress the baseline fluctuations. Principal component analysis (PCA) was used as an unsupervised clustering analysis technique to cluster the polyherbal oils based on their HPTLC, IR and UV fingerprint data. The principal components (PCs) were extracted from the obtained matrix of data using singular value decomposition algorithm as implemented in MATLAB software.

Analysis of thermal behavior
Thermal behavior of polyherbal oils was evaluated by differential scanning calorimetry (DSC) on Bahr DSC 310 calorimeter (Germany).

The calibration was done by Indium. Temprature program consisted of two segments, a cooling segment from 25 to -20 °C at the rate of 5 °C/min followed by a heating segment from -20 °C to 25 °C at the same rate. An empty DSC pan was also used as the reference.

Assessment of refractive index
Refractive index of oils was determined at 40 °C using an automatic ATAGO Rx7000-α digital refractometer (ATAGO, Japan).

Acid and peroxide values determination
Standard procedures of the American Oil Chemist Society were employed for indices values (AOAC, 1997), acid value (national standard No. 4178) and peroxide value (Rancimat according to national standard No. 7513).

Results and Discussion
Others were prepared via evaporation of aqueous herbal extracts in boiling vehicle oil or dispersion of herbal constituents in a heating vehicle oil on a water bath. The preparation procedure of each polyherbal oil sample was carried out in accordance with pharmaceutical manuscripts of Persian traditional medicine [12]. Some of these oils have different colors in day light (figure 1), as well as different taste and odors but organoleptic tests are not completely reliable to authenticate these oils. Thus, all samples were subsequently undergone to simple and suitable evaluation and fingerprinting methods.

Unlike standardization processes for orthodox drugs which deal with quantifying a single active ingredient, the paradigm of standardization of traditional Persian herbal formulations (which are mostly multi-compound and multi-ingredients preparations) is a real challenge for food and drug administration agencies. On the other hand, selection of an individual ingredient for determining either efficacy or quality of these formularies is in contrary to PTM principles.

In pharmaceutical concepts of PTM, no single active component was responsible for the observed efficacy, but synergistic or agonistic activities of all ingredients of the formulation

with each other, created a unique healing property for each of these formulations [5,13].
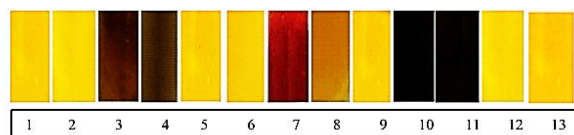

Figure 1. Colors of polyherbal oils in day light (photographed in a same condition with a 10 megapixel camera in a chamber with white background). From left to the right; 1: sesame oil; 2: olive oil; 3: *Ocimum basilicum* L. oil; 4: *Lippia citriodora* (Lam.) Kunth; 5: *Myrtus communis* L. oil; 6: *Vitis vinifera* L. oil; 7: *Peganum harmala* L. oil; 8: *Citrus medica* L. oil; 9: *Boswellia* spp. Oil; 10: *Petroselinum crispum* Mill. oil; 11: *Anethum graveolens* L. oil; 12: *Cucurbita pepo* L. oil; 13: *Zataria multiflora* Boiss. oil

HPTLC fingerprint can provide a pattern of almost all ingredients in the drugs and can be used to determine absence or presence of desired markers or active components. This method can also evaluate the ratios quantifiably, and ensures the consistency and stability of these kinds of herbal formulation [14].

Figure 2 has represented the anisaldehyde treated HPTLC fingerprint profiles of the prepared polyherbal oil samples compared to the vehicles (olive and sesame oil). Selected samples showed narrow differences between oil samples and related vehicles. These differences implied that various amounts of active components may be extracted into the sesame or olive oils as vehicles. In combination of pictured HPTLC profile, the densitometric ratio of different ingredients of polyherbal oils can be used to determine their identity and quality.

An HPTLC fingerprinting procedure was described by Sukumar *et al*. for quantification of lignans as markers in Sesame oil and 3 of its polyherbal formulations but they did not discussed other herbal ingredients rather than sesame oil [15]. Nidhi *et al*. used HPTLC densitometric method to quantify psoralen and plumbagin from their polyherbal oil formulations [16].

In both studies, they used methanol in conjunction with some extraction procedure to extract some of the ingredients from polyherbal oil formulations. But in our study, the whole diluted polyherbal oil formulations was applied directly on the HPTLC plates and it was possible to have an assumption on quality and ingredients of the vehicle as well as added herbs. The aim of this study was not quantifying any of the ingredients but finding a rapid authentication procedure for these polyherbal oils. On the other hand, as can be seen in figure 2 there is no tailing for any of the ingredients spot and it is assumed that the HPTLC procedure discussed in this study in conjunction of applying marker standards may be used successfully to quantify the ingredients of these oils. We used different reagent to visualize the HPTLC plates and the highest number of spots was observed on Liebermann–Burchard treated plates under UV$_{366}$ lamp.
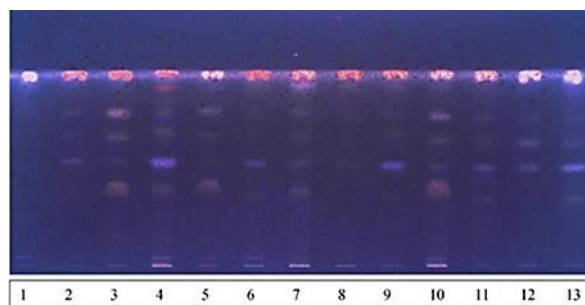

Figure 2. HPTLC Fingerprint profile of studied oil samples (treated with anazeldehyde and seen at 366 nm). From left to the right; 1: Sesame oil; 2: Olive oil; 3: *Ocimum basilicum* L. oil; 4: *Lippia citriodora* (Lam.) Kunth; 5: *Myrtus communis* L. oil; 6: *Vitis vinifera* L. oil; 7: *Peganum harmala* L. oil; 8: *Citrus medica* L. oil; 9: *Boswellia* spp. Oil; 10: *Petroselinum crispum* Mill. oil; 11: *Anethum graveolens* L. oil; 12: *Cucurbita pepo* L. oil; 13: *Zataria multiflora* Boiss. oil

As seen in figures 3 and 4, almost all samples showed some weak absorbance in the UV-C (200±280 nm). A Single sharp pick at 313.5 (possibly n→π* transitions of COOR) in all sesame and olive oil based samples were observed which was in UV-B (280-320 nm) range. The mentioned peak for harmel oil was shifted to the lower wavelength (305 nm).Oil samples showed an acceptable shielding power in
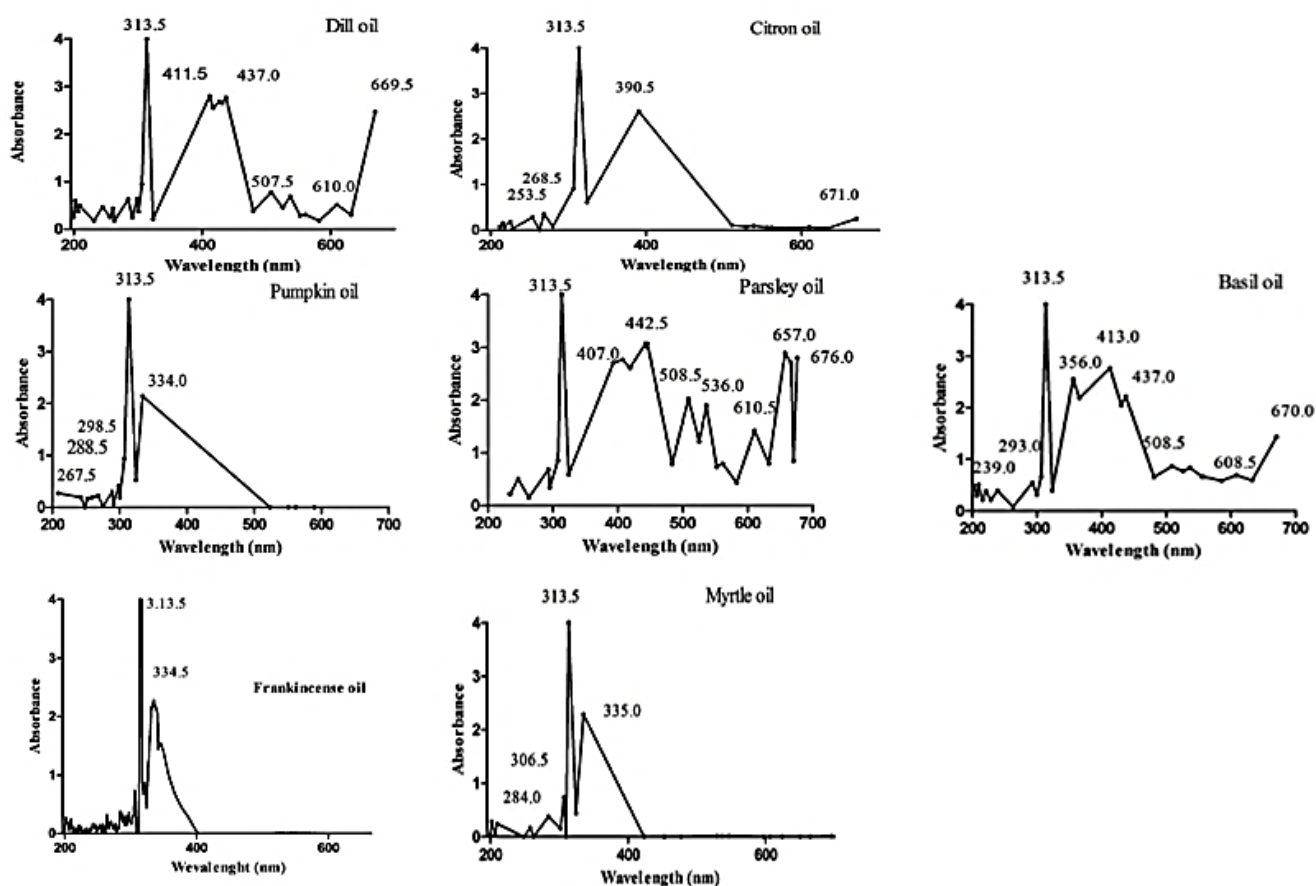
Figure 3. The UV absorbance spectra of sesame based oil samples

the UV-A (320-400 nm) range presented by high absorbance ability.

High absorbance level at 428-452 (blue absorption maximum), 630 and 670 nm (near red absorption maximum) were produced by high concentration of green pigments [17-19], especially chlorophyll a and chlorophyll b contents in dill, parsley, basil and lemon verbena polyherbal oils.

The carotenoids showed broad absorption with three maxima or shoulders in the blue spectral range between 400 and 500 nm. These data were in consistency with the oils colors. Since these polyherbal oils were complicated mixtures of several active constituents, it was difficult to relate each absorbance peak to a particular electronic transition or constituents but as seen in figures 3 and 4, the total pattern of UV–VIS spectra of the oils were different with each other and could be used for finger printing of these polyherbal oils. UV–VIS spectrophotometers, have been employed previously in the so-called "without identification", "blind" or "fingerprinting" procedures. These techniques which provide complex signals and nonspecific information usually cannot be used to verify the existence or absence of particular chemical compounds, or measure individual chemical or physical properties, but they can be useful to obtain comprehensive, multivariate description of these polyherbal formulations [20].Lichtenthaler *et al*. used UV-VIS spectroscopy to quantify chlorophylls and carotenoids in total plant extracts.
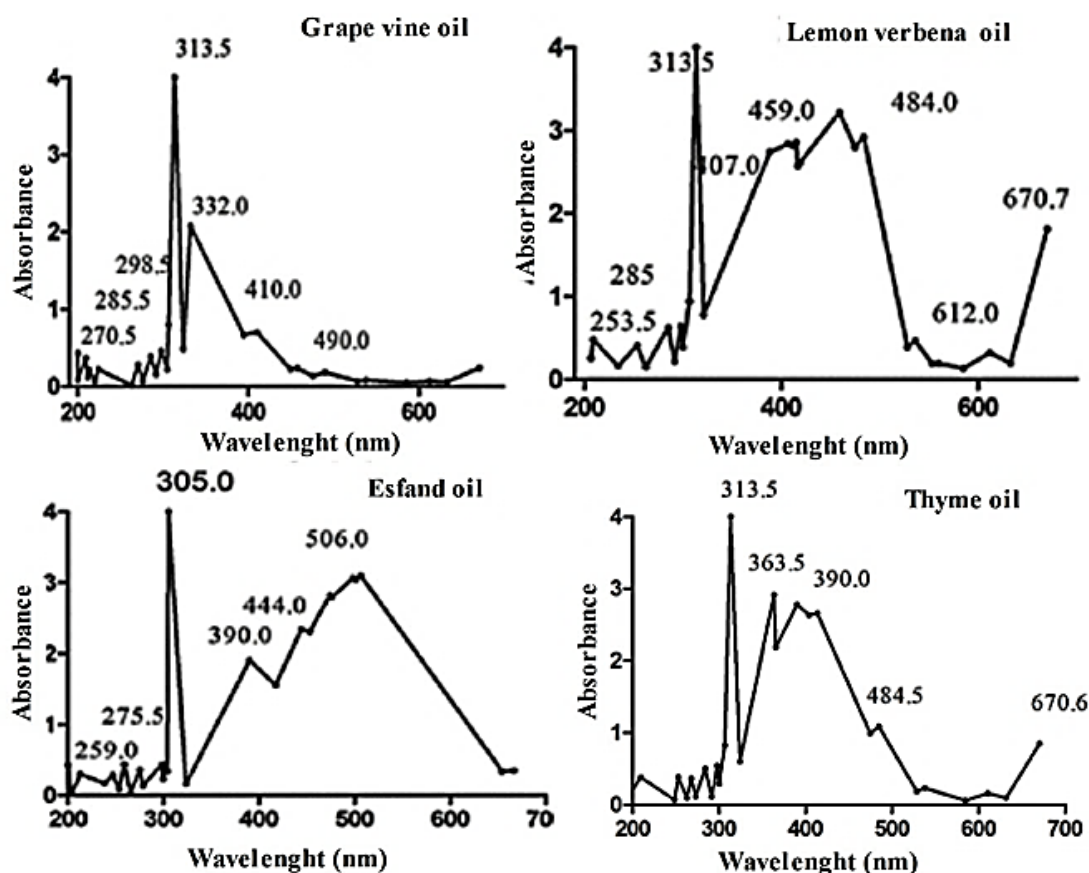
Figure 4. The UV absorbance spectra of olive oil based samples

They also, mentioned that the maximum absorbance of these pigments strongly depended on the type of solvent [21]. Thus for fingerprinting the oil, we have not extracted the pigments and chromophores from the polyherbal oils by any solvent. The maximum absorbance peaks at 400-500 nm and 630-670 nm were still sharp enough in diluted samples with dichloromethane (1:6), and the absorbance were ≤ 1 (data not shown). It could be suggested that the UV–VIS spectrums of diluted polyherbal oils may be used for quantification analysis but, the method needs to be validated.

The IR spectra of the polyherbal oils which were obtained without blank (vehicle) corrections were somehow identical to each other due to the low concentrations of the herbal components compared to the fatty acids and other components of their vehicles, sesame oil olive oil (data not shown). But, for those spectra which were obtained by vehicles oil as blank, the spectra were more distinguishable and the spectra could be used as fingerprinting tools although the major peaks of all spectra were almost in the same regions (figure 5). The major peaks in all spectra were at 750, 1160, 1275, 1746, 2855 and 2930 cm^{-1}. The sharp peak at 750 cm^{-1} represented bending of sp^3 hybridized $(CH_2)_n$ bonds while the peaks around 2855 and 2930 cm^{-1} generated by stretching sp3-hybridized C-H bonds. The peak around 1746 cm^{-1} stood for non-aldehyde C=O stretch. In order to evaluate the efficacy of each fingerprinting techniques used in this study for authenticating the polyherbal oil samples, MATLAB software was used. Based on HPTLC, IR and UV spectra data, the plot of the first two PCs for fingerprint data have been shown in figure 6.
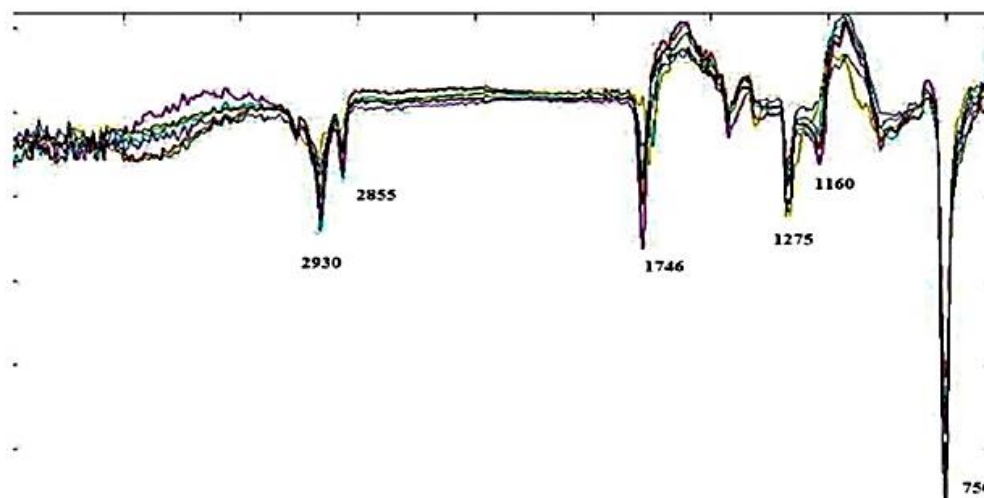
Figure 5. FT- infrared spectroscopy fingerprints of sesame oil based after standard normal variety (SNV)
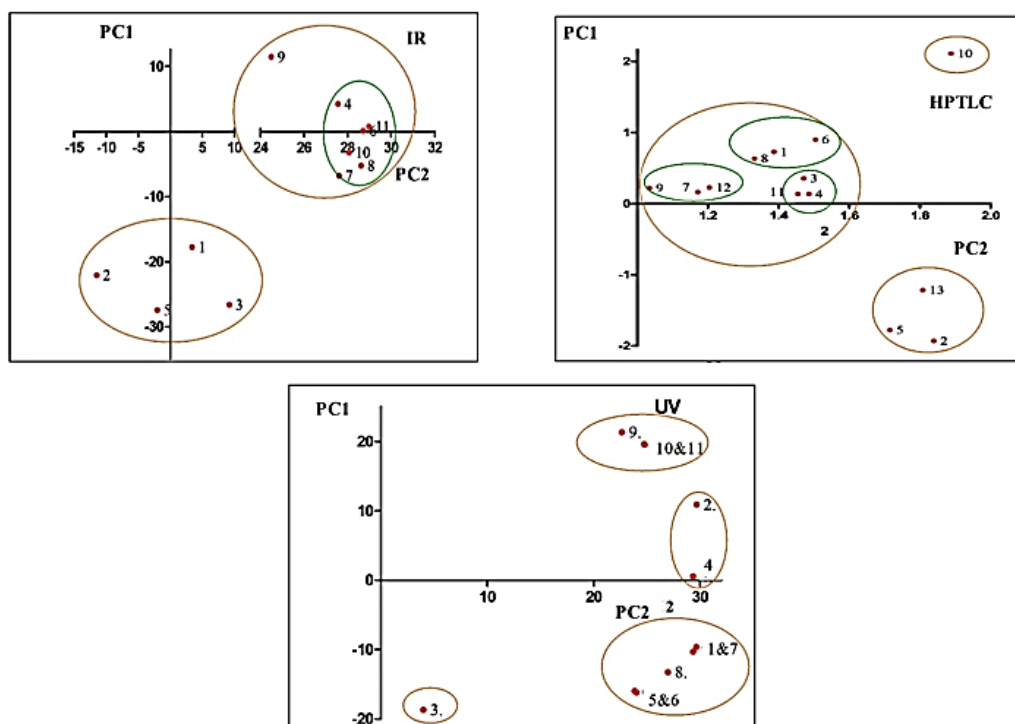

Figure 6. PC1 versus PC2 plots resulted from principal component analysis of HPTLC, IR and UV fingerprinting data for the traditional oil;   1: *Zataria multiflora* Boiss.; 2: *Vitis vinifera* L.; 3: *Peganum harmala* L.; 4: *Citrus medica* L.; 5: *Lippia citriodora* (Lam.) Kunth; 6: *Petroselinum crispum* Mill.; 7: *Ocimum basilicum* L.; 8: *Anethum graveolens* L.; 9: *Cucurbita pepo* L.; 10: *Boswellia* spp.; 11: *Myrtus communis* L.; 12: sesame; 13: olive

As it can be seen, four clusters were generated in UV plot, two clusters in IR plot and three clusters in HPTLC plot.

The clusters generated in UV plot were in accordance with the oils colors in visible light (figure 1). The most overlaps of the oil spots occurred in UV plot (an overlap for thyme and basil oils, an overlap for lemon verbena and parsley oil and another overlap for boswellia and myrtle oils). The biggest cluster in UV plot, belongs to those spectra containing green pigments peaks (high absorbance level at 428-452 and 630 and 670 nm). The oils in the second cluster (boswellia, myrtle and pumpkin oils), share maximum absorbance level at 313 and 334-335 nm. Esfand oil was completely separated from the others due to the presence of red pigments with the maximum absorbance at 506 nm. The clusters are not displaying any relation with their preparation method. Overall, it could be concluded that although several clusters were found in UV plot, they were not capable of discriminating between the polyherbal oils due to the occurrence of several overlaps.

In the IR plot, two clusters and one subcluster were generated. Despite using blank correction in data acquisition, the generated clusters were in consistence with their used vehicle oil. These clusters were also, in accordance with preparation methods for the polyherbal oil (with one exception in each cluster). It is possible that during the oil preparation some new by products or complexes were produced as a result of heating the vehicle oils and the herbal components. Also, as it can be seen in figure 5, the major peaks in all IR spectra were similar which might be due to the interactions of the herbal components with the oils such as production of hydrogen bonds and dipole-dipole interaction with the functional groups of the herbal metabolites and the vehicle constituents which were not omitted by blank correction. Overall, according to the pattern of cluster production and despite the absence of overlaps, IR fingerprinting of the oils should be used with more cautions to authenticate these polyherbal oils.

Three clusters and some subclusters were produced in HPTLC plot according to the generated fingerprints based on the used coding system. All of the oils HPTLCs contained spots for the herbal components as well as the vehicle components. Surprisingly, there was no correlation between the cluster generation with neither the oil vehicles nor the methods of polyherbal oil preparation. No overlapping for HPTLC fingerprinting system of the samples was observed. Therefore, it seems that the designed coding system was successfully able to produce a discriminative unique fingerprint for each sample. It seems that using the coding system with more features such as colors and density of the HPTLC spots, might lead to even a more convenient fingerprinting in future studies. Overall, it can be concluded that among UV, IR and HPTLC fingerprinting, the last one can be more reliable than others to authenticate polyherbal oils.

DSC thermograms of olive and sesame oil based herbal oils have been presented in figures 7 and 8, respectively. DSC pattern of all polyherbal oils based on sesame oil were almost similar. As seen in figure 7, there was an endothermic peak at -14 to -9 °C which was related to the melting of the frozen oil. The minor difference between these temperatures results from the differences in contents of the oils. The endothermic peak was followed by an exothermic one around 2 to 4 °C which may be due to the crystal changes of the samples during freezing and heating phases. A similar pattern was also observed for olive oil based samples, but the endothermic and exothermic peaks were shifted to the higher temperatures and the exothermic peaks were sharper (figure 8). This could be due to the higher stability of sesame oil in comparison to olive oil. Overall, DSC thermograms show that there is no proved sign of instability in herbal oil samples in the temperature range examined in this experiment.

Acid value represents the amount of COOH groups in a chemical compound [22]. Acid value is an important indicator of vegetable oils quality which shows the degree of rancidity [23] in oils (converting triglycerides into fatty acids and glycerol, causing an increase in acid number).
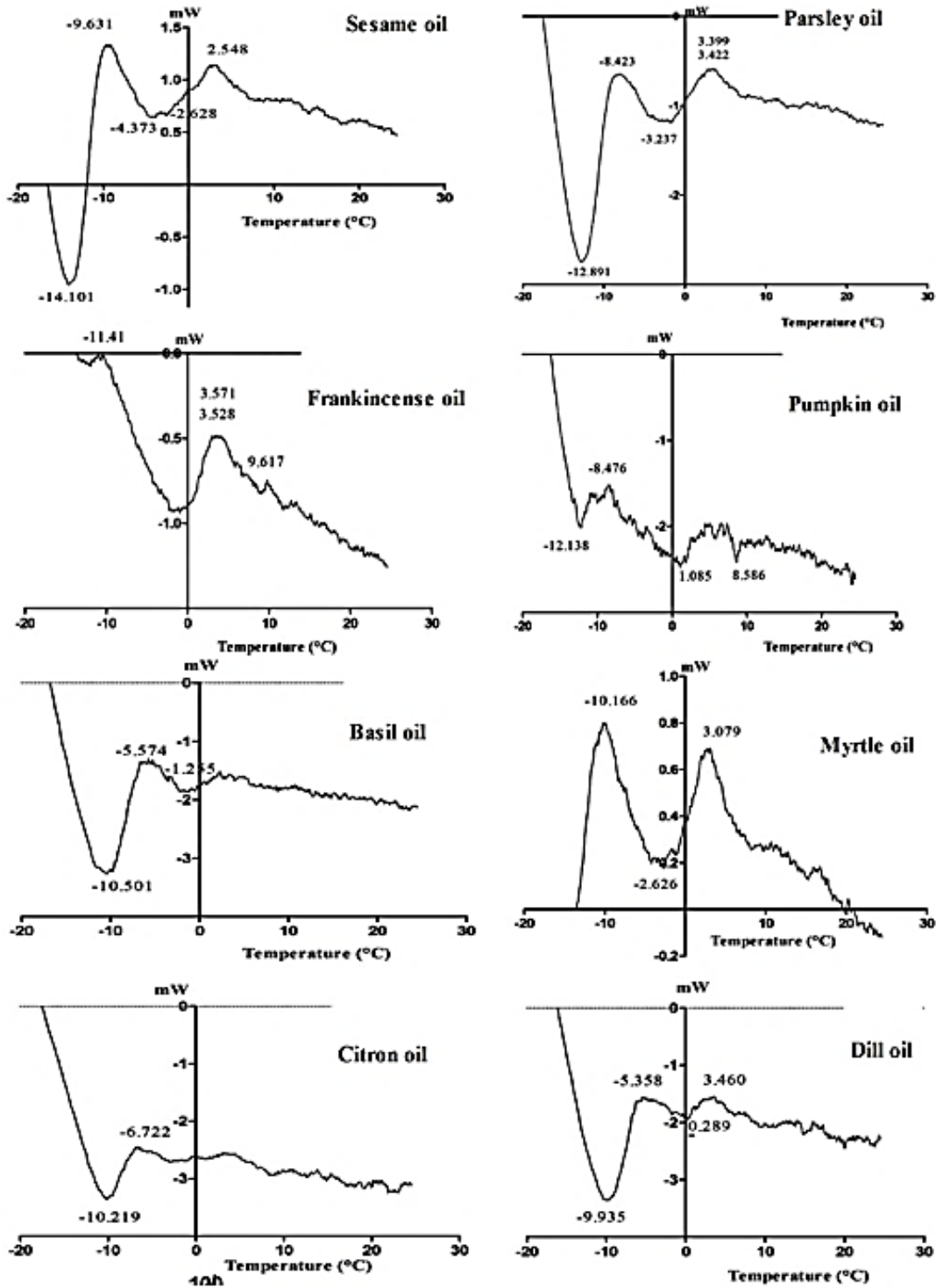
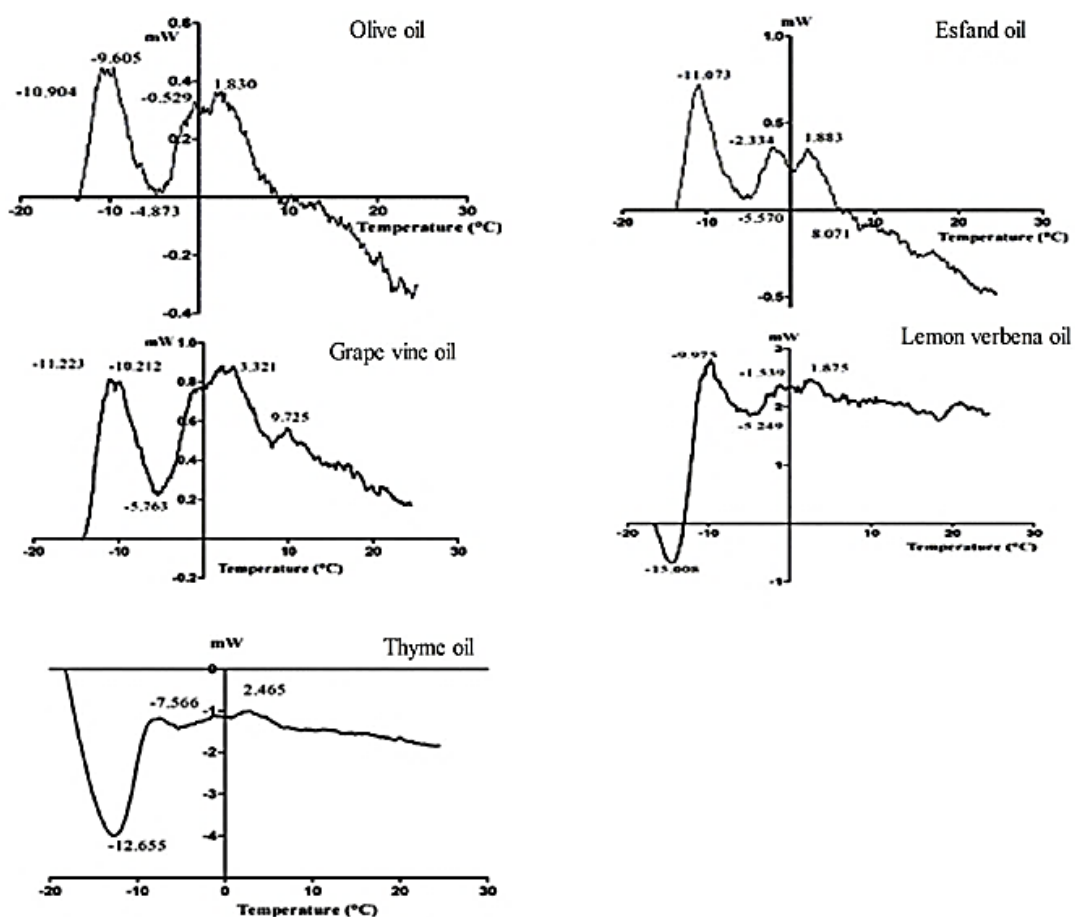**Figure 7**. DSC thermograms of sesame oil based polyherbal oils

Figure 8. DSC thermograms of olive oil based polyherbal oils

Acid values of freshly prepared polyherbal oils increased compared to sesame or olive oils as vehicles (table 2). This increase was likely due to addition of several phenolics (containing COOH) from herbs to the oily vehicles during processing of the polyherbal oils. Moreover, this increase was much higher for those which were processed by evaporation of the aqueous herbal extract in boiling vehicle oil (harmel oil, dill oil, frankincense oil, grape vine oil) which showed that the presence of water in conjunction with high temperature induced higher degrees of triglycerides hydrolysis during processing of the mentioned polyherbal oils. The peroxide value, which represented the initial oxidation and is measured in milliequivalents of active oxygen per kilogram of the oil, [24] was determined much higher in prepared oil samples compared to the respective oily vehicles. This increase of peroxide value was more dramatic for some of the samples such as lemon verbena, parsley and dill oils which looked greenish and rich in polyphenols and chlorophylls. It seems that the higher degree of unsaturation due to the presence of herbal polyphenols in the oil had increased the peroxide values. It is possible that phenolics in these polyherbal oils like unsaturated fatty acids of the oily vehicles took oxygen during processing and gave rise to the formation of peroxides and had interfered with the test results.

Table 2. Physicochemical evaluation of traditionally prepared oils

No.	Oil sample/base	Acid value	Peroxide value	Refractive index
1	Harmel oil/Olive oil	3.58	13.38	1.4625
2	Lemon verbena oil/Olive oil	2.06	34.35	1.4622
3	Pumpkin oil/Sesame oil	0.29	7.06	1.4655
4	Citron oil/Sesame oil	0.66	2.31	1.4653
5	Parsley oil/Sesame oil	0.34	21.27	1.4657
6	Dill oil/Sesame oil	1.55	10.92	1.4653
7	Grape vine oil/Olive oil	1.68	33.17	1.4622
8	Frankincense oil/Sesame oil	2.10	17.65	1.4695
9	Basil oil/Sesame oil	0.34	12.35	1.4657
10	Myrtle oil/Sesame oil	0.22	14.54	1.4653
11	Thyme oil/Olive oil	3.24	17.62	1.4623
12	Olive oil/Base	0.77	12.26	1.4624
13	Sesame oil/Base	0.22	4.51	1.4653

According to the graphs provided in a report by Bensmira *et al*., heating (150-200 °C) the sunflower seed oil containing lavender or thyme increased acid and peroxide values compared to the sunflower seed oil at 25 °C. Although these increases were much higher for the heated but untreated sunflower seed oil (150-200 °C). They related their findings to abundance of natural antioxidants transferred into sunflower seed oil following thyme or lavender treatment [25]. These founding are inconsistence with the results of the study.

Acid values of 4.0 mg KOH/g oil and peroxide values of 15 milliequivalents of active oxygen/kg oil for cold pressed and virgin edible vegetable oils have been reported acceptable although the acceptable acid value was 10.0 mg KOH/g oil for virgin palm oils [26]. According to European Union Commission Regulation EEC/1989/03, virgin olive oils must show acid values of 2 and peroxides value of 20 [27-29]. As seen in table 2 the acid values of all prepared polyherbal oils were in the acceptable range defined by FAO. But some of the oils in table 2 (lemon verbena, parsley and grape vine oils) showed outranged peroxide values. A high level of peroxide in oils is a major concern from rancidity and toxicology point of view. Edible oils oxidation products such

as peroxides have been reported to promote atherosclerosis and coronary heart diseases [25]. Thus, if it is proven that the observed high peroxide value of the mentioned oils was a result of rancidity (and not a result of high unsaturated phenolics), they are not suggested to be orally administered.

As far as we know, this is the first report on refractive indices of the mentioned polyherbal oils thus we could not compare the results in table 2 with previous data. But the acceptable refractive index (ND 40 °C) for vehicle oils, sesame oil (1.465-1.469), virgin and refined olive oil (1.4677–1.4705) and pomace olive oil (1.4680–1.4707) had been reported previously [25]. The data are in consistence with the results of this study although the refractive index of the original olive oil used in the present study was lower than what was mentioned in scientific texts. Concerning these refractive indices, the differences between vehicle oils and prepared polyherbal oils were narrow and many samples were as clear as the respective vehicle.

Although most of the phytochemical screening methods applied in the research are not specific, this work was the first report on evaluating and authenticating of these sorts of poly herbal oils and gives some useful data to authenticate them. On the other hand, one purposes of this study was to compare reliability and applicability of simple and rapid methods which can be used in ordinary quality control laboratories.

In this work, only one sample from each traditional oil preparation was employed for HPTLC fingerprinting. Due to the variation in concentrations of herbal materials in the vehicle oil, conditions of preparation and origins, the fingerprint profile of the same herbal oil could change from sample to sample. However, these poly-herbal oils are usually prepared in line with the procedures which have been described in traditional Persian literature. Thus, the amounts of herbs which are used to prepare these polyherbal oils are usually known and unique. On the other hand, variation in HPTLC fingerprints due to the differences in sources of plants and climate changes and so on are a common issue.

Although the preparations method is usually unique in traditional literature, this is possible to use plants from different sources and compare the results in future projects.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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