Reformulation of Traditional Chamomile Oil: Quality Controls and Fingerprint Presentation Based on Cluster Analysis of Attenuated Total Reflectance–Infrared Spectral Data

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Abstract

Herbal oils have been widely used in Iran as medicinal compounds dating back to thousands of years in Iran. Chamomile oil is widely used as an example of traditional oil. We remade chamomile oils and tried to modify it with current knowledge and facilities. Six types of oil (traditional and modified) were prepared. Microbial limit tests and physicochemical tests were performed on them. Also, principal component analysis, hierarchical cluster analysis, and partial least squares discriminant analysis were done on the spectral data of attenuated total reflectance–infrared in order to obtain insight based on classification pattern of the samples. The results show that we can use modified versions of the chamomile oils (modified Clevenger-type apparatus method and microwave method) with the same content of traditional ones and with less microbial contaminations and better physicochemical properties.

Keywords

traditional chamomile oil, Persian medicine, attenuated total reflectance–infrared spectra, principal component analysis

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Oils are one of the pharmaceutical dosage forms used widely in traditional Persian medicine.[1] Persian medicine dates back to thousands of years,[2,3] but it was developed by notable Persian scholars like Akhawayni (?-983 AD), Rhazes (865-925 AD), Haly Abbas (949-982 AD), Avicenna (980-1032 AD), Jorjani (1042-1137 AD), and others in the early medieval and Islamic age.[4-8] Later, this period was called the Persian or Islamic Golden Age.[7] These physicians and also other Persian scholars used various types of dosage forms to prepare drugs, mostly prepared by medicinal herbs. Persians wrote a group of medical texts namely *Qarabadins* as pharmaceutical books explained pharmaceutical dosage forms, preparing methods, and related information. They were the base of current pharmacopeias.[9] One of the popular dosage forms in these books currently used by traditional practitioners is medicinal oils called as *dohn* (its plural form is *adhan*) in medieval Persian language.[10] Although, medicinal oils have different types, they can be divided into the 2 following main types: (1) direct extraction

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(from the medicinal herbs) via distillation of aromatic plants parts or compression of oil-bearing parts and (2) indirect extraction, including extraction of plants components by a vegetable oil vehicle. Second group are nonoily herbs and therefore their components should be extracted by an oily vehicle (such as sesame, caster, and olive oils).[11] In *Qarabadin* books, we can find some different methods of indirect extraction. Usually, the extractions were done by putting the mixture of herbs in the vehicle oil exposed to the sun for a long time (40 days for chamomile oil) or by boiling herbal parts in the water and then vaporized the obtained aqueous extract in vehicle oil (sesame oil or chamomile oil) by heating. These methods have been used for centuries but no scientific investigations were done for them to investigate which method is better or if we can optimize and improve these methods via current knowledge and equipment.

Fingerprinting of the spectra resulting from analytical methods like high-performance thin layer chromatography, high-performance liquid chromatography, infrared spectroscopy, and so forth on the herbal samples is a new approach to evaluate and standardize such products.[12] Among them, attenuated total reflectance–infrared method is a good and cost-efficient method to give us a fingerprint of whole content of a sample. Using principal component analysis on infrared transmittance values is common for quality control and comparing the similarity of 2 samples.[13]

Regarding the described points, we selected chamomile oil as one of the most common and widely used oils to evaluate the methods of indirect extraction.[2] Chamomile (*Matricaria chamomila* L) is an Iranian native medicinal plant. It has cosmetic, therapeutic, and nutritional values, and is used in different forms such as tea, raw material, decoctions, as well as commercial products all around the world.[14] The chamomile flowers have about 1% yield essential oil containing bisabolone oxide A, bisabolol oxide A, chamazulene, and so on (there is matricine in the plant, and by heating during essential oil extraction it is converted to chamazulene). Also, the flowers have many poly phenol compounds like flavonoids, in particular apigenin and its derivatives.[15]

In Persian medicine, there are 4 types of drugs and foods: pure foods (*ghazai-e-motlagh* in Persian language), medicinal foods (*ghazai-e-davaei* in Persian language), nutritional drugs (*dava-e-ghazaei* in Persian language), and pure drugs (*dava-e-motlagh* in Persian language). Chamomile is categorized in medicinal foods group in traditional Persian medicine.[16] The definition of this group is similar to functional foods in current concepts. Chamomile oil is one form of preparation that has been used (orally and topically) for cooking[17] as well as massage therapy with oil,[18,19] cosmetics, general good health.[20] It is also used as remedy for various types of diseases such as migraine, otitis, sciatica, joint pains, arthritis,[21] and inflammation.[22] It also finds application as a nourishing agent for the hairs of head and eyebrow in traditional Persian medicine.[10,11,23] It is widely used nowadays in Iran, especially by current traditional healers.[11] There are some clinical trials that show the effect of traditional chamomile oil on some complications like severe carpal tunnel syndrome and knee osteoarthritis.[21,22]

Table 1. Particle Size Distribution of the Chamomile Flower Powder.

Mesh Range (Number)	Mean	Standard Deviation
<40	11.69	0.13
40-60	22.68	0.16
60-70	32.34	0.23
70-100	21.93	0.09
100-150	7.14	0.11
150-200	4.02	0.11
>200	0.52	0.01

In this investigation, we aimed to find formulations of traditional chamomile oil in traditional Persian medicine and after that, modified versions of these methods were designed. Then, preparation of both traditional and modified methods was done followed by their comparison in terms of microbial and physicochemical properties. Meanwhile, a convenient strategy to evaluate their contents via principal component analysis and hierarchical cluster analysis on infrared transmittance values was devised. This approach was designed in order to achieve the best formulary of chamomile oil based on novel chemometric methods.

Method

Literature Review

In advance, we referred to historical books on pharmacy (*Qarabadin* books) in Persian medicine. Two main Q*arabadins* were considered to obtain chamomile oil formulas. These 2 books are *Qarabadin-e-Kabir* written by Aghili Shirazi in 1772 AD[24] and *Qarabadin-e-Salehi* written by Mohammad Saleh Ghaeni Heravi in 1766 AD.[25]

Preparing the Plant for the Study

Chamomile was purchased from a traditional herbal shop (*Attari*) in Shiraz. The plant was collected from Kazeroon, a city near the Shiraz and one of the cultivation centers of chamomile in Iran. Also, a herbarium sample of the plant was sent to the Herbarium Center at School of Pharmacy, Shiraz University of Medical Sciences to be identified and deposited. A voucher number (PM 407) was accordingly prepared for the plant in the Herbarium Center.

In the next step, the flowers of the chamomile were separated to be used for the preparation of the oils. Then, these flowers were crushed via a mixer (Asan Toos Shargh, Iran) to make fine powders.

A 100-g sample of the prepared powder was gathered to evaluate particle size distribution of the powder. This sample was put into a serial sieves apparatus (with mesh sizes 200, 150, 100, 70, 60, and 40). This series of sieves was put in a sieve shaker (DG Scientific Product Co, Minor, Taiwan), and run with speed 10. The powder in each sieve was weighed every 5 minutes so that its weight variation decreased below 5%. At that moment (20 minutes), the weights of powders in each sieve were measured. It was repeated 3 times and particle size distribution of the powder was calculated (Table 1).

Chamomile Oil Preparation

Based on traditional Persian books on pharmacy,[24,25] 2 general traditional methods are presented for preparation of chamomile oil. First is with direct heat (via fire heat) and the second procedure is with indirect heat (via sun heat). We remade these 2 chamomile oils

and also tried to modify these historical methods with current knowledge and facilities. Next, we explain how these oils (6 types traditional and modified) were prepared.

Oils Preparation With Direct Heat

Method 1: Traditional direct heat method. According to historical method mentioned in Qarabadin books,[24,25] 600 g of chamomile flower powder was boiled in 3.6 L of water for 3 hours. Then, the powder was removed and the remaining water (aqueous extract of chamomile) was boiled with 0.5 L of sesame oil (Golkaran Co, Iran) for 2 hours (until all the water was vaporized and oil remained). The remained oil is the traditional chamomile oil prepared via traditional direct heat method.

Method 2: Clevenger-type apparatus method. It is a modified method of traditional direct heat method. In method 1, much of essential oil is lost during boiling chamomile flower in the first step. Also, the amount of remaining essential oil in the preparation is not under control and repeatable. To solve this problem, method 2 was designed. In this method, 200 g of chamomile flower powder and 2.5 L of water were put in a Clevenger-type apparatus (5 L balloon). The essential oil was gathered and saved. Then, the powder was removed from the water in the balloon. Remaining water (chamomile aqueous extract) was boiled in 167.5 mL of sesame oil for 2 hours until the water was vaporized and oil remained. Then, essential oil obtained in the first step via Clevenger-type apparatus was added to the oil and the final product was prepared.

Method 3: Modified Clevenger-type apparatus method. This method is the same as method 2, but the powder was put in small packages (each contained 5 g of powder), made by filter paper. This method was designed because in method 2 small amount of the plant powder could stick to the balloon wall and burn. It can cause differentiation in essential oil content. But, in method 3, the powder is packaged in filter paper and is not exposed to the balloon glass wall.

Method 4: Soxhlet method. In this method, 60 g of the powder was packaged by filter paper and put in to a Soxhlet (500 mL volume) apparatus. Also, 1 L water was put into the 2 L balloon of Soxhlet and the apparatus was run. After 3 hours, the water in the balloon was placed in another vessel and was boiled with 50 mL sesame oil until the water completely vaporized and only the oil remained.

Oils Preparation With Indirect Heat

Method 5: Traditional indirect heat method. According to historical books,[24,25] 280 g of chamomile flower powder was put in a glass vessel with 949.2 mL of sesame oil and the vessel was exposed to sun light for 40 days. Then, oil was filtrated and powder was removed. The remained oil was the traditional chamomile oil prepared via traditional indirect heat method.

Method 6: Microwave method. Method 5 takes too long a time and also the sun's energy cannot be controlled and is different across a year. In method 6, we used microwave energy as the source of energy. According to our pretests, we achieved the optimal time and power of microwave apparatus: 280 g of the powder and 949.2 mL of sesame oil was put in a vessel and exposed to microwave (Samsung, GE4020W) with 450 W energy and 2450 MHz frequency for 7 minutes. Then, the powder was removed and filtrated oil obtained as final product.

Microbial Limit Tests

Total Count. The sample of each oil types was filtered through a 0.22-μm filter (BIOFIL) having 50 mm thickness (mixed cellulose ester membrane) under aseptic condition (sterile filtration method). Isopropyl myristate was used to dilute the oils and also to wash filters surface in this method. After that, the filters were mammocked and put in the soybean-casein digest agar culture medium and were finally incubated for 48 hours. After incubation, the number of colonies were counted.

Specific Tests. The selected oils of method 3 (direct heat) and method 6 (indirect heat) that had the minimum total microbial counts were tested for specific objectionable contaminations with 5 pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella*, and *Candida albicans*), which should not be present in topical and oral preparations.[26,27]

The oils samples were aseptically filtered with the same method done for total count. For exploring *S aureus,* the pieces of the filters were put into the Braid-Parker agar and manitol salt agar mediums and incubated for 72 hours at 37°C. For *E coli*, the pieces of the filter were soaked in the fluid lactose medium and incubated for 24 hours at 37°C; then transferred into the eosin methylene blue agar and MacConkey agar mediums and incubated for 48 hours at 37°C. The medium used for *P aeruginosa* was cetrimide agar, the filter pieces were put into it and incubated for 48 hours at 37°C. For *Salmonella*, the pieces of the filter were soaked in the lactose broth medium and after 24 hours, 1 mL of it was transferred to 9 mL selenite-cystein medium and 1 mL into 9 mL tetrathionat medium. The mixtures were incubated for 24 hours. Then, 1 loop of each mixture was transferred to xylose-lysine-desoxychalate agar and brilliant green agar mediums. Finally, it was incubated for 48 hours at 37°C. The yeast extract glucose medium contains antibiotic (chloramphenicol). For exploring *Candida albicans*, a piece of the filter was put on the medium and incubated for 5 days at 25°C. Subsequently, total colony count was examined for each.

Physicochemical Tests

The below physicochemical tests were done on the sesame oil and all 6 prepared chamomile oils according to the British Pharmacopeia principles.[27]

Acid Value. Ten grams of the cooled oil samples were weighed in a conical flask (250 mL). Then, 100 mL hot ethyl alcohol and 1 mL phenolphthalein (as indicator) were added. After boiling the mixture for 5 minutes, the mixture was titrated by standard sodium hydroxide. The mixture was shaking vigorously during titration. Finally, acid value was calculated using the following formula:

$$\text{Acid value} = V_s \times 0.1 \times 28.2/W$$

where, V_s is denouncing volume (mL) of used standard sodium hydroxide and W the weight (g) of each oil sample (10 g).

Peroxide Value. Oil samples (2.5 g) were weighed into a conical flask (500 mL) containing 30 mL peroxide solution (acetic acid:chloroform 60:40) and 0.5 ml of saturated potassium iodide. After 1 minute, 30 mL distilled water and starch (as indicator) were added to the mixture making it appear as blue. Then, the mixture was titrated with standardized sodium thiosulphate solution until the blue color disappeared through shaking. Finally, peroxide value was calculated using the following formula:

$$\text{Peroxide value} = 1000 \times V_s \times N/W$$

where V_s is the volume (mL) of used standard sodium thiosulphate solution, W the weight (g) of each oil sample (2.5 g), and N the normality of the standard sodium thiosulphate solution.
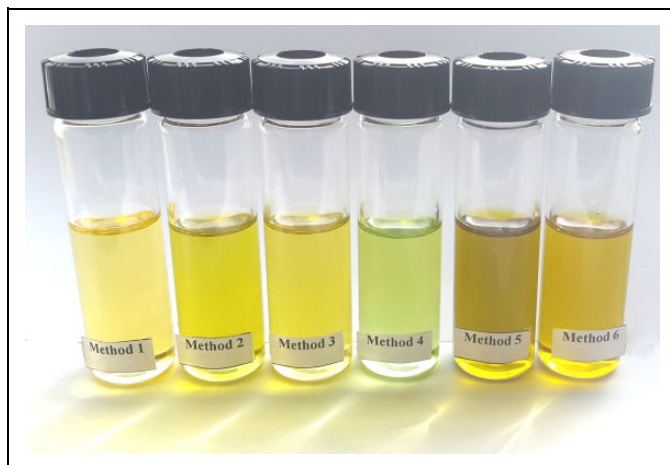
Figure 1. Different chamomile oils prepared using methods 1 to 6 (method 1, traditional direct heat method; method 2, Clevenger-type apparatus method; method 3, modified Clevenger-type apparatus method; method 4, Soxhlet method; method 5, traditional indirect heat method; method 6, microwave method).

Iodine Value. Oil samples (0.32 g) were delivered into a conical flask (500 mL) with glass stopper. Then, 10 mL of carbon tetrachloride was added. After mixing the solution, 25 mL of Wij's solution was added and the glass stopper was replaced after wetting with potassium iodide solution. The mixture was put in a dark room and was swirled half an hour to be mixed properly. Simultaneously, a blank solution was carried out (with same procedure but without oil sample). Then, 20 mL of potassium iodide solution and starch (as indicator) were added to make a blue color appear in both mixtures (sample and blank). The mixtures were titrated with standardized sodium thiosulphate solution until blue color disappeared through shaking the mixtures with the stopper on. Finally, iodine value was calculated using the following formula:

$$\text{Iodine value} = 12.69 \, (V_b - V_s)N/W$$

where V_b is the volume (mL) of standard sodium thiosulphate solution required for the blank, V_s the volume (mL) of standard sodium thiosulphate solution required for the sample, N the normality of the standard sodium thiosulphate solution, and W the weight (g) of the sample (0.32 g).

Saponification Number. In this procedure, 2 g of sample oils were weighed into a conical flask (200 mL) with 25 mL of potassium hydroxide in ethanol (0.5 mol/L). Then a cooling pipe was fixed on the top of the flask. The flask was gently heated while shaking. Regarding, the solution was refluxed and after 1 hour, the mixture was immediately cooled and titrated with hydrochloric acid (0.5 mol/L). The same procedure was performed for the blank (without oil sample). Finally, saponification number was calculated using the following formula:

$$\text{Saponification number} = 28.05 \, (V_b - V_s)/W$$

where V_b is the volume (mL) of standard hydrochloric acid required for the blank, V_s the volume (mL) of standard hydrochloric acid required for the sample, and W the weight (g) of the sample (2 g).

Table 2. Bacterial Colonies (CFU/g) of Each Prepared Chamomile Oil Types[a]

Sample	Bacterial Colony (CFU/g)
Sesame oil	<10
Method 1	20
Method 2	<10
Method 3	<10
Method 4	20
Method 5	>100 + fungi
Method 6	20

Abbreviation: CFU, colony-forming unit.
[a]Method 1, traditional direct heat method; method 2, Clevenger-type apparatus method; method 3, modified Clevenger-type apparatus method; method 4, Soxhlet method; method 5, traditional indirect heat method; method 6, microwave method.

Principal Component Analysis on Attenuated Total Reflectance–Infrared Spectral Data

In order to investigate the similarities between different extraction methods, infrared spectroscopy was used to obtain the fingerprint pattern of each sample. For this purpose, different formulations were subjected to a Bruker vertex-70 instrument by means of attenuated total reflectance sampling apparatus. Before the sampling procedure, the baseline for data acquisition was corrected using sesame oil as the medium. The transmittance values for all samples were obtained in the middle infrared range of 940 to 4000 cm^{-1}. The data were thereafter exported to Matlab software for further statistical analysis. Standard normal variate was performed as a preprocessing technique on the data matrix in order to suppress the baseline fluctuations of spectra. The resulting data matrix was also subjected to autoscaling preprocessing technique. To cluster the samples based on their components, principal component analysis was used as an unsupervised clustering analysis technique in 2 ways. Standard normal variate preprocessed and standard normal variate/autoscaled preprocessed data were used in 2 independent studies. Accordingly, the principal components were extracted from the resulting matrix of data using singular value decomposition algorithm. In principal component analysis, the principal components are ranked based on their eigen values in such a way that the first principal component is bearing the most variation of the data set. The second principal component is calculated to be orthogonal toward the first one. A plot of the first 2 principal components is therefore representative for data scattering in 2-dimensional space.[28,29]

Hierarchical Cluster Analysis

In order to cluster the 6 samples based on the infrared spectral data, hierarchical cluster analysis and principal component analysis (standard normal variate–modified data) were carried out. The resulting matrix was then subjected to Matlab (Mathworks Inc) in order to perform hierarchical cluster analysis. Cluster definition was done by means of Euclidean distance as a measure of similarity using unweighted pair group method.[30]

Partial Least Squares Discriminant Analysis

In order to confirm the validity of both clustering approaches of this study, a more discriminative classification method was used. Partial

Table 3. The Results of Physicochemical Tests on Chamomile Oil Types[a]

Sample	Acid Value	Peroxide Value	Saponification Number	Iodine Value
Sesame oil	0.16 ± 0.02	11.19 ± 0.12	208.43 ± 1.20	105.00 ± 0.51
Method 1	2.27 ± 0.03	10.78 ± 0.16	205.63 ± 1.11	106.96 ± 0.47
Method 2	2.42 ± 0.04	11.64 ± 0.13	206.22 ± 0.98	105.88 ± 0.35
Method 3	2.01 ± 0.03	11.52 ± 0.11	206.47 ± 1.07	105.32 ± 0.42
Method 4	2.05 ± 0.05	11.93 ± 0.12	206.33 ± 1.08	106.75 ± 0.53
Method 5	3.10 ± 0.03	20.86 ± 0.09	209.81 ± 1.02	104.83 ± 0.54
Method 6	2.21 ± 0.04	13.78 ± 0.12	207.33 ± 1.04	106.05 ± 0.38

[a]Method 1, traditional direct heat method; method 2, Clevenger-type apparatus method; method 3, modified Clevenger-type apparatus method; method 4, Soxhlet method; method 5, traditional indirect heat method; method 6, microwave method.
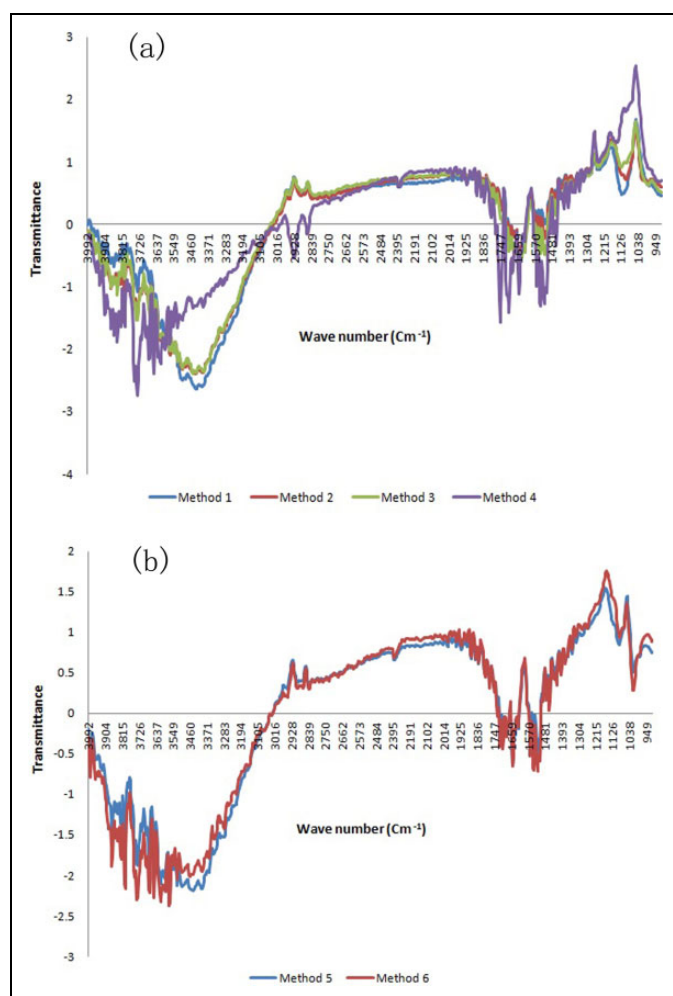

Figure 2. Infrared spectra of chamomile oils prepared with direct heat methods (a) (method 1, traditional direct heat method; method 2, Clevenger-type apparatus method; method 3, modified Clevenger-type apparatus method; method 4, Soxhlet method); and with indirect heat methods (b) (method 5, traditional indirect heat method; method 6: microwave method).

least squares discriminant analysis is a supervised classification approach. Based on this method linear regression models are calculated by projecting the predicted and observed variables to a new space.[31] To perform this calculation, the clustering result of the 2 used methods was used as the class model (class I = samples 1, 2, and 3, class II = sample 4, and class III = samples 5 and 6). The matrix of infrared spectra together with the vector of classes was entered to classification tool box.[32] To obtain the optimum number of latent variables for partial least squares cross-validation using venetian blinds method was used.

Results and Discussion

Particle Size Distribution of the Plant Powder

Particle size distribution of the chamomile powder is shown in Table 1. It shows a normal standard distribution of particles.

Oils Prepared Using Different Methods

Four chamomile oil types were prepared in the category of oils preparation with direct heat and also 2 chamomile oil types in the category of oils preparation with indirect heat. Figure 1 shows these prepared oils in one view. Following results compare them to reach better production for formulation of chamomile oil.

Microbial Tests

Table 2 shows measured amounts of microbial colonies (colony-forming units per gram) for each prepared oil type. As a result, among prepared oils with direct heat, the methods 2 and 3 had lower counts of microbial colonies; while preparing oil with indirect heat method, method 6, had lower microbial count in comparison with method 5.

Specific microbial tests on *S aureus*, *E coli*, *P aeruginosa*, *Salmonella*, and *C albicans* showed that the oils prepared with methods 2 and 3 had no objectionable contaminations. These contaminations are not to be seen in oral and topical preparations.

Physicochemical Tests

The results of 4 physicochemical tests for 6 chamomile oil types are shown in the Table 3. As it is seen, the amount of peroxide value, saponification number and iodine value in all the chamomile types are similar and close to the sesame oil before heating or any process. Only, peroxide value of method 5 is too high. It is due to the high amount of microbial colonies that caused changes in the physicochemical properties of the oil. On the
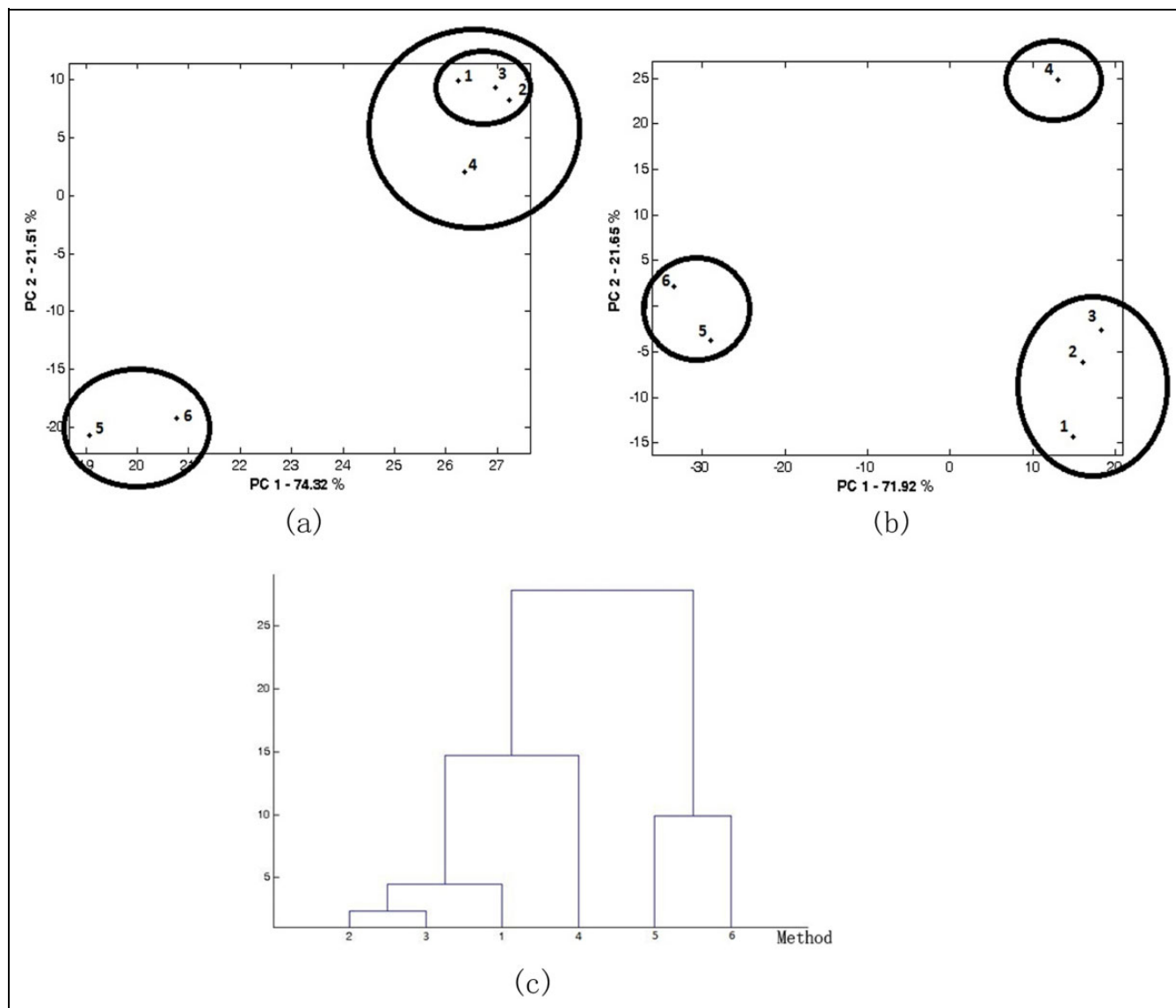
Figure 3. The plot of the first 2 PCs for SNV preprocessed data (a); for SNV/autoscale preprocessed data (b); and HCA studies on data matrix of PCs for SNV preprocessed data (c) (1, traditional direct heat method; 2, Clevenger-type apparatus method; 3, modified Clevenger-type apparatus method; 4, Soxhlet method; 5, traditional indirect heat method; 6, microwave method). PC, principal component; HCA, hierarchical cluster analysis; SNV, standard normal variate.
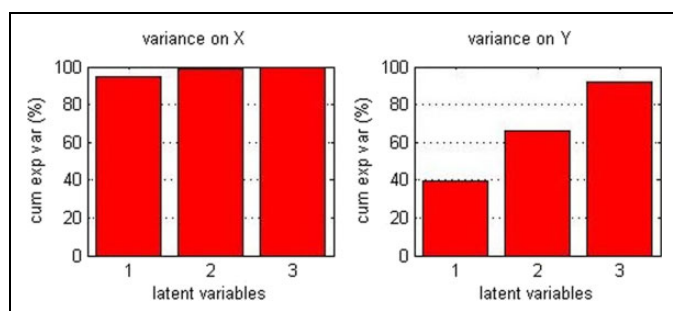

Figure 4. Using venetian blinds cross-validation for finding the optimum latent variables. Three variables led to the most variation in data matrix.

other hand, although acid value in the chamomile types had increased, it is same in all the types. This value also is high in method 5 because of high microbial colonies activities. All in all, between prepared oils with direct heat methods, method 3 has closer and more similar values to the sesame oil. Also, method 6 has better results in comparison with method 5 as prepared oil.

Principal Components Analysis, Hierarchical Cluster Analysis, and Partial Least Squares Discriminant Analysis on Infrared Transmittance Values

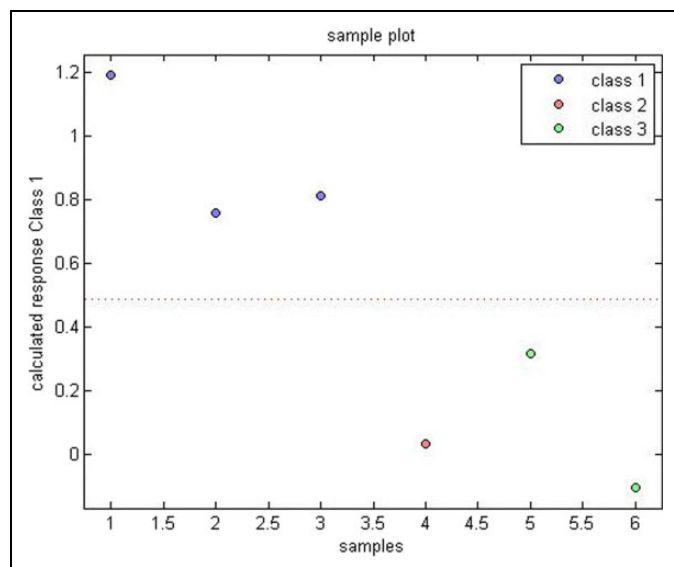The infrared diagrams of prepared oils with direct and indirect heat are shown in Figure 2.

Figure 5. PLS-DA result was in accord with the defined classes based on PCA and HCA (class 1, methods 1 to 3; class 2, method 4; and class 3, methods 5 and 6). PLS-DA, partial least squares discriminant analysis; PCA, principal components analysis; HCA, hierarchical cluster analysis.

The plot of the first 2 principal components is depicted in Figure 3a and b. Figure 3a results from modified data by standard normal variate and Figure 3b from modified data by standard normal variate/autoscale.

As seen in the figure, principal components 1 and 2 with 93.57% of infrared diagrams' information show methods 1, 2 and 3 in prepared oils with direct heat and methods 5 and 6 as prepared oils with indirect heat have similar patterns and are put nearly together in 2-dimensional spaces. On the other hand, cluster analysis of principal components analysis data (standard normal variate–modified data) showed clearly this position and similarity (Figure 3c). Among methods 1 to 4 (direct heat methods), only method 4 is not similar to other methods. It can be due to the lower heat for extraction in Soxhlet apparatus in method 4 in comparison with other methods.

According to partial least squares discriminant analysis, 3 variables led to highest variance in both X and Y data (Figure 4). The 3 latent variables were used for modeling of the data using Bayes assignation criterion. The model with the zero error rates was obtained and the classification method was able to discriminate each group of data (Figure 5). Since partial least squares discriminant analysis is considered as a supervised learning method, this finding verifies that the results obtained by the 2 used unsupervised classification models (principal components analysis and hierarchical cluster analysis) are trustworthy.

Discussion

The results show that we can formulate ancient preparations similar to other standard drugs. Statistical analysis on the

infrared spectra of the oils in this study is a new approach to compare the whole content of the oils not only by a marker. Principal components analysis is a good method to analyze resemblance of the attenuated total reflectance–infrared spectra of the samples as manifestation of the whole content of them.[13] It is a fast and cost-efficient method for quality control and comparison of the polyherbal preparations with a standard sample.

As it was seen in our study, method 5 (traditional indirect heat method) has some limitations such as microbial and physicochemical ones, but by replacing the solar energy by microwave in case of method 6, the analyzed criteria of the formulation was improved and principal components analysis shows these 2 oils to have the same infrared spectrum and therefore probably the same content. On the other hand, we could have improved the formulation of chamomile oil prepared with traditional direct heat method (method 1) to a more standard and repeatable method (using modified Clevenger-type apparatus—method 3). The principal components analysis shows these 2 oils to have the same content and we can replace method 1 by method 3. We chose to evaluate infrared spectra to find a cost-efficient and easy method for comparison. Future analysis on specific markers on these oils (eg, chamazulene in essential oil and apigenin in flavonoids) could give us more information and help us to set up this method and statistical analysis to compare the formulations together. Partial least squares discriminant analysis was used as a supervised classification method to obtain the error rate of principal components analysis and hierarchical cluster analysis. The reasonable error rate of partial least squares discriminant analysis revealed the differentiation power of the used methods in this study.

Conclusion

Our study shows that using modified Clevenger-type apparatus method for preparing chamomile oils with direct heat method and microwave method for preparing chamomile oil with indirect heat method are recommended instead of historical methods.

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Ethical Approval

Ethical approval is not required for this study as no human or animal subjects were involved.

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