

Comparative Study of Physical and Chemical Properties of the Oil Extracted from Fresh and Storage Aniseed and Determination of the Optimal Extraction Method

Anas Rajab

Department of pharmaceutical chemistry & drug control, Faculty of Pharmacy, Syrian Private University (SPU)

Available Online: 10th September, 2016

ABSTRACT

Several methods of extraction of volatile oils from the *Pimpinella anisum* L. seeds were experimented, and the best method was determined when the seeds were soaked with organic solvents at room temperature. The hexane is considered a good solvent to extract total and volatile aniseed oil. The following physical and chemical properties of extracted aniseed oil were studied: refractive index, density, acidity, acid value, moisture, volatile substances, saponification value and absorptivity in ultraviolet UV. It was found that aniseed oil is cannot be used as cooking or food oil. The long-term stability was studied for aniseed. It was found that the main total quantity of aniseed oil and its components was decreased interval of time.

Keywords: Aniseed, long-term, stability, volatile oil, gas chromatography, extraction.

INTRODUCTION

Anise is derived from Anisum (Latin) and its scientific name is *Pimpinella anisum* L. Its seeds called Aniseed^{2,5}. Anise is one of the oldest medicinal plants known to human, The ancient egyptians planted it and named it "Nikon" and it was used in the therapeutic prescriptions by the Greeks and Romans. It was described by Greek philosopher Dioscorides , a natural scientist, as a food flavour which helps with the digestion of heavy meals^{2,3}. Aniseed is used in combination with drugs to treat cough, sore throat, tonsillitis, and headache. It also facilitates childbirth and increases milk yield in nursing mothers. In addition, aniseed is good for healing abdominal cramps, removing bloating because it helps with digestion, and activates the immune system^{1,4-6,8-11,14}. It has been established in recent scientific researchs that aniseeds has male hormone effect if taken in small amounts, but the effect is inhibited if taken in large amounts^{13,14}. The aniseed volatile oil is used as a flavoring agent in chemical and food industry such as in beverages, pastries and confectionery industry as well as in the cosmetics industry^{13,16}. Aniseed volatile oil components are affected by the plant genetic factor, it is also affected by several physiological and environmental factors such as ripeness, climate, soil and storage conditions as well as the extraction method; and the difference in the composition of volatile oils is quantitative rather than qualitative⁸. Anise is derived from Anisum (Latin) and its scientific name is *Pimpinella anisum* L. Its seeds called Aniseed^{2,5}. There are several important methods to extract aniseed oil^{6,9,11,12,14,15}.

Percolation with water vapor.

Extraction using Organic Solvents: There are two ways for extraction by organic solvents, the first method uses Soxhlet extraction apparatus at solvent boiling point, and the second method depends on soaking in the organic solvents at room temperature. The physical and chemical properties of plant oils is determined by: Refractive Index, density, moisture, volatile compounds, acidity, acid value, Saponification Value, and absorptivity in ultraviolet UV^{7,16}. Long-term stability studies on plants is considered recent approaches to follow active components in volatile oils, these types of studies are happened under conditions (25°C ± 2°C / 60% RH ± 5% RH) in stability chamber¹⁷.

study Aim

The aims of this research is:

finding the best method for separation aniseed oil.

determine its physical and chemical properties for found that aniseed oil is used as food oil.

Study of aniseeds stability under regular storage conditions (25°C ± 2°C / 60% RH ± 5% RH)

Experimental

MATERIALS AND INSTRUMENTS

Materials

Aniseed was collected from Aleppo countrysides fields in Syria.

Hexane 99.8%, benzene 99.5%, petroleum ether (b.p. 40-80° C), diethylether 99.7%, ethanol 99.9%, Potassium hydroxyl 85%, Hydrochloric acid 32%, Anethole 98%, Anisaldehyde 98%, Estragole 97% and linalool 97%, were obtained from MERCK (Germany).

Instruments

UV/VIS Spectrophotometry, Jasco, model V-530, system

Table 1: Difference of extraction of anise volatile oil by different extraction methods

| | Separation method | Percentage of aniseed volatile oil % |
|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------------------------------|
| Extraction of aniseed volatile oil using hexane solvent | Extraction of aniseed volatile oil with soaking in the hexane at room temperature | 4.825 |
| | Extraction of aniseed volatile oil using Soxhlet apparatus | 4.644 |
| Extraction of aniseed volatile oil using percolation with water vapor | | 3.119 |

Table 2: change of Percentage of extracted aniseed oil by changing the type of solvent

| solvent | percentage of total aniseed oil % |
|---------------------------------|-----------------------------------|
| tetrachloromethan | 8.58 |
| petroleum ether (b.p. 40-80 C°) | 8.73 |
| hexane | 8.77 |
| bezene | 10.5 |

Table 3: The physical and chemical properties of total aniseed oil

| | |
|----------------------------------------------------|--------|
| Refractive Index (n_d^{20}) | 1.4961 |
| density g/cm^3 (d^{20}) | 0.9677 |
| Acidity (weight / weight %) | 0.47 |
| acid value (KOH/g) | 0.94 |
| Saponification Value (KOH/g) | 69.6 |
| moisture and volatile compounds (weight / weight%) | 5.31 |

software, made in Japan.

GAS Chromatography, Shimadzu, model GC-9A (FID), made in Japan.

Climatic chamber, Angelantoni, model CH1500, made in Italy.

Rotator Evaporator, Normschliff, model VV1, made in Germany.

Drying oven, MMM Croup, model Ecocell 55, made in Germany.

Balance, ± 0.05 mg, Sartorius, model ED224S, made in Germany.

Extraction methods of aniseed volatile oil

Two methods to extraction of aniseed volatile oil were performed. The aim of this research is to select of the best method to extract the aniseed volatile oil.

Extraction method of aniseed volatile oil using percolation with water vapor

50 g of aniseed were grinded and soaked in distilled water for an hour, then percolated with water vapor until depletion of oil from the specimen. After that, the extraction of volatile oil is completed from the aqueous extract; it was performed using diethyl ether. The etheric extract was evaporated using the rotating evaporator under low pressure at 40° C to get the anise volatile oil.

Extraction methods of aniseed volatile oil using organic solvents

Extraction method of aniseed volatile oil using Soxhlet apparatus

50 g of grinded aniseed were placed in a paper capsule in the middle part of Soxhlet apparatus. About 250 ml of hexane were added to the flask. The apparatus was assembled and the heating was made by electrical heater till the refluxed solution became colorless. After that, distillation of the resulting extract was performed under normal air pressure to get a hexane drop contains aniseed volatile oil. The hexanic extract was evaporated by the rotating evaporator under low pressure at 40° C to get the volatile oil.

Extraction method of aniseed volatile oil by soaking in hexane at room temperature

50 g of grinded aniseed were weighed and soaked in 250 ml hexane for one week at room temperature. After filtration, the hexane was removed using rotating evaporator at 40°C and the percentage of remaining oil was calculated (the total oil of aniseed). After that, the total oil was solved in hexane again, and then the resulting product was distilled using rotating evaporator under low pressure at 40° C. The hexane was removed rotating evaporator to get the volatile oil.

Studying the solvent influence on the quantity of total aniseed oil

50 g of fresh and dried aniseed were soaked in 250 ml hexane for one week at room temperature, and filtration of the extracted aniseeds was repeated twice again in the same solvent (hexane). The extracts were collected then the solvent was removed using rotating evaporator at 40°C. The percentage of total oil to the weight of seeds was calculated. The previous steps were repeated for the solvents: petroleum ether (b.p. 40-80°C), benzene and tetrachloromethane.

Determination of physical and chemical properties of aniseed oil

The physical and chemical properties were determined for aniseed oil by soaking in hexane at room temperature.

Determination of Refractive Index

The measurement was carried out at 20°C. The following correction equation was applied for the temperature differences¹⁶.

$$n^T = n^{T1} - (T - T1) F, T_1 < T$$

$$n^T = n^{T1} - (T1 - T) F, T_1 > T$$

Where

F: Correction factor = 0.00035

T₁: Temperature at measurement

T: = 20°C

Determination of density

The density was calculated (weight of oil to weight of distilled water) using 10 ml of volumetric flasks at 20°C.

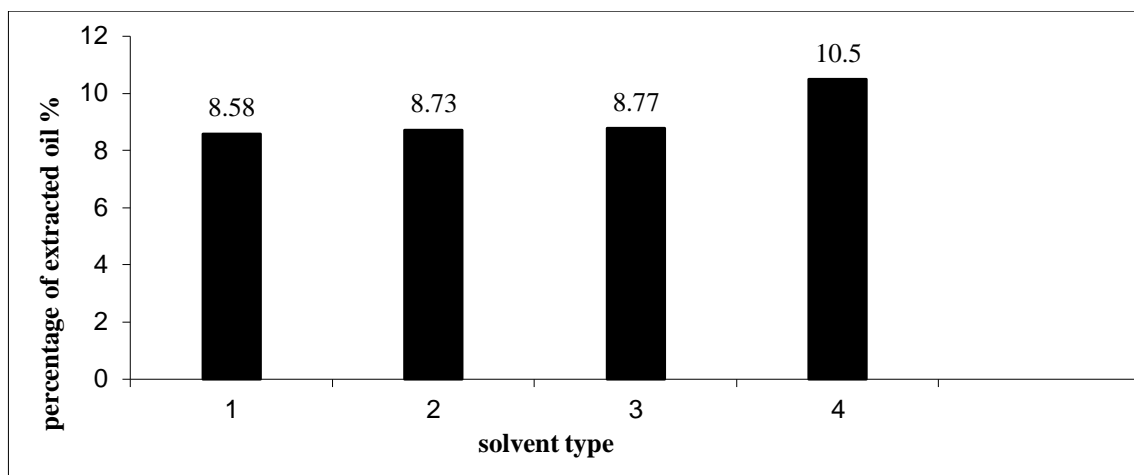


Figure 1: Percentage change of extracted aniseed oil by changing the type of solvent

Table 4: Decreased of main components quantity in the aniseed oil with time under the long-term stability conditions (25°C, 60%RH).

| Time (month) | total oil % | volatile oil % | Anethole in volatile oil % | Anisaldehyde in volatile oil % | Estragole in volatile oil % | Linalool in volatile oil % |
|--------------|-------------|----------------|----------------------------|--------------------------------|-----------------------------|----------------------------|
| 0 | 8.77 | 4.83 | 86.99 | 0.564 | 2.971 | 0.0211 |
| 6 | 8.24 | 4.39 | 86.73 | 0.557 | 2.930 | 0.0203 |
| 12 | 7.91 | 4.08 | 86.55 | 0.552 | 2.898 | 0.0187 |
| 18 | 7.62 | 3.81 | 86.38 | 0.548 | 2.876 | 0.0168 |
| 24 | 7.15 | 3.38 | 86.35 | 0.542 | 2.862 | 0.0161 |

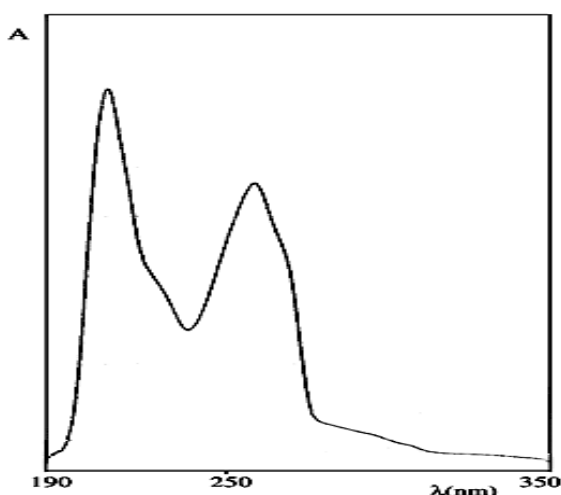


Figure 2: UV spectrum of the aniseed oil

The following correction equation was applied for the temperature difference^{4,7}.

$$d^T = d^{T_1} - (T - T_1) K, \quad T_1 < T$$

$$d^T = d^{T_1} + (T_1 - T) K, \quad T_1 > T$$

Where:

F: Correction factor = 0.00068

T₁: Temperature at measurement

T = 20°C

Determination of moisture and volatile substances

20g of oil was dried in oven (its temperature increased 10°C/min until 105°C), and placed in the oven for 3 hours at same temperature (105 °C) and cooled in air-free

conditions. The weight of sample was calculated. The previous steps were repeated until fixing the weight. The following equation was applied¹⁶:

$$\text{Moisture and volatile compounds \%} = (\text{difference of two weights} / \text{oil weight}) \times 100$$

Determination of acidity and acid value

Acidity value is number of milligrams of potassium hydroxide that is added to neutralize the acidity of 1 gram of oil.

The calculation of acidity and free acidity were carrying out by solving 10 g of oil in 50 ml of neutralized mixture contain equal volumes of ethanol and diethyl ether. The resulting mixture was calibrated with potassium hydroxide in ethanol (0.1N) in the presence of phenolphthalein 1%. The percentage of acidity in oil was calculated from this equation¹⁶:

$$\text{Free acidity} = a M N / 10 P$$

Where:

a: Volume of Reagent (ml)

M: Molecular weight of oleic acid = 282 g/mol

N: Normally Reagent

P: Mass of Oil (g)

The acidity value was calculated using this equation:

$$\text{Acidity value} = 56.1 a N / P$$

Where: 56.1 is the molecular weight of KOH

Determination of saponification Value

Saponification value is number of milligrams of potassium hydroxide that is needed to saponificate 1 gram of oil.

25 ml of potassium hydroxide in ethanol (0.5N) was added to 2 g of oil. The mixture was refluxed for one hour.

Titration of hot mixture with HCl (0.5N) was carried out in the presence of phenolphthalein 1%¹⁶.

Saponification Value = $56.1 N (a - b) / p$
Where:

A control sample was analysed and the saponification value was calculated from the following equation:

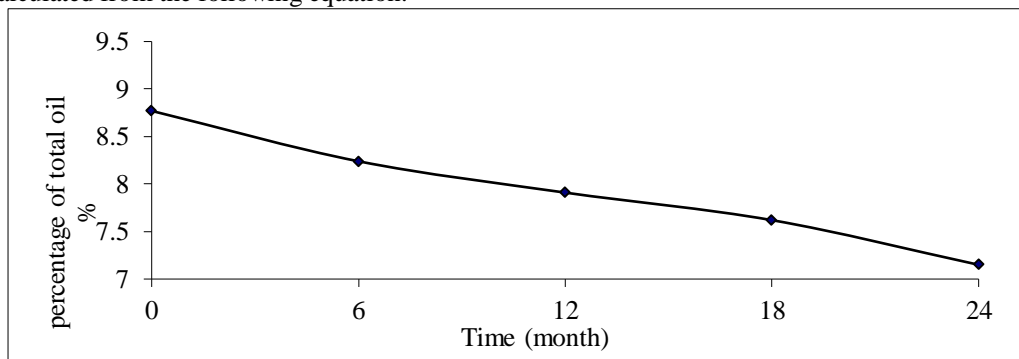


Figure 3: Decreased of aniseed volatile oil percentage with time under the long-term stability conditions (25°C, 60%RH).

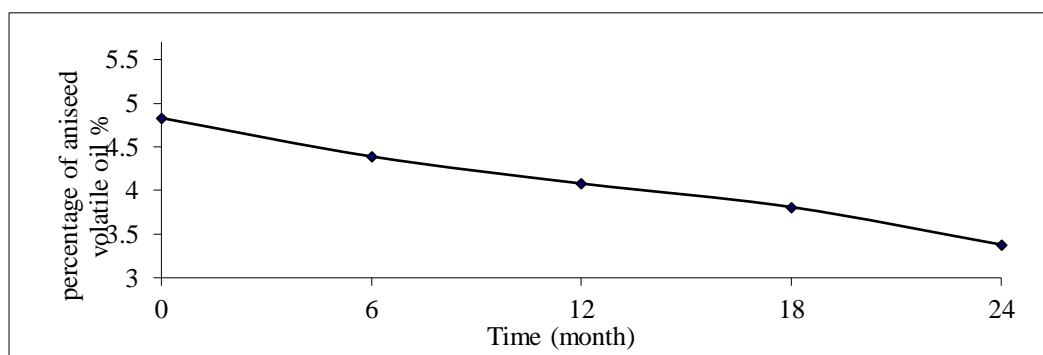


Figure 4: Decreased of anethole percentage in volatile oil with time under the long-term stability conditions (25°C, 60%RH).

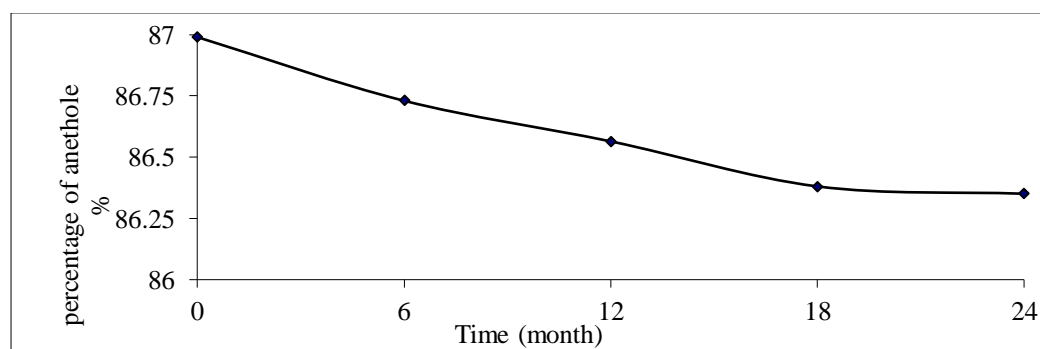


Figure 5: Decreased of anisaldehyde percentage in volatile oil with time under the long-term stability conditions (25°C, 60%RH).

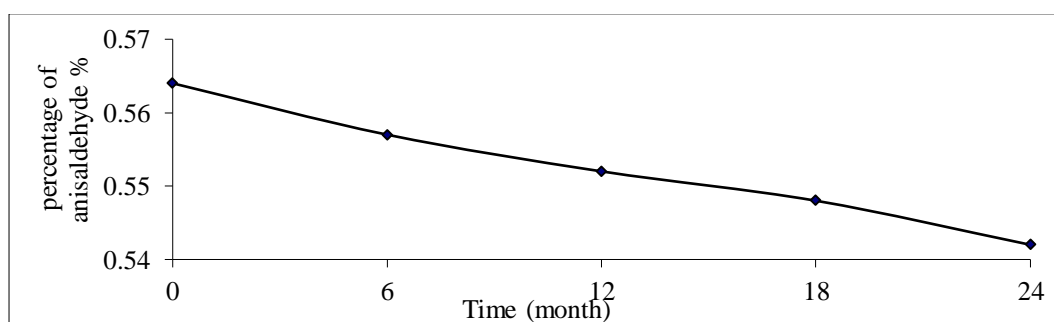


Figure 6: Decreased of estragole percentage in volatile oil with time under the long-term stability conditions (25°C, 60%RH).

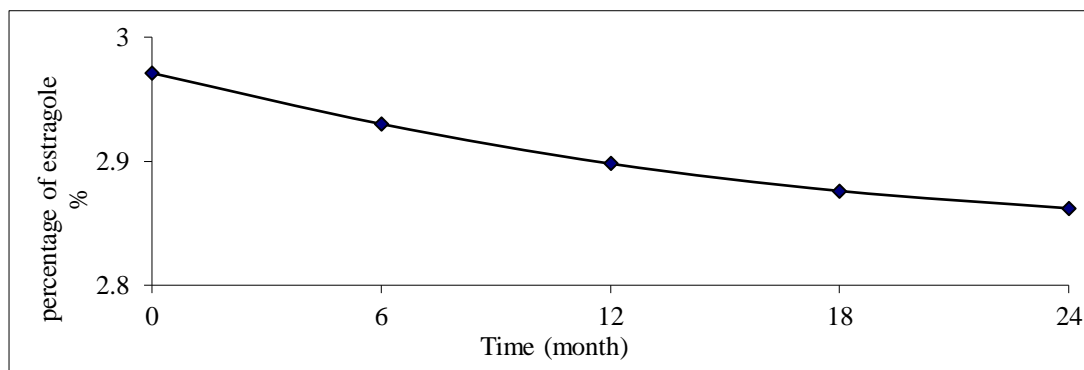


Figure 7: Decreased of linalool percentage in volatile oil with time under the long-term stability conditions (25°C, 60%RH).

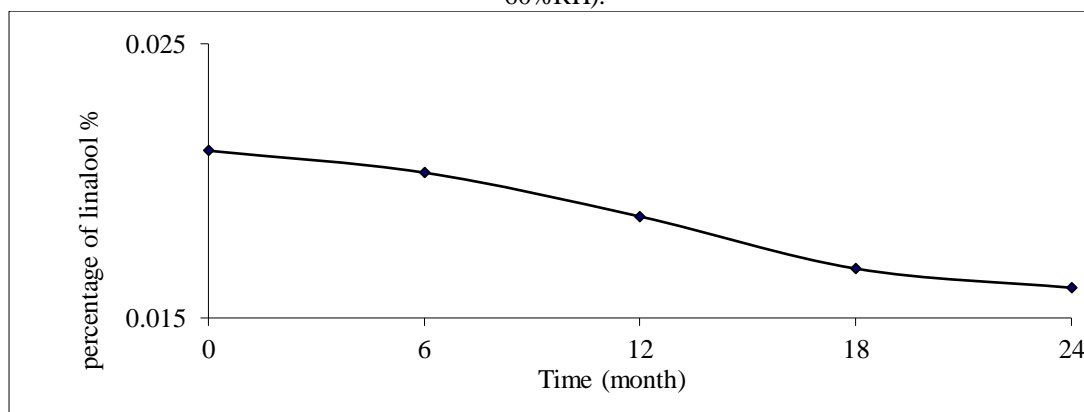


Figure 8: Decreased of linalool percentage in volatile oil with time under the long-term stability conditions (25°C, 60%RH).

a: Volume of HCl for titration the excess of HCl.

b: Volume of HCl for titration the HCl in the control sample.

N: Normality of HCl (N)

P: Mass of the oil (g)

Absorptivity in Ultraviolet (UV)

The solution (1%) of aniseed oil in ethanol was prepared and UV scan was carried out at a range of (190-400 nm), and the result was compared with reference values⁷.

Study of long-term effect on the quantity of main components in the aniseed oil

Samples of aniseed were placed in stability chamber under regular storage conditions (25°C ± 2°C / 60% RH ± 5% RH) for 2 years. Then, GC analyses of the aniseed volatile oil were carried out each 6 months¹⁷. The component of volatile oils was identified qualitatively and quantitatively using gas chromatography GC by comparison with standards, and the following conditions applied
Chromatographic column is Carbowax 20M, 2 mm × 2 mm.

Micro-sized injected 5 µl

Nitrogen carrier gas flow capacity of 0.5 ml / min

Degree Injector heat 200° C The degree of the primitive temperature 90° C is maintained for a period of 4min

The final temperature 210° C is maintained for a period of 15min

Heating speed of 2 degrees per minute within the range 90°C to 210°C

The percentage was calculated for anethole, anisaldehyde, linalool and estragole in the volatile oils using gas chromatographic technique (GC) that were compared with standard compounds. The areas of peaks were compared with the others that belong to standard compounds.

RESULTS AND DISCUSSION

Select of the best method for separation of aniseed volatile oil

Several methods were performed to extract anise volatile oil. The results in table (1) show the different yield of anise volatile oil by different extraction methods. By comparing the results in Table (1), we found the extraction of anise volatile oil using organic solvents has higher yield from percolation with water vapor. In addition, Also the extraction using soaking method with organic solvents at room temperature has higher yield from using the Soxhlet apparatus.

Studying of the Solvent Influence on the Quantity of the total Aniseed Oil

The percentage of total extracted aniseed oil was calculated by changing the solvents (hexane, petroleum ether (40-80°C), tetrachloromethane and benzene) and fixing the solvent volume and aniseed weight. The results are shown in Table (2).

The figure (1) shown percentage change of extracted aniseed oil by changing the type of solvent.

(1): tetrachloromethan, (2): petroleum ether (b. p. 40-80 C°), (3): hexane, (4): benzene

Table (1) and Figure (1) show that among the four solvents, benzene is the strongest for the extraction of aniseed oil, followed by hexane and then petroleum ether (b.p. 40-80 C°) and then by tetrachloromethan.

It has been found the benzene and hexane are able to extract the main components of aniseed oil when using the different chromatographic methods. Therefore, hexane is considered the best solvent for extraction of aniseed oil, benzene should be avoided due its high toxicity.

Determination of physical and chemical properties of total aniseed oil

The physical and chemical properties of total aniseed oil was determination and the results are shown in Table (3). Maximum absorption was at 210 nm and 259 nm in the UV absorption spectrum of the aniseed oil (Figure 2).

Study of long term effect on the quantity of essential components in the aniseed oil

Under the long-term stability conditions (25°C, 60%RH), the contents of quantity of main components in the aniseed oil was decreased (table 2). The following Figure show the changing of each the total aniseed oil, aniseed volatile oil and main components in the aniseeds with the time: Figure (3): Decreased of total aniseed percentage with time under the long-term stability conditions (25°C, 60%RH). Table (4) and Figures (3), (4), (5), (6), (7), and (8) shows that decreased of total aniseed oil, aniseed volatile oil, anethole, anisaldehyde, estragole, and linalool quantity in the aniseeds with time under the long-term stability conditions (25°C, 60%RH), where:

The total aniseed oil decreased 18.47%

The volatile aniseed oil decreased 30.02%

Anethole decreased 0.73%

Anisaldehyde decreased 3.9%

Estragole decreased 3.6%

Linalool decreased 23.69%

CONCLUSION

In conclusion, extraction of volatile Aniseed oil was performed by using different solvents. The best method of extraction was by soaking the seeds in an organic solvent at room temperature. Hexane is a good solvent to extract total anise oil because of higher yield and less toxic properties. From the physical and chemical properties of the aniseed oil, it was found that aniseed oil is used as a therapeutic drug, but it cannot be used as cooking or food oil. Studying the long-term stability conditions (25°C, 60%RH) for two years, it was found that the percentage of

aniseed oil and its main components were decreased with time.

REFERENCES

1. Salem M., 2012, "Medicinal herbs", Almarreek pub.; Reyad- KSA
2. Labania M., 2012, "spices", Alsaboni pub.; Cairo.
3. The Arab Organization for Agricultural Development, 2008, "Medicinal, aromatic and poisonous plants", League of Arab States; Alkartoom.
4. Dhemoth A. S., 1997, "Medicinal plants and treatment methods (Part I)", Albatraa pub.; Amman.
5. Hakeem W.; Hasan Aga M. E.; Badawi S.; Alkadi E.; Darkalt A.; Alshater Z.; Ebrahim Th.; Karbesa M., 2012, "Atlas of medicinal and aromatic plants in the Arab world", the Arab Center for the Studies of Arid Zones and Dry lands; ACSAD; Damascus.
6. Hasan Aga M. E.; Alshamaa E.; Alnor M. S., 2012, "Drugs and Phytochemistry", Damascus University pub.
7. Dahhan M., 1992, "Oils Technology", Aleppo University pub.
8. Huet, R., 1991, "Les Huilles essentielles d'agrumes Fruits", 46(5):551576.
9. Choi H.S.; Sawamura M.; Kondo Y., 2002, "Jornal of Food Science (Vol.67)", Pages : 1713-1718.
10. Felix D.; Melle. J. P.; Routaun Boka, 1997, "Handbook of plant and fungal toxicants", New-York.
11. Gil M. I.; Ferreres F.; Barberan T., 1999, "Effect of post-harvest storage and processing on the antioxidant constituents (flavonoide and vitamin C) of fresh-cut spinach", J. Agric. Food chem. 47, pages: 2213-2217.
12. Miething Holger; Seger Volkmar, 1989, "Separation of non-polar compounds by droplet counter-current chromatography", J.Chromatographia, 478(2), 433-7.
13. Wallis T. E., 1991, "Textbook of pharmacognosy", Delhi-India.
14. Trimen. H; Bentley. R, 1992, "Medicinal plants", Allied Book center, Dehradun India.
15. Wagner H. ; Blatt S. , 1996, " Plant drug analysis" spreinger- verlag , Berli-Heidelberg
16. IUPAC, 1965, "Standard Methods of the oils and fats" , 5th ed., Butter worth's, London.
17. Nassani M., 2003, "Stability Study of Pharmaceutical Products Requirements and Principles", Journal of GXP Compliance, 7(3), 53-65.