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Potential Studies of Physical and Chemical Properties of Neuro-protective Walnut and Turmeric Oil - An Analytical Approach

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Research Article

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ABSTRACT

This project deals with the extraction and analysis of essential oils from walnut and turmeric. The oil samples were extracted by cold extraction, hot extraction and steam distillation methods using organic solvents such as petroleum ether, chloroform, acetone and methanol. The extracted oils were subjected to thin layer chromatographic studies to understand the nature and number of components present. They were also analysed in UV-VIS spectrophotometer for the presence of chromospheres & tested for antibacterial potential with two bacteria *E.Coli* and *Staphylococcus* sp.

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INTRODUCTION

Health benefits of Spices

Spices have a traditional history of use, with strong roles in cultural heritage, and in the appreciation of food and its links to health. Demonstrating the benefits of foods by scientific means remains a challenge, particularly when compared with standards applied for assessing pharmaceutical agents. Pharmaceuticals are small molecular-weight compounds consumed in a purified and concentrated form. Food is eaten in combinations, in relatively large, unmeasured quantities under highly socialized conditions. The real challenge lies not in proving whether foods, such as herbs and spices, have health benefits, but in defining what these benefits are and developing the methods to expose them by scientifically [1-4].

Typically, herbs and spices are used only in small amounts, so use them as often as you can to make the most of their health benefits. Along with vegetables, fruits, legumes, whole grains, nuts and seeds help to achieve a beneficial daily amount of protective plant foods.

Nuts

It's the seed of a tree. The fruits of many hardwood trees have four layers: an outer skin, a soft layer rich in sugars, a hard shell, and a seed. When we eat the soft layer, we call it a fruit. When we eat the seed we call it a nut **(figure 1).**

Figure 1. Different parts of a fruit

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Nut is a general term for the dry seed or fruit of some plants. While a wide variety of dried seeds and fruits are called nuts, only a certain number of them are considered by biologists to be true nuts. Nuts are an important source of nutrition for both human beings and wildlife.

Nutritional benefits

Several epidemiological studies have revealed that people who consume nuts regularly are less likely to suffer from coronary heart disease. Recent clinical trials have found that consumption of various nuts such as almonds and walnut scan lower serum LDL cholesterol concentrations. Although nuts contain various substances thought to possess cardioprotective effects, scientists believe that their acid profile is at least in part responsible for the hypolipidemic response observed in them. In addition to possessing cardioprotective effects, nuts generally have a very low glycemic index (GI). Consequently, dietitians frequently recommend nuts be included in diets prescribed for patients with resistance problems such as diabetes mellitus [5].

Essential oil

An essential oil is any concentrated, hydrophobic liquid containing volatile compounds from plants, which are called aromatic herbs or aromatic plants. They are also known as volatile or ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as oil of clove. Oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oils have specific chemical properties, and they give characteristic fragrances

Experimental

The first part of our experiment deals with procurement of Walnut (Juglansregia) and Turmeric (Curcuma Longa) samples from local markets in Muscat. Crushed in to small pieces of samples by using pestle and mortar. Take different amount of the materials for hot and cold extraction [6,7].

Cold extraction of walnut oil

200 g of crushed walnut sample was weighed, transferred to a 1000 ml conical flask and extracted with petroleum ether, chloroform, acetone and methanol. To petroleum ether powdered walnut was added and kept for 48 hours. After 48 hours it was filtered by simple filter funnel. The filtrate was distillated by water bath to evaporate petroleum ether (boiling point is 60-80°C). 50 ml beaker was weighted the remaining liquid was added to it and it was evaporated over

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water bath until all the solvent removed and got pure walnut oil. The amount of walnut oil was weighted. The same procedure can be used to extract the walnut oil by using chloroform, acetone or methanol **(Figure 2).**

Figure 2. Cold Extraction of walnut oil from petroleum ether.

Extraction of Walnut oil by solvent Extraction (Hot distillation condensers)

10 g of the crushed walnut was taken in a thimble. The Soxhlet apparatus was fixed and hot extraction was carried out, while in round bottom flask, petroleum ether was present. This operation was carried out for 2 hrs. When the color of petroleum ether changed to yellow; the solution was taken out. Distillation experiment carried out with hot extraction solution. The Solution is then concentrated on water bath. The amount of oil was weighted. The same procedure was repeated by changing the solvent as chloroform, acetone or methanol **(Figure 3).**

Figure 3. Cold Extraction of walnut oil from petroleum ether

Cold extraction of turmeric oil

500 g of crushed turmeric seeds was weighted and added into 1000 cm^3 conical flask. Petroleum ether was added and kept for 48 hours. After 48 hours, it was filtered by using filter funnel. The filtrate was distillated by water bath to evaporate petroleum ether (boiling point is 60-80°C). 50 ml beaker was weighted and the distillate was added and it

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was evaporated over water bath until all the solvent was removed and got pure turmeric oil. The amount of oil was weighted. The same procedure can be used to extract the turmeric oil by using chloroform, acetone or methanol.

Hot extraction of turmeric oil

10 g of turmeric powder was taken in a thimble and the Soxhlet apparatus was fixed and hot extraction was carried out taking in a round bottom flask by using the solvent petroleum ether. This operation was carried out for 2 hrs. When the color of petroleum ether changed to yellow, the solution was taken out. Distillation was carried out with hot extraction solution. The solution is then concentrated on water bath. The amount of oil was weighted. The same procedure was followed by changing the solvent such as chloroform, acetone or methanol.

Extraction of Turmeric oil by steam distillation

535 g of Turmeric powder was weighted, and placed in a round bottom flask, and 250 ml of distilled water was added. The distillation flask was heated using heating mantle. The distillate was collected. The distillate sample was taken in a separating funnel and 20 ml of chloroform was added to separate the organic layer from water layer. This process was repeated three times. Anhydrous Sodium Sulphate (in small amount) was added to the collected organic layer to absorb the traces of water and then the mixture was filtered. The filtrate sample was warmed on a boiling water bath to remove the chloroform **(Figure 4)**. The oil obtained was weighted to determine the yield and stored for further analysis [8-10].

Figure 4. Steam distillation of turmeric oil

Study on physical and chemical properties of Oils

Measurements of Density, color and acid values:

The density of walnut oil and turmeric oil was measured by measuring the mass of a known volume of the samples. Density was measured by using the following equation, Density = Mass/ Volume.

The colors of the walnut oil and turmeric oil were by using a UV chamber. A small spot of walnut oil was put in a pre-coated TLC plate. Then the TLC plate was kept in a UV chamber. The color was determined. The color of the turmeric oil was also determined by the same method.

Acid value is the number of mg of KOH required to neutralize the free acids present in 1 g of fats or oils. The acid value of the oils was found out as follows. Dissolved 10.2 g of walnut oil was dissolved in 50 ml of a mixture of equal volume

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of ethanol and dilute ether. 1 ml of phenolphthalein was added and titrated with 0.01 N NaOH until the solution remained faintly pink after shaking for 30 second.

Acid value = (n x 5.6)/w

n = Number of ml of sodium hydroxide consumed

w =Weight in grams of the substance

(Official values: Acid value should be less than 2)

The acid value of the turmeric oil was also determined.

Determination of solubility of the oils

The good solvent for oil is alcohol and hence it was used it in this experiment to know the solubility of oil, froth formation and formation of insoluble Mg and Ca Soap. 5 ml of the walnut oil was taken in a 250 ml beaker. 15 ml of alcoholic sodium hydroxide solution was added. The beaker was covered with a watch glass and heated the mixture on a boiling water bath. Then it was stirred with a glass rod from frequently. The process was completed when the oil was dissolved. The solution of oils was diluted with 15 ml of water .Using this solution the following experiments were carried out:

Froth Formation

1 ml of the prepared soap solution was taken in a test tube; 2 ml of water was added and shaken. The formation of forth was noticed which indicated soap formation.

Formation of insoluble Mg and Ca soap

1 ml of the soap solution was taken into two separate test tubes. To one of the test tubes Calcium Chloride ($CaCl_2$) solution was added and to the other test tube few drops of Magnesium Sulphate ($MgSO_4$) solution was also added. The formation of insoluble Ca and Mg soaps were noted.

The same procedure was used to determine the solubility of Turmeric oil.

UV-VIS spectral studies of oils

Visible spectrophotometer methods of analysis is one of the earliest instrumental techniques, but even today this analysis is considered to be one of the top ranking methods because of their overall utility and accuracy [11-13].These methods of analysis are based on measuring the absorbing of electromagnetic radiation by colored compounds in the visible part of the spectrum. If the analytes are colorless, they are converted into colored compounds by reaction with suitable reagent. Thus, these methods of analysis are based on the following aspects [14,15].

- Complex-formation reaction.
- Oxidation-reeducation process.
- A catalytic effect. In the last few decades, this technique has been extensively used for determination of the metals. For spectrophotometric methods of analysis, the following requirements must be fulfilled.
- Application of Beer's Law Limit.
- Stability of the color.
- Sensitivity, selectivity & reproducibility of the method.

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Beer's Law relates the absorbance with concentration of the analytes by the following expression

A= ε b c

Where the A is the absorbance, is the molar absorptivity, b is the path length of the absorbing medium (expressed in cm), C is the concentration of the absorbing solute (moles/liter). In general way, a straight line is obtained on plotting the absorbance against the concentration of the analytes.

Apparatus

A shmadzu UV-visible spectrometer (model NO.160A, Japan)

Procedures:

1 ml of extracted sample was dissolved in 24 ml of CCl_4 and then the spectrum of the compound was taken against CCl_4 as blank **(Figures 5-9).**

Figure 5. Shimadzu UV-visible spectrometer (model NO.160A, Japan)

Figure 6. Walnut oil from Chloroform

Figure 7. Walnut oil from petroleum ether

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Figure 8. Turmeric oil from methanol

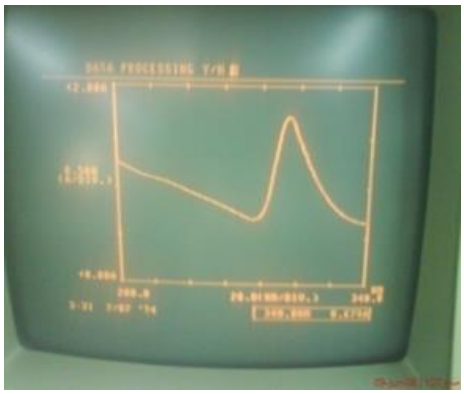

Figure 9. Turmeric oil from petroleum ether

PROCEDURES

1 ml of extracted sample was dissolved in 24 ml of CCl_4 & then the spectrum of the compound was taken against CCl_4 as blank. A shmadzu UV-visible spectrometer (model NO.160A, Japan) was used for analysis.

Thin layer chromatographic analysis of Essential Oils

Thin layer chromatography is a techniques used to study the components present in a sample mixture [16]. In this method, pre-coated TLC plates with silica gel (stationary phase) and different mobile phases viz. petroleum ether, chloroform, chloroform-methanol (90:10),(80:20), chloroform-acetone(90:10), and (80-20) were used to get an idea on the types and the number of components present in the sample [16-18].

Materials

Chromatography tube,-TLC plates (pre-coated),-chloroform, Acetone, Methanol, Petroleum ether, Iodine.

TLC of Walnut oil

Method:

A small amount of walnut oil was applied using glass capillary about 2 cm above the lower edge of activated Silica gel TLC plates. The spots were dried in an oven. The plate's strips were dipped in mobile solvent in the glass chromatographic jars by ascending chromatographic technique. In all cases, the mobile phase (solvent) was allowed to migrate up to 10 cm from the starting line of plate strips. The silica TLC plates were dried again and the components were visualized as colored spots by spraying with the detecting reagent **(Figure10&11).** The components were identified on the basis of their RF values.

TLC of Turmeric oil

The same procedure was used for Turmeric oil to study the TL

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Figure 10. Chromatogram of walnut oil

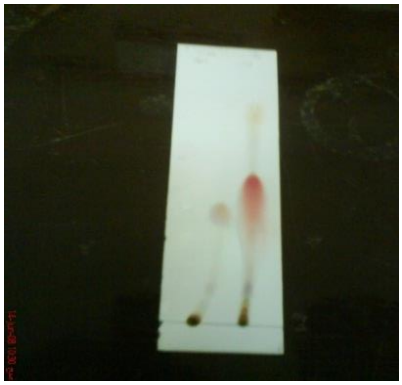

Figure 11. Chromatogram of turmeric oil

Materials and Method of evaluation of Antibacterial activity

1. Sterilized Nutrient Agar plates
2. Sterile filter paper discs
3. Ethylene glycol
4. Strain of bacteria (*E.Coli*)

Disc Diffusion method was used.

Overnight cultured bacterial strains were spread on Nutrient agar in Petri dishes. Extracted oils dipped in filter paper disks they were transferred by using sterilized forceps and placed on the agar surface and pressed down to ensure complete contact with medium The plates were incubated for 48 hours at 37˚C. At the end of 48 h, the zones of inhibition by the essential oils over the bacteria were measured **(Table 1-8).**

RESULTS

Table 1. Extraction of Walnut oil with different solvents by Cold Extraction method

Solvent	Weight of Walnut	Weight of essential oil	Yield (%)
Petroleum Ether	400 g	80.2 g	20.05%
Chloroform	400 g	19.4 g	4.85%
Acetone	400 g	7.4 g	1.85%
Methanol	400 g	1 g	0.25%

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Table 2. Extraction of Walnut oil with different solvents by Hot Extraction method

Solvent	Weight of Walnut	Weight of essential oil	Yield (%)
Petroleum Ether	10 g	4.2 g	42%
Chloroform	10 g	1.2 g	12%
Acetone	10 g	0.4 g	4%
Methanol	10 g	0.8 g	8%

Table 3. Extraction of Turmeric oil with different solvents by Cold Extraction method

Solvent	Weight of turmeric powder	Weight of essential Oil	Yield (%)
Petroleum Ether(60-80c)	500 g	19.5 g	3.9%
Chloroform	500 g	40.8 g	8.2%
Acetone	500 g	25.6 g	5.12%
Methanol	500 g	50 g	10%

Table 4. Extraction of Turmeric oil with different solvents by Hot Extraction method

Solvent	Weight of Turmeric Powder	Weight of essential Oil	Yield (%)
Petroleum Ether(60-80c)	10 g	2.1 g	21%
Chloroform	10 g	1.2 g	12%
Acetone	10 g	0.8 g	8%
Methanol	10 g	1 g	10%

Table 5. Extraction of turmeric oil by steam distillation

Spices	Wt. of oil(g) extract	Yield (%)
Turmeric Powder	0.245 g	0.046%

Table 6. Results of physical and chemical properties of oil

Properties	Walnut oil	Turmeric oil
Color	Bright yellow	Bright yellow
Appearance	oily	oily
Density	1.2	1.31
Acid value	0.0056	0.025
Froth Formation	Froth formed	Froth Formed
Mg salt formation	Insoluble salt formation	Insoluble salt formation
Ca Salt formation	Insoluble salt formation	Insoluble salt formation

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Table 7. TLC Results of Walnut oil

Solvents used for extraction	Mobile phase	Number of spots in Hot extraction	Number of spots in Cold extraction
Petroleum Ether	Petroleum Ether	Spot(1)R_{f1}=0.50880 Spot(2)R_{f2}=0.571698	**Spot(1)R_{f1}=0.50880 Spot(2)R_{f2}=0.571698**
$CHCl_3$	$CHCl_3$	Spot(1)R_{f1}=0.305 Spot(2)R_{f2}=0.74729	**Spot(1)R_{f1}=0.3729 Spot(2)R_{f2} =0.7729**
CHCl3	$CHCl_3$:Methanol 45:5	Spot(1)R_{f1}=0.933 Spot(2)R_{f2} =0.5611	**Spot(1)R_{f1}=0.2611 Spot(2)R_{f2} =0.6833**
Acetone	$CHCl_3$	Spot(1)R_{f1}=0.0536 Spot(2)R_{f2} =0.2438 Spot(3)R_{f3}=0.5714	**Spot(1)R_{f1}=0.0743 Spot(2)R_{f2} =0.0516 Spot(3)R_{f3}=0.2027 Spot(4)R_{f4}=0.2643 Spot(5)R_{f5}=0.816**
Acetone	$CHCl_3$: Acetone 45:5	Spot(1)R_{f1}=0.09 Spot(2)R_{f2} =0.088 Spot(3)R_{f3}=0.703	**Spot(1)R_{f1}=0.08 Spot(2)R_{f2} =0.088 Spot(3)R_{f3}=0.2102 Spot(4)R_{f4}=0.903**
Methanol	**$CHCl_3$: Methanol 45:5**	**Spot(1)R_{f1}=0.0563 Spot(2)R_{f2} =0.067 Spot(3)R_{f3}=0.1945 Spot(4)R_{f4}=0.364 Spot(5)R_{f5}=0.709**	Spot(1)R_{f1}=0.0563 Spot(2)R_{f2} =0.069 Spot(3)R_{f3}=0.1945 Spot(4)R_{f4}=0.364 Spot(5)R_{f5}=0.709

Table 8. TLC Results of Turmeric oil

Solvent used for extraction	Mobile phase	Number of spots in Hot extraction	Number of spots in Hot extraction
Petroleum Ether	Petroleum Ether	Spot(1)R_{f1}=0.5088 Spot(2)R_{f2} =0.5716	**Spot(1)R_{f1}=0.5088 Spot(2)R_{f2} =0.5716**
$CHCl_3$	$CHCl_3$	Spot(1)R_{f1}=0.0298 Spot(2)R_{f2} =0.339 Spot(3)R_{f3}=0.1684 Spot(4)R_{f4}=0.408	**Spot(1)R_{f1}=0.0298 Spot(2)R_{f2}=0.229 Spot(3)R_{f3}=0.1980Spot(4)R_{f4}=0.377**
CHCl3	$CHCl_3$:Methanol 45:5	Spot(1)R_{f1}=0.0203 Spot(2)R_{f2} =0.1528 Spot(3)R_{f3}=0.6439	**Spot(1)R_{f1}=0.0703 Spot (2)R_{f2} =0.1428 Spot(3)R_{f3}=0. 6439**
Acetone	$CHCl_3$:Aceton 75:5	Spot(1)R_{f1}=0.08559 Spot(2)R_{f2} =0.3574 Spot(3)R_{f3}=0.91618	**Spot(1)R_{f1}=0.07823 Spot(2)R_{f2} =0.4426 Spot(3)R_{f3}=0.8509 Spot(4)Rf4=0.99705**
Acetone	$CHCl_3$: Acetone 90-10	Spot(1)R_{f1}=0.06730	**Spot(1)R_{f1}=0.07730 Spot(2)R_{f2} =0.2825**
Methanol	**$CHCl_3$: Methanol**	**Spot(1)R_{f1}=0.05909 Spot(2)R_{f2} =0.1426 Spot(3) R_{f3}=0.7666**	Spot(1)R_{f1}=0.05909 Spot(2)R_{f2} =0.1426

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Result of antimicrobial activity Studies

The walnut oil did not show any antimicrobial activity under the concentrations studied. It was observed that the turmeric oils had significant antimicrobial activity against *E.Coli* bacteria and the zone of inhibition was for turmeric oil was 1.5 cm.

DISCUSSION

Essential oils were extracted from nuts and spices by three methods viz. hot extraction, cold extraction (both by using organic solvents) and also by steam distillation. For walnut, the cold as well as hot extraction methods gave better yields of oil with petroleum ether as extraction solvent.

For turmeric, methanol is the best solvent for the extraction of oil by cold extraction and in hot extraction method, petroleum ether was found to be the suitable solvent. Turmeric oil also was extracted by the steam distillation because it contained moisture which will hamper the extraction by organic solvents.

Thin layer chromatographic technique was used to know the number and nature of the components present in the extracted oils. In walnut oil, it was found in the extract, obtained by cold method that contained 5 major components which was extracted by acetone. The one obtained from methanol by both hot and cold methods, also had five components. When the other solvents were used as mobile phases, less than five components were eluted on TLC plates.

In both hot and cold extraction techniques, the turmeric oil extracted with chloroform showed better separation (with chloroform as the mobile phase) and showed the presence of four components.

It was noticed that the walnut oil (petroleum ether and chloroforms extractions) showed chromophore absorption at the λ=290 nm, likewise the turmeric oil extracted with petroleum and methanol had peaks corresponding to λ= 300 nm.

In the antibacterial analysis, *E.Coli* bacteria was used and found that turmeric oil had better antibacterial activity than walnut oil .The walnut oil didn't exhibit any antibacterial activity with the concentrations studied.

Acid value is a measure of oxidation of oil. Both walnut oil and turmeric oil on exposure to air underwent rapid oxidation and became rancid (spoilage) and their acid values were 0.0056 and 0.025 respectively. But comparatively, since turmeric oil has higher value which indicated that it was more susceptible for rancidity.

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CONCLUSION

The plant oils have wide application in the day to day life of human beings. Many oils are used as food and medicines all over the world. Scientific studies clearly show the composition and utility of these oils in addition to improve the existing methods of their extraction.

For this study, two species viz. walnut and turmeric were taken and the oils have been extracted by three methods - steam distillation, cold extraction and hot extraction. It was found that steam distillation is not an effective method while the extraction by organic solvents is very effective. The analysis of the above two oils gave an idea on their composition, types of compounds present, and preliminary information about their antibacterial potential.

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