ORIGINAL ARTICLE

QUALITATIVE DETERMINATION OF HERBAL OILS USED IN VARIOUS HERBAL AND COSMECEUTICALS FORMULATION

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ABSTRACT

Objective

The object of present study is to analyze the various oils used in the preparation of herbal formulations and available in the market. However, it is uncertain whether these raw materials comply with the standards prescribed in the pharmacopeias.

Methods

In this study three oils of plant source i.e.**Syzygium aromaticum**, and **.**have been obtained from the market and various quality control tests including Physico-chemical characteristics, thin layer chromatography (TLC), spectrophotometric assay (British Pharmacopoeia) have been performed to determine their compliance with the officials standards. The TLC has been used for the identification of the active ingredients on comparison of their RF values with the reference standard.

Result

The qualitative and quantitative evaluation has been carried out on the selected herbal oils. Some of them were found to comply with official standards while some did not comply probably due to adulteration or being spurious or substandard raw material. Though this type of study provide useful data that would be helpful for manufacturer in the authentication of these oil obtained from plant sources for formulation purpose.

Keywords:Syzigium aromaticum,s, **,** TLC, Quality Control Tests.

INTRODUCTION:

The Importance of medicinal plants has been innate from generation to generation. Medicinally rich plants are endorsed all around the world. The herbal system of medicine is now extended and well established. Herbals are frequently incorporated in medicines, cosmaceuticals and nutraceuticals. But the quality control parameters are still questionable. Globally there is a dramatic increase in the popularity and acceptance of herbal medicines while the availability and quality of raw material are still problematic. A demographic study shows that, Pakistan is enriched in naturally available medicinal plants that is abundant in Hazara, Malak, and Kurram, muree hills, azad Kashmir, northern areas and Balochistan or are cultivated on farmlands in Sindh, Punjab, Balochistan, Khyber Pakhtoon Khwan and Kashmir.[1, 2]

The selected oils have been obtained from plants rich in pharmacological activities and found in various commercially available pharmaceutical

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andcosmological products either as an active

orexcipients include, **Syzigium aromaticum** (clove oil),**Eucalyptus globulus** (Eucalyptus oil) and (olive oil).

Syzigium aromaticum:
Clove oil contain 23 identified constituents the major was eugenol (76.8%), followed by â-caryophyllene (17.4%), á-humulene (2.1%), and eugenyl acetate (1.2%) clove oil also act as chelating agent and shows antioxidant activity.[3]

The study shows that the Clove oil act as a favorable anesthetic for temperate Australian intertidal fishes as mortality is extremely low.[4,5] Eugenol have considerable antifungal activity against clinically relevant fungi (Candida, Aspergillus).[6] In-vitro study of clove oil reveals the cytotoxic activity towards human fibroblasts and endothelial cells. Clove oil was considered to be highly cytotoxic at concentrations as low as 0.03% (v/v).[7]

Eugenol is the main active ingredient of clove and has been chosen as a marker compound for the chemical evaluation or QC of clove. Quantitative analysis of the 15 clove samples showed that the content of eugenol varied significantly, ranging from 163 to 1049 ppb. The method of determination of eugenol by HPLC is accurate to evaluate the quality and safety assurance of clove.[8] In the recent study, An HPTLC densitometric method for the simultaneous determination of cinnamaldehyde and eugenol as well as trace amounts of piperine in pepper-contaminated cinnamon was developed The accuracy of the method was checked by conducting a recovery study at three different levels. The linearity was found to be in the ranges 52.54–735.56, 533.2–8531.2 and 50–300 ng/spot, respectively, with correlation coefficients of 0.9985 ± 0.04, 0.9982 ± 0.06 and 0.9937 ± 0.11 for cinnamaldehyde, eugenol and piperine.[9]

Eucalyptus globulus:
The chemical constituents of the essential oil obtained from the leaves of Eucalyptus globulus Labill are 1, 8-eucalyptol (72.71 %), α-pinene (9.22 %), α-terpineol (2.54 %), (-)-globulol (2.77 %), α-terpineol acetate (3.11 %), and alloaromadendrene (2.47 %).[10]

Eucalyptus oil has antiviral activity against herpes simplex. Eucalyptus oils used as benign pest control against bacteria, fungi, insects, nematodes, weeds and mites.[11] An Invitro study reveals the antibacterial study of eucalyptus oil againstEnterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella choleraesuis, Shigella flexneri, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, S. saprophyticus, and S. xylosus.[12] Eucalyptus oil also used as cosurfactant on the transdermal delivery of hydrocortisone (model drug) from eucalyptus oil microemulsion.[13,14]

According to British Pharmacopoeia (BP) and European Pharmacopoeia standard Eucalyptus oil must contain not less than 70.0% w/w 1, 8-cineole (eucalyptol). In a recent study, thirty different eucalyptus oil samples were scanned on the FOSS NIRSystems 6500 Rapid Content Sampler using a reflectance vessel as sample presentation method. The range of cineole contents content in eucalyptus oil, is between 52.5 to 99.0% w/w.[15]

Olea europea:
An in vitro and in vivo study indicates that olive oil phenolics are powerful antioxidants. The unusual pungenttaste and high stability of extra-virgin olive oil is due to its phenolic compounds, i.e., hydroxytyrosol and oleuropein.[16]

Virgin olive oil (VOO) possesses the most abundant natural antioxidants i.e. hydrophilicphenols. Instead of tocopherols and carotenes are also present. The hydrophilic phenols found in VOO are phenolic alcohols and acids.[17,18] The polyphenolic compounds found in olive oil are capable of several biologic activities such as lower incidence of coronary heart disease.[19,20] Olive oil posses several important biological properties include antioxidant, anti-inflammatory, chemo preventive and anti-cancer activities.[17]

Lately, a new spectrophotometric assay for the determination of the polyphenolic content of olive

oil has been introduced. It is a substrate-recycling assay for phenolic compounds that employs tyrosinase in the presence of excess NADH. The amount of total polyphenols was determined in virgin olive oils by both with the Folin-Ciocalteu reagent and with the enzymatic method. The results suggest a better estimation of the polyphenol content, as compared with the colorimetric method finally, the enzymatic method demonstrates that there is a linear relationship between the olive oil phenolic content and the antioxidative capacity of oil extracts.[21] Another new procedure for determining free fatty acids (FFA) in olive oil based on spectroscopic Fourier transform infrared-attenuated total reflectance spectroscopy measurements is proposed. The best results obtained in the 1775–1689 cm^{-1} range, using 3 PLSR components. In both concentration ranges 0.1 to 2.1%, at a confidence interval of $\alpha = 0.05$, no significant differences between the reference values and the calculated values were observed.[22]

MATERIALS AND METHODS:

Collection and identification of crude drugs

Three commercially available herbal oil were collected from the local market of Karachi that included Syzigium **aromaticum** (clove oil),**Eucalyptus globulus** (Eucalyptus oil) and Olea europaea (olive oil). These drugs were sampled according to their availability. The Samples of the drugs were coded as: Clove oil (C1-C3),Eucalyptus oil (E1-E3) and Olive oil (O1-O3). The authentic reference standards of the constituents of the crude drugs were obtained from Sigma Aldrich Co.

Evaluation of crude drugs:

The herbal oils were subjected to various quality control tests mentioned in the British Pharmacopoeia (BP 2016) to conform their authenticity. The techniques used for the evaluation of crude drugs included physicochemical parameters (relative density, optical rotation, acid value) and thin layer chromatography (TLC).

METHODOLOGY:

Determination of Relative Density:

The relative density of a plant extract or essential or fixed oil is determined to check the identity and purity of plant material. It is the ratio of the mass of a certain volume of a substance to the mass of an equal volume of water at a specific temperature.[23]

Determination of Refractive Index

It is a simple method to determine the purity of plant material because each plant material has specific refractive index and it can be determined with the help of a refract meter. In this apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined. The refractive index of a medium with reference to air is equal to the ratio of the sine of the angle of incidence of a beam of light in air to the sine of the angle of refraction of the refracted beam in the given medium.[23]

Determination Optical Rotation:

It is another trustworthy method to test the purity of plant extract, fixed oil and essential oil. Polari meter is the instrument used to determine optical rotation of any substance. Optical rotation is the property showed by chiral substances that has the plane of polarization of polarized light. Optical rotation is measured to be positive (+) for dextrorotatory substances (i.e. Those that rotate the plane of polarization in a clockwise direction) and negative (-) for laevorotatory substances.[23]

Thin Layer Chromatography (TLC):

This method is particularly important for qualitative assessment of plant raw material. Thin layer chromatography is a technique in which solute get separated between a stationary phase (silica or alumina) and a mobile phase which may be polar (ethanol, water, ethyl acetate) or non polar (toluene, ether) etc. The phenomena of separation may be adsorption or partition depending upon the stationary phase or solvent system used.[24]

Acid Value:

It is an analytical Method to measure the constants of fats and oil. It is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. The acid value determines the

cleavage of the triacylglycerol's into free fatty acids, which has an adverse effect on the quality of many lipids. Acid value is an indication about edibility of the lipid. It can be determines by the following formula.[23]

$I_A =$

Here,

n= ml of titrants

m= mass of substance

RESULTS AND DISCUSSION:

Syzygium aromaticum

The following quality control tests have been performed to check the authenticity of different clove oil samples i.e. C-1, C-2 and C-3.

Relative density:

The relative density of sample of clove oil is given in Table 1. The Relative density of the clove oil sample C-1 to C-3 is in the range of 0.435 – 1.063. The British Pharmacopoeia range is 1.030 to 1.063.[23] Thus, the sample C-1 and C-2 comply with the range mentioned in the BP monograph while C-3 is outside the range which may be due to some impurity/contamination.

Table-1. Relative Density of Syzygium aromaticum samples

S. No.	Sample	Weight of empty RD bottle	Weight of water	Weight of sample	RD
1	C1	24.0685	25.1333	26.3083	1.046
2	C2	24.0685	25.1333	25.7494	1.063
3	C3	11.1804	21.1116	9.191	0.435

Optical rotation:

The optical rotation of clove oil is reported in table 2. The range of optical rotation is between 0° to -2° in British Pharmacopoeia.[23] Thus samples of clove oil C-1 and C-2 show optical activity that complies with the range mentioned in BP monograph while C-3 is optically in active it means it is impure and is of substandard quality. On this basis the sample oil C-3 can be rejected.

Table-2. Optical rotation of Syzygium aromaticum samples

S. No.	Sample	Optical rotation
1	C1	-1.45
2	C2	-1.56
3	C3	Inactive

Refractive index:

The refractive index of clove oil samples, C-1 to C-3, is given in table 3. The range of Refractive index (RI) given in British pharmacopoeia is 1.528 to 1.537. The RI value of sample C-1 and C-2 complies with the range mentioned in the BP while C-3 does not comply with the values which proves that it is impure and may be of substandard quality. Thus, it fails to pass the quality control test.

Table-3. Refractive Index of Syzygium aromaticum samples

S. No.	Sample	Refractive index	Temp^o C
1	C1	1.538	25.0
2	C2	1.534	25.8
3	C3	1.474	24.7

Thin layer chromatography:
The **of** the sample of clove oil (C-1 to C-3) is given in Table 4. TLC of clove oil (Figure 5.18) showed the sample spot with slightly varying value in the range of 0.75-0.82 corresponding to the standard spot of eugenol with 0.74. Thus, the samples comply with the BP standard as mentioned in the monograph. The chromatogram obtained with the test solution shows a quenching zone (eugenol) that is similar in position to the quenching zone in the chromatogram obtained with the reference solution.

Table-4. Value of Syzygium aromaticum samples

Sanno.	Sample	value
1.	Standard	0.74
2.	C1	0.75
3.	C2	0.82
4.	C3	0.81

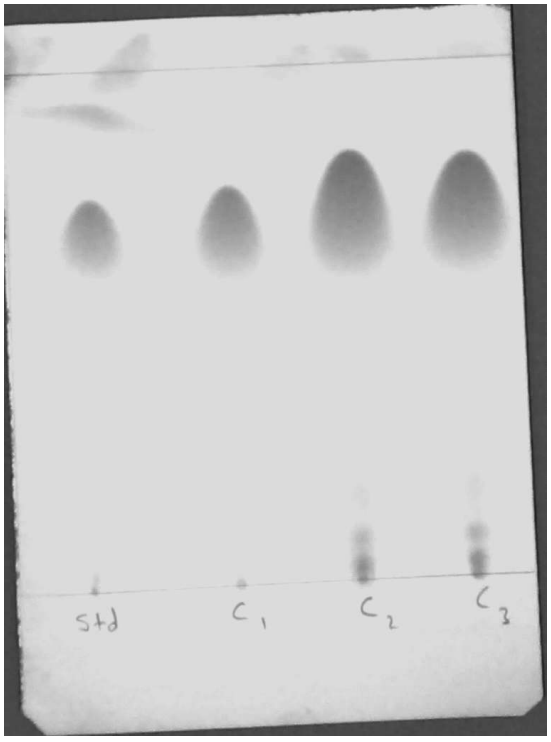

Figure 5.18 TLC of Syzigium aromaticum STD C1C2C3

Figure-1. Comparison between the values of the standard and sample

Eucalyptus globulas (Eucalyptus oil):
Following of the quality control test have been performed to check the authenticity of different eucalyptus oil samples i.e. E-1, E-2 and E-3.

Relative Density:
The relative density (RD) of eucalyptus oil samples E-1, E-2 and E-3 are given in Table-5. The relative density of eucalyptus oil samples was found to be in the range of 0.895-0.926. The sample E-2 and E-3 comply with the range mentioned in British Pharmacopoeia that is 0.906 to 0.927 while the RD of E-1 is below the range (0.895).[23]

Table-5. Relative Density of Eucalyptus globulas samples

S. No.	Sample	Weight of empty RD bottle	Weight of water	Weight of sample	RD
1	E1	24.0685	25.1333	22.6875	0.895
2	E2	24.0685	25.1333	23.2759	0.926
3	E3	24.0685	25.1333	22.9165	0.911

Optical rotation:

The Optical rotation of eucalyptus oil sample is given in Table 6. The range of optical rotation mentioned in British Pharmacopoeia is between 0° to +10. The samples of eucalyptus oil show optical rotation between 4.130 to 9.386.[23] Thus, all the samples are optically active and comply with the BP specifications.

Table-6. Optical Rotation of Eucalyptus globulas samples

S. No.	Sample	Optical rotation
1	E1	9.386
2	E2	4.130
3	E3	5.264

Refractive index:

The refractive index of eucalyptus oil samples is given in Table 7. The range of Refractive index given in BP is 1.458 to 1.470. Refractive index of the Eucalyptus oil samples was found to be in the range of 1.457 – 1.465. Thus, all the samples are complying with the specifications.

Table-7. Refractive Index of Eucalyptus globulas samples

S. No.	Sample	Refractive index	Temp C
1	E1	1.466	25.0
2	E2	1.457	25.8
3	E3	1.465	24.7

Olea europaea (Olive Oil):

Following of the quality control test have been performed to check the authenticity of different olive oil samples i.e. O-1, O-2 and O-3.

Relative Density:

The Relative density of oil samples O-1, O-2 and O-3 is given in Table-8. The range of relative density in British Pharmacopoeia is about 0.913.[23] Thus; all the samples were found to comply with the specification and were of good quality.

Table-5. Relative Density of Eucalyptus globulas samples

S. No.	Sample	Weight of empty RD bottle	Weight of water	Weight of sample	RD
1	O1	24.0685	25.1333	22.8713	0.910
2	O2	24.0685	25.1333	23.0221	0.916
3	O3	24.0685	25.1333	22.9969	0.915

Acid value

The acid values of olive oil samples O-1, O-2 and O-3 are given in Table-9. The standard acid value mentioned in BP (2013) should not be more than 0.3. Thus, the samples of olive oil (O-1 and O-2) comply with the standard value, while O-3 is slightly above the range that may be due to some impurity or any enzymatic degradation due to unfavorable storage condition.

Table-9. Acid Value of Olea europaea samples

S. No.	Sample	Acid value
1	O-1	0.26
2	O-2	0.31
3	O-3	0.50

Specific Absorbance:

The absorbance at 270 nm of different olive oil sample (O-1, O-2 and O-3) is given in Table10. The specific absorbance of olive oil is maximum 1.20 at 270 nm mentioned in British Pharmacopoeia.[23] The absorbance of olive oil samples (O-1 and O-2) are found to be comply with the range while O-3 sample is slightly above the official range. It may be due to some impurity or adulteration.

Table-10. Specific Absorbance of Olea europaea samples

No.	Sample	Weight of sample c	Absorbance A	Specific Absorbance	λ_{max}
1	O1	1.0899	0.600	0.550	270 nm
2	O2	1.2021	0.715	0.594	270 nm
3	O3	1.4521	2.005	1.377	270 nm

CONCLUSION:

The main dilemma in Pakistan is the quality or standard of herbal product or the raw material used in the formulation of commercial products. Unfortunately, there is no awareness in herbal drug manufacturer to check the quality of their products or the raw material used in the manufacturing of these products. In order to establish the quality, safety and efficacy of these drugs, ten herbal raw materials have been selected for this study. The qualitative and quantitative evaluation has been carried out on the selected herbal oils. Some of them were found to comply with quality standards while some did not comply probably due to adulteration or being spurious or substandard raw material. One of the important aspects of this study is to create awareness of quality testing of herbal oils among herbal, cosmaceuticals and pharmaceutical industries of the country that would be beneficial for the therapeutic outcomes of commercially available herbal products.

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