Research Article

Methods for the Authentication and Characterization of Canola (*Brassica napus*; Brassicaceae), Corn (*Zea mays*; Poaceae) and Cotton Seed (*Gossypium hirsutum*; Malvaceace) Oils

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Abstract

The study highlighted the authentication, identification and characterization of canola oil (*Brassica napus*; Brassicaceae), corn oil (*Zea mays*; Poaceae) and cotton seed oil (*Gossipium hirsutum*; Malvaceace) using Gas Chromatography-Mass Spectroscopy (GC-MS) (for phytoconstituents identification), Fourier Transform Infrared (FTIR) Spectroscopy (for structure elucidation) and Ultra Violet (UV) spectroscopy (for solubility studies). In GC-MS analysis18, 19 and 26 compounds were identified in canola oil, corn oil and cotton seed oil, respectively, which were interpreted in FTIR spectroscopy. In UV spectroscopy, correlation coefficient (R^2) of canola, corn and cotton seed oils at 275 nm were 0.9815, 0.9893 and 0.9972, respectively and the accuracy of the method was under range as per standard guidelines. The selected oils were miscible in chloroform, ether, hexane, toluene and petroleum ether. Canola, cotton seed oil was slightly miscible in ethanol (95%) whereas corn oil was practically immiscible but found miscible with benzene. As per ICHQ3C guidelines, benzene exist in class 1 and class first solvent should be avoided because of their carcinogen nature. Chloroform, hexane and toluene solvent exist in class 2 whereas ethanol and ether in class-3, which are toxic in nature if used above the permissible daily exposure (PDE) limit. Data obtained from the intra and inter-day solubility study showed that the precision of selected oils slightly varied in chloroform, petroleum ether. The results of canola, corn and cotton oils from GC-MS, FTIR and UV-spectroscopy provided the required information which will be very helpful for formulating these oils into stable dosage forms that will be suitable for human consumption, as otherwise it is very difficult to administer an oil as such.

Keywords: *Brassica napus*; *Gossypium hirsutum*; *Zea mays*; GC-MS; UV spectroscopy

Introduction

Extraction from the natural resources like oils, phytoconstituents and dietary nutrients offer very useful therapeutic properties against human disorders [1]. However, safety of bioactive phytoconstituents has received attention of the researchers and pharmaceutical industries to develop potential therapeutic formulations [2]. As the consumption of oils such as canola (*Brassica napus*; Brassicaceae), corn (*Zea mays*; Poaceae) and cotton seed (*Gossypium hirsutum;* Malvaceace) have been associated with wide therapeutic properties such as antipsoriatic, antioxidant, anti-inflammatory, anti-androgenic (5-α reductase inhibitors) but, the challenge is to identify phytochemicals and their chemical structures to integrate them into dosage forms for the novel therapeutic approach [3-6].

Extraction of oils is usually carried out with the help of solvents,

so there is a need to remove the solvent from the extract otherwise solvent can cause serious adverse health effects in the long run use [7]. So the selection of solvent for the extraction of fixed oils is very important in order to avoid adverse health effects. Solubility of a drug determines its concentration at the site of absorption and also affects its bioavailability [8]. Therefore, it is another important parameter before incorporating them into a suitable pharmaceuticals dosage form. The identification of adulterants is time consuming and need sophisticated instruments. Gas Chromatography-Mass Spectroscopy (GC-MS) is an easy and reliable technique to identify the presence of phytoconstituents and adulterant in oils. This is an effective tool to identify percentage area, retention time, number and chemical formula of the compounds present in oil and is an indicator to detect adulterant, if any [9,10]. Moreover, Fourier Transform Infrared (FTIR) spectroscopy is used to elucidate the chemical structure of an unknown sample and is commonly used in pharmaceutical and food industry to study edible oils, mainly for the quantitative and qualitative determination of specific compounds [11-13]. In literature, validated UV spectrophotometric method which is used for the solubility studies of oils is not clearly mentioned. Otherwise, UV spectrophotometric is quick, sensitive, non-destructive and cost effective tool for the solubility study of any compound in solvents like chloroform, hexane, ether and toluene [14]. As per ICHQ3C guidelines, the solvents which are used for the development of pharmaceutical excipients and drug products are categorized into three classes. Solvents of class 1 are considered as human carcinogens therefore, these solvents should be avoided in the pharmaceutical formulations. While, class 2 and 3 solvents are less toxic in nature when used in Permitted Daily Exposure (PDE) limit, above this limit, the solvents can cause toxic

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effects [15]. PDE is defined as the maximum acceptable intake per day of residual solvent in pharmaceutical products. Residual solvent is that amount of solvent remaining in the final product after drying, which is an also known as organic volatile impurity [7]. Thus, the present study is an attempt to perform GCMS, FTIR and UV-spectroscopy of canola oil (*Brassica napus*; Brassicaceae), corn oil (*Zea mays*; Poaceae) and cotton seed oil (*Gossypium hirsutum*; Malvaceace) to collect the pre-formulation data.

Materials and Methods

Oils and chemicals: *Brassica napus* and *Zea mays* oils were obtained from Deve Herbs India, while *Gossypium hirsutum* was obtained from RV Essential India. Chloroform and toluene were procured from Sisco Research laboratories Pvt. Ltd India. Hexane, petroleum ether and PEG 400 were purchased from Hi-Media Laboratories Pvt. Ltd. India. Ethanol (95%) was collected from Changshu Hongsheng Fine Chemical Co. Ltd. China. The solvents and co-solvents were of suitable analytical grade.

Derivatization of fixed oils for Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis: Canola oil (*Brassica napus*; Brassicaceae), corn oil (*Zea mays*; Poaceae) and cotton seed oil (*Gossypium hirsutum*; Malvaceace) are fixed oil. Therefore these oils were derivatized for GC-MS analysis. Firstly, 10 ml of n-heptane was added into the 0.170 g of sample and mixed by the vortex shaker machine. Then, 4 ml of 3.5% methanolic KOH was added into the above prepared mixture and shaken for 2 minutes. This mixture was placed on water bath at 70 °C for 2 minutes and repeated this step five times. The upper layer was withdrawn into a beaker and was evaporated till sample get dried. Then, 0.5 ml of n-heptane was added to the residue, mixed well and 0.2 microlitre of sample was injected for analysis.

GC-MS (instrumentation and sampling) technique: For the present study, GC–MS-QP2010 Plus computerized system (Shimadzu Corporation, Kyoto, Japan) was used to analyze canola oil, corn oil and cotton seed oil. The instrument comprised of AOC-20i auto injector, AOC-20 s head space sampler and a mass selective detector. Dimensions of the capillary column (Rtx-5MS) were 30 m (length) × 0.25 mm (diameter) × 0.25 μm (film thickness) and the packing material was cross bond, 5% diphenyl/95% dimethyl polysiloxane (Restek Corporation, Bellefonte, USA). These conditions were used to obtain GC-MS spectra: the temperature of ion source was 230°C and temperature of interface was set at 260°C for 2.5 min. Ionization was made by electronic impact at 70 eV with m/z range 40 to 650. With the flow rate 1.21 ml/min, helium (>99.999%) was employed as gas in split mode (10:1). Injection temperature was set at 250°C and 1.0 μl injection volume was used for the analysis. The temperature of oven was maintained for 3 min at 100°C and raised at the rate of 10°C/min up to 280°C and hold for the next 19 min.

Fourier Transform Infrared (FTIR) spectroscopy: FTIR (Spectrum RX; Perkin Elmer, Waltham, MA, USA) technique was employed to elucidate the chemical structure of canola, corn and cotton oils by KBr pellet method.

Ultra-violet spectroscopy method: UV absorbance spectra of canola corn and cotton seed oil were obtained using UV spectrophotometer (UV-VIS Spectrophotometer 117, Systronics India Limited). The maximum absorbance was observed at 275 nm using 10 mm quartz cuvette. ICH guidelines for standardization of pharmaceuticals were used to validate the method.

Preparation of standard solution: For calibration curve using Ultra-violet spectroscopy, 2000 μg/ml of oil standard stock solution was prepared and different dilutions were done for the preparation of further solutions.

Linearity: Calibration curve was prepared using five concentrations (n=3) of canola, corn and cotton seed oil in the range of 10 mcg/ml to 50 mcg/ml. It was calculated by linear regression analysis.

Accuracy: It was assessed by measuring the amount found against (using Ultra-violet spectroscopy) the actual amount taken and then calculated with the formula given below [14].

$$\text{Accuracy} = \frac{\text{Amount detected}}{\text{Amount added}} \times 100$$

Solubility analysis of canola, corn and cotton seed oil: In the present study, chloroform, ethanol (95%), hexane, toluene, petroleum ether and PEG-400 (15% v/v) were used to evaluate the solubility of canola, corn and cotton seed oil by adding required volume of oil to pre-determine the amount of solvent with constant stirring on magnetic stirrer.

Shake flask method for intra and inter-day solubility analysis: This method was used to determine the intra and inter-day solubility study of canola, corn and cotton seed oil in chloroform and petroleum ether. Samples were analyzed every 24 h for three days by recording absorbance at 275 nm using UV spectrophotometer. Continuous shaking was carried out using orbital shaker (Scigenics Biotech, India). The results were expressed in the form of precision (%RSD). The precision is considered as the variance or standard deviation of a series of measurements [14].

$$\text{Precision (\%RSD)} = \frac{\text{Standard deviation of measurement}}{\text{Mean value of measurements}} \times 100$$

Results

GC-MS analysis: The data GC-MS analysis of canola oil (*Brassica napus*; Brassicaceae), corn oil (*Zea mays*; Poaceae) and cotton seed oil (*Gossypium hirsutum*; Malvaceace) revealed the presence of 18,19 and 26 compounds respectively with their percentage area, molecular formula and molecular weight, which are available in the updated version of GC-MS (Shimadzu Corporation, Kyoto, Japan) (Table 1-3). Some of the phytoconstituents identified in these oils have been reported to possess multiple therapeutic effects by showing memory enhancing, anti-psychotic, anti-depressant, anti-microbial, anti-inflammatory, anti-oxidant activity etc., (Table 4).

FTIR Study

FTIR spectroscopy of canola oil: A broad peak is observed near 3467 cm^{-1} indicates the phenolic –OH group presence. The C-H stretching of alkane may be due to the presence of asymmetric –CH$_2$ is shown at 2925 cm^{-1} whereas the aromatic C-H stretch was found at 3009 cm^{-1}. Out of plane bending because of =C-H as peak is shown at 914 cm^{-1}. Four or more -CH$_2$ was shown at 722 cm^{-1} which shows the presence of long open chain of fatty acids of canola oil. Aromatic C=C is shown at 1458 cm^{-1} which coincides with CH$_2$ bending of methylene group. The presence of –CH$_3$ bending is shown at 1377 cm^{-1} and 1399 cm^{-1}. A strong peak is observed at 1747 cm^{-1} indicating the presence of C=O group presence. The presence of stretching vibrations due to symmetric –CH$_2$ is indicated at 2854 cm^{-1}. The peaks between 1000

Table 1: GC-MS report of canola (*Brassica napus*; Brassicaceae) oil.

S.No.	Compounds Identified	Molecular Formula	Molecular Weight	Area%
1	Oleylaldehyde	$C_{18}H_{34}O$	266	14.07
2	Oleic acid, trimethylsilyl ester	$C_{21}H_{42}O_2$	354	7.69
3	Oleic acid chloride	$C_{18}H_{33}C_{10}$	300	2.69
4	Linoleic acid chloride	$C_{18}H_{31}C_{10}$	298	1.18
5	Glycidyloleate	$C_{21}H_{38}O_3$	338	2.43
6	Decyloleate	$C_{28}H_{54}O_2$	422	0.44
7	Trans-squalene	$C_{30}H_{50}$	410	7.15
8	Delta-tocopherol	$C_{27}H_{46}O_2$	402	3.78
9	Cholesterylmyristate	$C_{41}H_{72}O_2$	596	0.95
10	Stigmasteryl acetate	$C_{31}H_{50}O_2$	454	0.45
11	Gamma-tocopherol	$C_{28}H_{48}O_2$	416	0.24
12	Beta-tocopherol	$C_{28}H_{48}O_2$	416	13.87
13	Clionasterol	$C_{29}H_{50}O$	414	1.79
14	Vitamin-E	$C_{29}H_{50}O_2$	430	3.57
15	Alpha-tocopheryl acetate	$C_{31}H_{52}O_3$	472	0.31
16	Campesterol	$C_{28}H_{48}O$	400	9.48
17	Stigmasterol methyl ether	$C_{30}H_{50}O$	426	8.83
18	Beta-sitosterol	$C_{29}H_{50}O$	414	21.09
				100

Table 2: GC-MS report of corn (*Zea mays*; Poaceae) oil.

S.No.	Name of compound	Molecular Formula	Molecular Weight	Area%
1	Cetylic acid	$C_{18}H_{36}O_2$	284	0.7
2	Palmitic acid	$C_{16}H_{32}O_2$	256	3.45
3	Ethyl oleate	$C_{20}H_{38}O_2$	310	77.41
4	Stearic acid	$C_{18}H_{36}O_2$	284	3.38
5	Linolseaure	$C_{18}H_{32}O_2$	280	1.12
6	Linoleic acid	$C_{18}H_{32}O_2$	280	1.35
7	Olealdehyde	$C_{18}H_{34}O$	266	0.15
8	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.5
9	Trilinolein	$C_{57}H_{98}O_6$	878	0.29
10	Callitrisic acid	$C_{20}H_{28}O_2$	300	0.5
11	Palmitic acid chloride	$C_{16}H_{31}C_1O$	274	1.02
12	Hexadecanoic acid	$C_{19}H38O_4$	330	0.22
13	Hydrofol acid 150	$C_{18}H36O_2$	284	0.16
14	Dihydro-aplotaxene	$C_{17}H_{30}$	234	0.23
15	Linoleic acid chloride	$C_{18}H_{31}C_1O$	298	0.22
16	Dihydro-aplotaxene	$C_{17}H_{30}$	234	1.38
17	Oleoyl chloride	$C_{18}H_{33}C_1O$	300	1.68
18	Oleic acid chloride	$C_{18}H_{33}C_1O$	300	6.06
19	Beta-sitosterol	$C_{29}H_{50}O$	414	0.19
				100

Table 3: GC-MS report of cotton (*Gossypium hirsutum*) seed oil.

S.No.	Common name	Molecular Formula	Molecular Weight	Area%
1	Tetradecanoic acid	$C_{14}H_{28}O_2$	288	0.15
2	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.7
3	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.67
4	Cyclodecene	$C_{10}H_{18}$	138	2.23
5	Oleic acid	$C_{18}H_{34}O_2$	282	4.13
6	Octadecanoic acid	$C_{21}H_{42}O_4$	358	0.64
7	Ethyl oleate	$C_{20}H_{38}O_2$	310	64.87
8	Octadecanoic acid	$C_{21}H_{42}O_4$	358	4.08
9	Linoleic acid	$C_{18}H_{32}O_2$	280	1.2
10	Linoleic acid	$C_{18}H_{32}O_2$	280	1.4
11	Glycidylpalmitate	$C_{19}H_{36}O_3$	312	0.19
12	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.38
13	Hexadecadienoic acid	$C_{17}H_{30}O_2$	266	0.31
14	Dehydroabietic acid	$C_{20}H_{28}O_2$	300	0.31
15	Linoleic acid	$C_{18}H_{32}O_2$	280	0.74
16	Linoleoyl chloride	$C_{18}H_{31}ClO$	298	0.71
17	Palmitic acid chloride	$C_{16}H_{31}ClO$	274	0.64
18	Beta-monopalmitin	$C_{21}H_{38}O_2$	322	0.23
19	Cetiol V	$C_{28}H_{54}O_2$	422	0.28
20	Dihydroaplotaxene	$C_{17}H_{30}$	234	1.01
21	Oleic acid chloride	$C_{18}H_{33}ClO$	300	0.76
22	Oleic acid chloride	$C_{18}H_{33}ClO$	300	5.57
23	Squalene	$C_{30}H_{50}$	410	0.19
24	Glyceryltridodecanoate	$C_{39}H_{74}O_6$	638	1.9
25	Gamma-sitosterol	$C_{29}H_{50}O$	414	1.12
26	Cycloartenol	$C_{30}H_{50}O$	426	0.59
				100

Table 4: Pharmacologically reported therapeutic activity of identified phytoconstituents.

Compound	Pharmacological activity	References
Vitamin E	Anti-inflammatory, antioxidant	[16]
Oleic acid chloride	Anti-microbial, antioxidant	[17]
Trilinolein	Anti-arrhythmic, anti-ischemic, antioxidant	[18]
Gamma-sitosterol	Anticancer , anti-diabetic , anti-atherogenic effect	[19]
Campesterol	Anticancer, anti-atherogenic property, prevent prostate hyperplasia	[19,20]
Beta-sitosterol	Anticancer , 5-alpha reductase inhibitor	[20,21]
Palmitic acid	Cardioprotective effect	[21]
Hexadecanoic acid	Anti-inflammatory	[22]
Stigmasterol	Acetylcholinesterase inhibition,5-α reductase inhibitor	[23]
Squalene	Anti-oxidant, anti-cancer	[24]
Linoleic acid	Anti-inflammatory	[25]

cm^{-1} to 1300 cm^{-1} shows the presence of C-O stretch in alcohols, esters and carboxylic acids respectively (1237 cm^{-1}, 1099 cm^{-1} and 1163 cm^{-1}). The peak at 1237 cm^{-1} coincides with symmetric C-O-C stretching. The peak at 1793 cm^{-1} shows the presence of acid chloride which further indicates the presence of linoleoyl chloride and oleoyl chloride (Figure 1A).

FTIR spectroscopy of corn oil: The peak at 3009 cm^{-1} indicates the aromatic C-H stretching present in dehydroabietic acid. The C-H stretching of alkane may be attributed to the presence of asymmetric –CH$_2$ in fatty acids of corn oil and is shown at 2925 cm^{-1}. The presence of stretching vibrations due to symmetric –CH$_2$ was indicated at 2855 cm^{-1} which also coincides with –C-H stretch found in aldehyde (-CHO) present in cis-9-hexadecenal. Out of plane bending due to =C-H of compounds like beta-sitosterol was shown at 914 cm^{-1}. Four or more -CH$_2$ was shown at 722 cm^{-1} which shows the presence of long open chain present in fatty acids of corn oil. Aromatic C=C of dehydroabietic acid was shown at 1458 cm^{-1} which coincides with CH$_2$ bending of methylene group. The presence of –CH$_3$ bending was shown at 1377 cm^{-1} while a strong peak at 1747 cm^{-1} indicates the presence of C=O stretch for aliphatic aldehyde of cis-9-hexadecenal.

The peak at 1747 cm^{-1} shows the presence of C=O stretch for ester of oleic acid. The peaks between 1000 cm^{-1} to 1300 cm^{-1} shows the presence of C-O stretch in alcohols, esters and carboxylic acids (1099 cm^{-1}, 1163 cm^{-1} and 1238 cm^{-1} respectively). The peak at 1238 cm^{-1} coincides with symmetric C-O-C stretching. The O-H stretch is shown at 3400 cm^{-1}. The peak at 1793 cm^{-1} shows the presence of acid chloride which further shows the presence of linoleoyl chloride and palmitic acid chloride (Figure 1B).

FTIR spectroscopy of cotton seed oil: The aromatic C-H stretch was present at 3008 cm^{-1}. However, the C-H stretching of alkane was shown at 2925 cm^{-1}. Out of plane bending due to =C-H was shown at 914 cm^{-1}. Four or more -CH$_2$ was shown at 722 cm^{-1} which shows the presence of long open chain present in fatty acids of cotton seed oil. A broad peak was observed near 3463 cm^{-1} which indicates phenolic –OH group presence. Aromatic C=C was shown at 1458 cm^{-1} which coincides with CH$_2$ bending of methylene group. A strong peak was observed at 1747 cm^{-1} showing C=O group presence. The –C-H stretch found in aldehyde (-CHO) was shown at 2854 cm^{-1}. The peaks between 1000 cm^{-1} to 1300 cm^{-1} shows the presence of C-O stretch in alcohols, esters and carboxylic acids (1238 cm^{-1}, 1098 cm^{-1}, 1120 cm^{-1} and 1164 cm^{-1}). The peak at 1238 cm^{-1} coincides with symmetric C-O-C stretching. The peak at 1793 cm^{-1} shows the presence of acid chloride which further represents presence of palmitic chloride, linoleoyl chloride and oleoyl chloride respectively (Figure 1C).

Validation of UV spectroscopy for solubility studies of canola oil, corn oil and cotton seed oil: The suggested UV method permits cost effective and easy quantification of *Brassica napus* oil, *Zea mays* oil and *Gossypium hirsutum* seed oils. The maximum absorbance of canola, corn and cotton oil was shown at 275 nm. Shake flask method was employed for the determination of intra and inter-day solubility of selected oils in different solvents. The Correlation coefficient (R^2) was 0.9815, 0.9893 and 0.9972 for canola, corn and cotton seed oils respectively and has shown compliance with Beer-Lambert's law. Slope, intercept and repeatability of these three oils have been shown in Table 5. The accuracy was confirmed by recovery values of 90% to 110% as per Indian Pharmacopoeia. Solubility of these oils was estimated using UV spectroscopy method using various solvents (Table 6). Intra and inter-day precision of canola, corn and cotton has been displayed in Tables 7-9.

Discussion

Canola oil (*Brassica napus*; Brassicaceae), corn oil (*Zea mays*; Poaceae) and cotton seed oil (*Gossypium hirsutum*; Malvaceace) are reported to have potential pharmacological effects on human body but these oils do not show patient compliance if administrated as raw form [4]. Thus, the researchers and pharmaceutical industries are concentrating to formulate suitable dosages form such as tablets, emulsions, suspensions, gels, ointments etc. Before the development of formulation, there is great need to establish pre-formulation studies like phytoconstituent identification, chemical structure elucidation and solubility [26-28]. These analytical techniques are mainly used for authentication, identification and characterization of oils. Canola, corn and cotton are the edible oils, which also have potential therapeutic effects but these oils are associated with some incompliance to be administrated as raw form therefore, to overcome these problems researchers are exploiting for the development of novel formulation. GC-MS data of canola, corn and cotton oils revealed the presence of 18, 19 and 26 compounds respectively with their percentage area, molecular formula and molecular weight, which are available in the
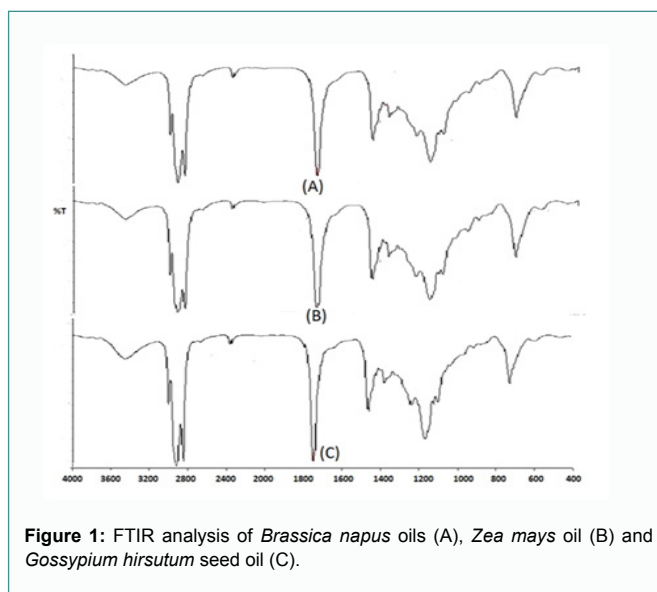

Figure 1: FTIR analysis of *Brassica napus* oils (A), *Zea mays* oil (B) and *Gossypium hirsutum* seed oil (C).

Table 5: UV method validation parameters.

S.No.	Oil	Slope	Intercept	Correlation Coefficient (R²)	Accuracy (%recovery)
1	*Brassica napus* oil	0.0063	0.417	0.9815	100.03
2	*Zea mays* oil	0.001	0.3758	0.9893	100.4
3	*Gossypium hirsutum* seed oil	0.0029	0.377	0.9972	100.7

Table 6: Solubility study of *Brassica napus*, *Zea mays* oil and *Gossypium hirsutum* seed oil in different solvents.

S.No.	Solvent	Canola oil	Corn oil	Cotton oil	PDE (mg/day) of solvent
1	Ethanol	Very soluble	Very soluble	Very soluble	≤ 50
3	Chloroform	Freely soluble	Freely soluble	Very soluble	0.6
4	Hexane	Freely soluble	Soluble	Soluble	2.9
5	Toluene	Soluble	Soluble	Soluble	8.9
6	PEG 400 (15% v/v)	Insoluble	Insoluble	Insoluble	Non mentioned
7	Tween-80 (15% v/v)	Slightly soluble	Slightly soluble	Slightly soluble	Not mentioned

Table 7: Intra-day and Inter-day precision of *Brassica napus* oil in different solvents.

S.No.	Solvent	Intra-day Precision (%RSD)	Inter-day precision (%RSD)
1	Chloroform	0.78	2.69
2	Ethanol	0.42	1.44
3	Tween 80 (15% v/v)	0.72	1.52

Table 8: Intra-day and Inter-day precision of *Zea mays* oil in different solvents.

S.No.	Solvent	Intra-day Precision (%RSD)	Inter-day precision (%RSD)
1	Chloroform	1.23	3.22
2	Ethanol	0.86	2.8
3	Tween 80 (15% v/v)	0.62	1.63

Table 9: Intra-day and Inter-day precision of *Gossypium hirsutum* oil in different solvents.

S.No.	Solvent	Intra-day Precision (%RSD)	Inter-day precision (%RSD)
1	Chloroform	0.98	1.68
2	Ethanol	1.27	2.5
3	Tween 80 (15% v/v)	0.79	1.09

updated version of GC-MS. In literature, to the best of our knowledge no attempt has been taken to identify phytoconstituents from the canola oil, corn oil and cotton seed oil. The present study is an attempt to identify the complete GC-MS analysis of canola oil, corn oil and cotton seed oil. The GC-MS analysis of canola showed the presence of therapeutically active compounds such as campesterol, linoleic acid, oleic acid, sitosterol, stigmasterol, tocopherol and Vitamin E. These compounds have been reported as memory enhancer, anti-psychotic, anti-depression, anti-microbial, anti-inflammatory, anti-oxidant etc [16-25]. In literature, canola, oil, corn oil and cotton seed oil are also reported to have these activities. Hence, it can be hypothesized that phytoconstituents found in these oils may act synergistically for the treatment of human disorders. Hence; it can be hypothesized that if these phytoconstituents found in oils may act synergistically for the treatment of human disorders. Furthermore, the oil has such phytoconstituents which are therapeutically active, but have not been studied against specific diseases. Therefore; it can be show potential effects against these disorders. Furthermore, previously canola oil contains erucic acid, which has been reported as potential heart damaging component and it is safe to use it at 2% w/v concentration [29-30]. Dupont et al., [31] 1989 extracted erucic acid from canola oil using cold pressed method followed by enzymatic reaction, without mention of GC-MS analysis to validate the presence of erucic acid in the oil. One of the components of corn oil (palmitic acid) has been shown to affect mitochondrial function by mediating oxidative stress or lipotoxicity at excess concentration [32]. Excessive administration of oleic acid and linoleic acid may accumulate on human lymphocytes and further induce apoptosis and necrosis [33]. Adulteration in oils can disturb percentage composition of oils so, the analysis of the percentage of phytoconstituents is very essential for balancing the therapeutic effect of constituents. Moreover, gossypol is one of the components found in raw cotton seed oil, which has been shown anti-fertility effect on males and also reported as male oral contraceptive agent [34-36]. In this study, gossypol is not found in GC-MS analysis of derivatized cotton seed oil sample procured by us. However, FTIR of non-derivatized cotton seed oil revealed the presence of chemical bond of gossypol. Therefore, it is necessary to know the percentage of phytoconstituents before the administration of these oils in humans.

FTIR is a system to elucidate the chemical structure of an unknown sample [37]. Furthermore, this technique is also used to determination of presence of phytoconstituents and quality control of the oils. In literature, a few reports are available to understand the structure of the canola oil, corn oil and cotton seed oil, which are not fully clear. In the present study, FTIR study has shown the presence of chemical bond of those phytoconstituents which are identified in GC-MS analysis. Therefore, FTIR data further validates the GC-MS reports.

Inter and intra-day repeatability solubility study of canola, corn and cotton seed oils indicated the slight variation in precision which shows that the selected oils meets the specific acceptance criteria. It has been observed that canola oil, corn oil and cotton oil were miscible in chloroform, hexane, ether, toluene and petroleum ether. Canola, cotton seed oil was slightly miscible in ethanol (95%) whereas corn oil was practically immiscible but found miscible with benzene. As per the ICH Q3C benzene exist in class 1 whereas chloroform, hexane and toluene exits in class-2 while ethanol (95%), ether in the class-3, which are thought to have limited application because of their toxic nature if use above the PDE limit. These solvents are usually used to develop pharmaceutical formulations [7,15]. However, at the drying step of the formulation development, these solvents supposed to be evaporated. But, it has been suggested that these solvents cannot be fully evaporated from the formulation due to few physical and chemical barriers. Therefore, it is very important to analyze the amount of organic solvents remaining in the final product after evaporation using the techniques like gas chromatography, single drop microextraction and solid-phase microextraction before administered into animals in pre-clinical studies [38,39]. The data from the GC-MS, FTIR and UV-spectroscopy of canola, corn and cotton oils would be beneficial for the further use of these oil by pharmaceutical and food industries [37,40,41].

Conclusion

GCMS, FTIR and UV spectroscopy have been used for the authentication, identification and characterization of canola, corn and cotton seed oils. The results of canola, corn and cotton oils from GC-MS, FTIR and UV-spectroscopy provided the required information which will be very helpful for formulating these oils into stable dosage forms that will be suitable for human consumption, as otherwise it is very difficult to administered an oil as such. Selection of solvent is primary step for formulation process and solubility studies with UV-spectrophotometer provides a useful tool to select safe and stable solvent for canola, corn and cotton seed oils before incorporating them into dosage forms.

References

1. Aziz ZA, Ahmad A, Setapar SH, Karakucuk A, Azim MM, Lokhat D, et al. Essential Oils: Extraction Techniques, Pharmaceutical And Therapeutic Potential-A Review. Curr Drug Metab. 2018;19(13):1100-10.

2. Palit P. Bioactivity-Guided Phytofractions: An Emerging Natural Drug Discovery Tool for Safe and Effective Disease Management. Nat Pro drug discovery. 2018;57-71.

3. Neigut S, inventor; Neigut, Stanley, assignee. Compositions and delivery systems for the topical treatment of psoriasis and other conditions of the skin. United States patent US 6,048,886. 2000.

4. Vander Jagt DL, Deck LM, Royer RE, Heidrich JE, inventors; STC. UNM, assignee. Therapeutic agents for skin diseases and conditions. United States patent US 9,925,153. 2018.

5. Ghaffar F, Khan I, Shah H, Iqbal K. 42. Nutritional, physicochemical, antimicrobial and DPPH free radical scavenging potential of the biotech and conventional hybrids of seeds and seed oils from Gossypium hirsutum L. Pure Appl Biol. 2018;7(2):765-74.

6. Moinard-Checot D, Chevalier Y, Briançon S, Fessi H, Guinebretière S. Nanoparticles for drug delivery: review of the formulation and process difficulties illustrated by the emulsion-diffusion process. J Nanosci Nanotechnol. 2006;6(9-10):2664-81.

7. Grodowska K, Parczewski A. Organic solvents in the pharmaceutical industry. Acta Pol Pharm. 2010;67(1):3-12.

8. Bala I, Bhardwaj V, Hariharan S, Kumar MN. Analytical methods for assay of ellagic acid and its solubility studies. J Pharm Biomed Anal. 2006;40(1):206-10.

9. Fang G, Goh JY, Tay M, Lau HF, Li SF. Characterization of oils and fats by 1H NMR and GC/MS fingerprinting: Classification, prediction and detection of adulteration. Food Chem. 2013;138(2-3):1461-9.

10. Gordon MH, Miller LA. Development of steryl ester analysis for the detection of admixtures of vegetable oils. J Am Oil Chem. 1997;74(5):505-10.

11. Setyaningrum DL, Riyanto S, Rohman A. Analysis of corn and soybean oils in red fruit oil using FTIR spectroscopy in combination with partial least square. Int Food Res J. 2013;20(4):1977-81.

12. Man YB. Analysis of canola oil in virgin coconut oil using FTIR spectroscopy and chemometrics. J Food Pharm Sci. 2013;1(1):5-9.

13. Matkovic SR, Valle GM, Briand LE. Quantitative analysis of ibuprofen in

pharmaceutical formulations through FTIR spectroscopy. Latin Am Appl Res. 2005;35:189-95.

14. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Tech. 2012;3(6):1000151.

15. Impurities: Guideline for Residual Solvents. 2019.

16. Azzi A. Many tocopherols, one vitamin E. Mol Aspects Med. 2018;61:92-103.

17. Alabi KA, Lajide L, Owolabi BJ. Biological activity of oleic acid and its primary amide: experimental and computational studies. J Chem Soc Nigeria. 2018;43(2):9-18.

18. Chan P, Kao PF, Tomlinson B. Cardiovascular effects of trilinolein, a natural triglyceride isolated from the herb sanchi (Panaxnoto ginseng). Acta Cardiologica Sinica. 2005;21(2):71-6.

19. Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr. 2000;130(9):2127-30.

20. Moghadasian MH. Pharmacological properties of plant sterols in vivo and in vitro observations. Life Sci. 2000;67(6):605-15.

21. Boubaker J, Ben Toumia I, Sassi A, Bzouich-Mokded I, Ghoul Mazgar S, Sioud F, et al. Antitumoral potency by immunomodulation of chloroform extract from leaves of nitraria retusa, tunisian medicinal plant, via its major compounds ß-sitosterol and palmitic acid in balb/c mice bearing induced tumor. Nutr Cancer. 2018;70(4):650-62.

22. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434-9.

23. Gade S, Rajamanikyam M, Vadlapudi V, Nukala KM, Aluvala R, Giddigari C, et al. Acetylcholinesterase inhibitory activity of stigmasterol &h exacosanol is responsible for larvicidal and repellent properties of Chromolaena odorata. Biochim Biophys Acta Gen Subj. 2017;1861(3):541-50.

24. Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 2009;14(1):540-54.

25. Santos C, Weaver DF. Topically applied linoleic/linolenic acid for chronic migraine. J Clin Neurosci. 2018;58:200-1.

26. Chaurasia G. A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. IJSR. 2016;7(6):2313.

27. Acharya PC, Shetty S, Fernandes C, Suares D, Maheshwari R, Tekade RK. Preformulation in Drug Research and Pharmaceutical Product Development. In Dosage Form Design Considerations. 2018;1-55.

28. Bandopadhyay S, Bandyopadhyay N, Deb PK, Singh C, Tekade RK. Preformulation Studies of Drug Substances, Protein, and Peptides: Role in Drug Discovery and Pharmaceutical Product Development. In Dosage Form Design Considerations. 2018;401-33.

29. Hulan HW, Kramer JK, Mahadevan S, Sauer FD, Corner AH. Relationship between erucic acid and myocardial changes in male rats. Lipids. 1976;11(1):9-15.

30. Dupont J, White PJ, Johnston KM, Heggtveit HA, McDonald BE, Grundy SM, et al. Food safety and health effects of canola oil. J Am Coll Nutr. 1989;8(5):360-75.

31. Lin L, Allemekinders H, Dansby A, Campbell L, Durance-Tod S, Berger A, et al. Evidence of health benefits of canola oil. Nutr Rev. 2013;71(6):370-85.

32. Ruan JS, Lin JK, Kuo YY, Chen YW, Chen PC. Chronic palmitic acid-induced lipotoxicity correlates with defective trafficking of ATP sensitive potassium channels in pancreatic ß cells. J Nutr Biochem. 2018;59:37-48.

33. Cury-Boaventura MF, Gorjão R, de Lima TM, Newsholme P, Curi R. Comparative toxicity of oleic and linoleic acid on human lymphocytes. Life Sci. 2006;78(13):1448-56.

34. Gadelha IC, Fonseca NB, Oloris SC, Melo MM, Soto-Blanco B. Gossypol toxicity from cottonseed products. Scientific World Journal. 2014:ID 231635.

35. He T, Zhang HJ, Wang J, Wu SG, Yue HY, Qi GH. Application of low-gossypol cottonseed meal in laying hens' diet. Poult Sci. 2015;94(10):2456-63.

36. Gadelha IC, Lima MA, Melo MM, Soto-Blanco B. Gossypol promotes the degeneration of chicken ovarian follicles in vitro. Braz J Poultry Sci. 2016;18(3):505-10.

37. Rohman A, Man YC. Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. Food Res Int. 2010;43(3):886-92.

38. Liu W, Zhang K, Yang G, Yu J. A highly efficient microextraction technique based on deep eutectic solvent formed by choline chloride and p-cresol for simultaneous determination of lignans in sesame oils. Food Chem. 2018;281:140-6.

39. Roy J. Pharmaceutical impurities--a mini-review. AAPS Pharm Sci Tech. 2002;3(2):E6.

40. Alghamdi SS, Khan MA, El-Harty EH, Ammar MH, Farooq M, Migdadi HM. Comparative phytochemical profiling of different soybean (Glycine max (L.) Merr) genotypes using GC–MS. Saudi J Biol Sci. 2018;25(1):15-21.

41. Mohamed MH, Wilson LD, Headley JV, Peru KM. Screening of oil sands naphthenic acids by UV-Vis absorption and fluorescence emission spectrophotometry. J Environ Sci Health A. 2008;43(14):1700-5.

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