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Extraction efficiency and characteristic evaluation of turmeric oleoresins; A potential functional ingredient

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Oleoresins are key flavoring and coloring ingredients in spices and herbs. Among spices, turmeric is most commonly used all around the world. Turmeric oleoresins have a promising future in pharmaceutical and food processing industries as functional ingredients. Apart from conventional extraction methods, by using modern extraction methods, a higher yield of oleoresins can be obtained with minimal effects on their characteristic parameters including phytochemicals, antioxidants, volatiles, non-volatiles and fatty acids profile. In the present research, turmeric oleoresins were extracted by 5 different extraction methods and were compared for extraction efficiency and characterization. Turmeric samples were dried, and their moisture content was further adjusted to 12% prior to extraction to get maximum extracts during extraction process which may be affected due to higher moisture content. Extraction was carried out by (i) conventional solvent extraction (hexane, acetone, petroleum ether, diethyl ether and iso-propanol), (ii) steam distillation, (iii) 3-phase partitioning, (iv) Supercritical fluid extraction/SCFE and (v) solvent extraction followed by cryogenic grinding. The results revealed that the highest extraction efficiency was observed in solvent extraction after cryogenic grinding (4.85%). Total phenolic content (TPC), total flavonoids content (TFC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and Lipoxygenase (LOX) inhibition assay were determined through UV-vis spectrophotometer, functional characterization was carried out by Fourier transformed infrared (FTIR) spectroscopy while fatty acids profiling was carried out by gas chromatography attached with flame ionization detector. Total volatiles and non-volatiles were also determined by oven drying method. Characteristically, oleoresins extracted through SCFE were found to be a rich source of TPC (20.57 mg GAE g-1), TFC (109.00 mg QE 100g-1) and total volatiles (2.23%) while highest non volatiles were observed in extracts obtained after cryogenic grinding. Similarly, the highest antioxidant activities assessed by DPPH and FRAP were indicated by oleoresins extracted through SCFE as 59.55% and 220.60 µM Fe^{2+} g-1, while highest LOX activity (58.33%) was observed in extract obtained by 3-phase partitioning. FTIR spectroscopic analysis of the selected sample indicated the presence of different functional groups with bond type at different wave numbers. GC-FID analysis of selected sample indicated the presence of 13 different fatty acids ranging from 1.9-18.6%. Pentadecane was found in lowest percentage while cis-10 pentadecanoic acid was found in highest concentration. It was concluded that although the extraction % of SCF extract was slightly lower (4.07%) but it was found best in term of purity and characterization with a strong potential to be used in food and other industries.

Keywords: Turmeric oleoresins, extraction, characterization, Phytochemicals, fatty acids profiling.

INTRODUCTION

A spice consists of seed, bark, fruit, root or other parts of plant which imparts specific color and flavor to food during food processing operations. Along with being a rich natural source of color and flavor, it also acts as anti-microbial agent and as a functional ingredient in food, cosmetics, and perfumery industry (Peter and Babu, 2012). Spices such as garlic, turmeric, mint, ginger and cinnamon etc. have been used as flavor enhancer and preservatives in food industries. Moreover, these have been a significant part of traditional medicine culture such as Ayurveda, Western herbalism and Chinese medicine (Liu *et al*., 2021). Turmeric as rich source of bioactive compounds and having strong antioxidant potential also acts as an important nutraceutical ingredient and imparts resistance against different diseases (Ashraf *et al*., 2016).

Turmeric extract is a rich source of functional ingredients and functional foods have promising effects on human health. Functional foods are effective against many diseases such as

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cancer, hypertension, constipation, diabetes, and other cardiovascular diseases (Zafar *et al*., 2020).

Different types of functional compounds are extracted from different spices and have a strong potential to be used in related industries. Some important ingredients extracted from spices are oleoresins. Coloring and flavoring properties of a spice are related to its oleoresins which consist of both volatile and non-volatile compounds. Oleoresins not only have applications in food industries but also have many health benefits and are used in many medicinal and pharmaceutical formulations (Rajashri *et al*., 2020). Based upon handling and utilization, oleoresins are considered as a promising functional ingredient in food and pharmaceutical industries. Oleoresins impart positive effects on living cells and tissues due to presence of several bioactive compounds in them. Oleoresins have anti-microbial, anti-fungal and immunity boosting properties which make these the best fit functional ingredient for industrial uses (Pal *et al*., 2020).

Turmeric is one of the most important spices generally used all around the world. Turmeric and other spices' extracts have been previously used in food and medicine industries not only as taste and flavor enhancers but also as shelf-life enhancers due to their anti-fungal and anti-bacterial characteristics (Sharma *et al*., 2014). Its utility as culinary ingredient is due to its specific flavor and color which is imparted to food during processing. Oleoresins extracted from turmeric powder have wide applications in food and pharmaceutical industries as functional ingredients. They are rich sources of essential oil and curcuminoids content generally ranging between 2-2.5% and 0.2-2% simultaneously in extracts from different turmeric varieties (Chattopadhyay *et al*., 2004).

Different extraction methods can be used to extract oleoresins from turmeric and other spices. Generally, organic solvents are used for extraction purposes which extract oleoresins from spices and dissolve them which can further be separated by vacuum drying. Steam pressure under controlled conditions and separation following centrifugation can also be used for extraction purposes (Lang and Wai, 2001).

According to a report published in "Central Bureau of Investigation" on November 5, 2020, the demand for oleoresins is increasing day by day especially in the European market. Oleoresins are preferred especially in food and beverage formulations because of their functional properties and low cost. Oleoresins' share in market was recorded as 1.44 US$ in 2018 and an annual growth rate of 4.7% is expected till 2025. European markets account for 1/3rd of the world's total oleoresins production. Black pepper, paprika and turmeric are the three most consumed oleoresins in pharmaceutical and food processing industries.

Oleoresins production in Pakistan is not up to the mark. We need to import oleoresins to meet their requirements in food, nutraceutical and pharmaceutical industries. By installing extraction units in high spices production areas, we can fulfill not only the local industrial requirement but can also export oleoresins to other countries. The only concerned issue regarding oleoresins extraction is that solvent residues become a part of oleoresins to some extent which need some special care to be removed during oleoresins' recovery process. The described work is an effort to compare different extraction methods to find out an optimal extraction method for oleoresins with higher efficiency, better quality extract and less residual impurities in final extract.

MATERIALS AND METHODS

The current research was conducted at National Institute of Food Science and Technology and Central High-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan.

***Procurement of Raw Materials and Chemicals*:** Fresh turmeric rhizomes were purchased from local market. Chemicals and reagents were purchased from local trusted supplier and standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

***Sample Preparation*:** Turmeric rhizomes were properly cleaned, washed, sliced and dried in dehydrator (Harvest Saver, R-5A, 1-800-369-4283). The moisture content of the turmeric powder was optimized to 12% and stored in airtight container at room temperature before extraction. For initial moisture analysis prior to dehydration and adjustment to 12%, the following method was used for moisture analysis.

***Moisture determination*:** Moisture in the sample was determined by the method described by AOAC, 2006. For this purpose, a weighed amount of sample was taken in a China dish and sample was dried in hot air oven (LabTech, LDO-150N, Korea) at 105 ºC ± 5 ºC until constant weight was obtained. Then the moisture content was determined by the following formula:

Moisture (%) = (Loss in weight/weight of sample) * 100
Where;
Loss in weight = Weight of sample before oven drying - Weight of sample after oven drying

***Extraction Process*:** Oleoresins' extraction was carried out by 5 different methods as described below.

***Extraction by Organic Solvents*:** For solvent extraction process, the method described by Vijyan *et al*. (2019) was followed. Hexane, acetone, petroleum ether, diethyl ether and iso-propanol were used for extraction through Soxhlet apparatus (LabTech, LEX-3030F, Korea) and 5-6 washings were given for each solvent for maximum extraction. Then extracts were concentrated by rotary vacuum evaporator (Labtech, N-N, Japan). The yield of oleoresins was calculated on percent basis (w/w) and the oleoresins were stored in refrigerator. Extract acquired by extraction through petroleum ether was found highest in percentage (4.74%) and was further selected for comparison with other extraction methods including (i) steam distillation, (ii) SCFE, (iii) 3-phase partitioning and (iv) extraction followed by cryogenic grinding.

***Extraction by Steam Distillation*:** For extraction by steam distillation, the method followed by Manzan *et al*. (2003) was used with slight modifications. For extraction by steam distillation, an autoclave (SANYO, MLS-3780, Japan) was used and condensed steam was collected in a beaker after condensation. Controlled pressure was applied to generate vapors. A bed of turmeric powder was placed inside the autoclave and steam was passed through that bed followed by condensation of steam. The mixture was kept for 1 day for settling and then essential oil and settled material was collected. The recovered oleoresins were calculated as g of oil per 100 g of rhizome powder.

***Supercritical Fluid Extraction*:** For supercritical fluid extraction method, the protocol described by Vijayan *et al.* (2019) was followed. Lab scale supercritical fluid extractor (SFT – 150 SFE System, France) was used for extraction of turmeric oleoresins. Turmeric powder was filled in the extraction vessel. Vessel was sealed properly and gas injectors were attached with the extraction vessel. CO_2 was used as carrier gas. 3000 psi pressure, 60 ºC temperature and 2 L per minute CO_2 flow rate was used and extraction was carried out for 4 hours. Oleoresin was collected in an attached vial while releasing the CO_2 pressure periodically.

***Extraction by 3-Phase Partitioning*:** For extraction by 3-phase partitioning, the method of Kurmundle *et al*. (2011) was followed. For extraction by 3-phase partitioning, 5.129 g of turmeric powder was taken, and a slurry was prepared by dissolving it in 50 mL of distilled water. Weighed amount of ammonium sulphate and t-butanol was added in mixture, gently stirred for 1 hour and then 1 hour stand time was given for formation of 3 phases. 3 phases were separated by centrifugation (Eppendorf, Centrifuge 5804 R, Germany) for 20 minutes at 2850 × g. Upper organic layer was collected, and solvent was evaporated to get pure oleoresin.

***Extraction Followed by Cryogenic Grinding*:** For extraction after cryogenic grinding, the method of Sharma *et al.* (2014) was followed. Turmeric rhizomes were sliced and liquid nitrogen was sprayed just before blending and finally the mixture was mixed properly. Low temperature during blending solidified the oil content and then turmeric powder was prepared for extraction. Then moisture content of the obtained powder was optimized to 12% using dehydrator (Harvest Saver, R-5A, 1-800-369-4283) and then extraction was performed via Soxhlet extraction method using petroleum ether as solvent.

***Characterization of extracted Oleoresins*:** Oleoresins were characterized by performing following tests

***Total Phenolic Content (TPC)*:** Folin-Ciocalteu (FC) method was used for TPC analysis and method described by Al-Reza *et al*. (2010) was followed. 0.2 mL of extract along 46 mL of distilled water and 1 mL of FC reagent was taken in a 50 mL flask. Then after 3 minutes, 3 mL of sodium carbonate solution (7.5 % w/w) was added and all the mixture was incubated at the orbital shaker (IKA®, KS 260B, Germany) at 30±2 ºC for an hour. The prepared mixture was at spectrophotometer (IRMECO, U2020, Germany) at 760 nm. Gallic acid was run as standard ranging from 0-10 mg mL-1 and finally results were calculated in mg GAE g-1.

***Total Flavonoids Content (TFC)*:** TFC determination of the extracted oleoresins was carried out by following the protocol of Chang *et al*. (2002). 1 ml sample was taken in a test tube and 1 molar 100 µL aluminum chloride was added from side wall followed by the addition of 100 µL potassium acetate. Total volume was made 4 mL by the addition of solvent in test tube. The mixture was incubated for 30 minutes at room temperature and at the ending point yellow color was developed. Absorbance of the samples was measured at 517 nm. Quercetin was used as standard and TFC content was determined as mg QE 100g-1.

***DPPH Analysis*:** DPPH method was used to analyze the antioxidant activity of the samples following Lee *et al.* (2007). 0.1 mL of oleoresin was diluted in methanol. 3 mL of DPPH radical solution (100 µmol L-1) prepared in methanol was added to the sample solution and mixture was incubated for 30 minutes. The samples' absorbance was measured by the UV visible spectrophotometer (IRMECO, U2020, Germany) at 517 nm. Trolox reagent solution at different concentrations (0-32 µmol L-1) was used as standard and DPPH radical activity was calculated.

***FRAP Analysis*:** FRAP assay was used to evaluate ferric reducing ability of oleoresins following Palvic *et al*. (2018). 0.1 mL of diluted oleoresin with methanol was taken and 3 mL of FRAP reagent was added. The mixture was incubated at 27±2 ºC for 8 minutes and then absorbance was measured at 593 nm via spectrophotometer (IRMECO, U2020, Germany). 0-700 µmol L-1 trolox reagent solutions were taken to obtain standard graph for calculation.

***Total Volatiles and Non-Volatiles*:** For total volatiles and non-volatiles content, the method described by Kshirsagar *et al.* (2009) was followed. 0.2 g of oleoresins were weighed accurately and placed in hot air oven (LabTech, LDO-150N, Korea) at 100 ºC for 45 minutes. The evaporating dish was cooled and weighed again. The loss in weight corresponded to volatile oil and left behind weight was taken as no-volatiles content. Yield was expressed as a percentage.

***Lipoxygenase (LOX) Inhibition Assay*:** LOX inhibition assay was carried out by following the modified procedure of Chaubey *et al*. (2018). 0.025 ml of oleoresin was taken and dissolved as 3 mg mL^{-1} in DMSO. 0.475 mL of lipoxygenase enzyme (400 IU mL^{-1}) was added to the mixture and incubated for 2 min. Then 0.2 mol L-1 borate buffer (pH-9) was prepared and linoleic acid was dissolved in it as 250 µmol L-1. 0.5 mL from this mixture was added to sample mixture and incubation was done at 30±2 ºC for 10 minutes. A blank was also run by mixing 0.025 mL dimethyl sulfoxide (DMSO) in place of oleoresin. 1 mg mL^{-1} of indomethacin was used as positive control, a standard inhibitor of LOX by acting as a natural substrate for lipoxygenase. The samples,

blank and standards were run at 234 nm and LOX inhibition activity was determined.

***Fourier Transformed Infrared Spectroscopy*:** Fourier transformed infrared spectroscopy was performed by following the method of Haiyee *et al*. (2009). FTIR spectra of oleoresins extracted by different methods were obtained using FTIR spectrophotometer (Bruker, Tensor 27, America). Absorbance was measured within the range of 600-4000 cm^{-1} wavelength. Before each sample, an empty scan was run for optimization of device and FTIR results for samples were generated on the average basis of 10 scans.

***Fatty Acid Profiling*:** Fatty acids present in oleoresins were determined by the method described by Kumar *et al*. (2016). GC-FID (GC-17, A Shimadzu, Japan) was used and standards of different fatty acids were fed to determine the concentration of fatty acids. DB WEX 30M 0.25 column was used, nitrogen was used as mobile phase and flow rate was 30 mL min^{-1}. Temperature was used 140 ºC for 5 minutes and was raised to 240 ºC at the rate of 4 ºC min-1 and temperature was retained up to 240 ºC for 30 minutes. Sample injection was done at 250 ºC and fatty acids were calculated based upon the detection of samples and standards.

RESULTS

Oleoresins were extracted by different methods and their characterization was performed to evaluate best extraction method to get high quality acceptable end-product with maximum benefits.

***Oleoresins Extraction by Different Extraction Methods*:** Different organic solvents were used to compare extraction potential of different solvents. The results for different solvents are indicated in Fig 1. The oleoresin with highest extraction yield (petroleum ether) was further used for extraction comparison with other extraction methods and for characterization. While comparing with all extraction methods, the highest extraction % was observed for the solvent extraction followed by cryogenic grinding of turmeric rhizome and the lowest was observed by super-critical fluid extraction. Extraction results for different methods are described in Table 1.

Table 1. Turmeric oleoresins extraction by different extraction methods

Extraction Methods	Oleoresins (%)
Organic Solvent	4.74±0.01a
Steam Distillation	4.76±0.02a
SCFE	4.07±0.04c
3-Phase Partitioning	4.51±0.11b
After Cryogenic Grinding	4.85±0.25a

SCFE: Supercritical fluid extraction, Organic Solvent = Petroleum ether; In table, mean values with same alphabets show non-significant relation ($p>0.05$) and mean values with different alphabets show significant relation ($p<0.05$).

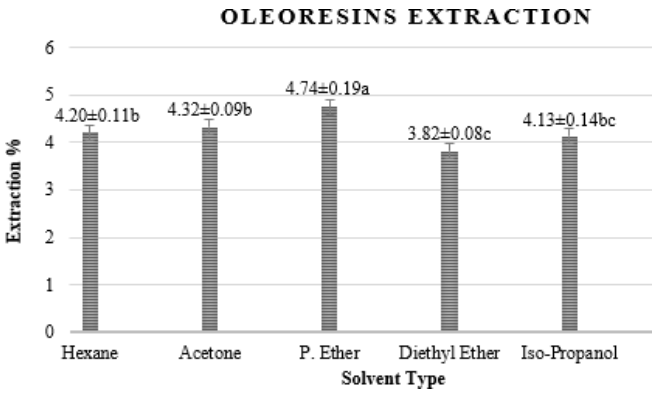

Figure 1. Graphical representation of turmeric oleoresin Extracted by different organic solvents.

Where, P. = Petroleum

***Characterization of Extracted Oleoresins*:** Extracted oleoresins were subjected to different characterization tests including phytochemical tests TPC, TFC, antioxidants tests DPPH, FRAP, total volatiles and non-volatiles and lipoxygenase inhibition assay to evaluate the stability of

Table 2. Characterization of oleoresins extracted by different methods

Extraction methods	TPC (mg GAE g^{-1})	TFC (mg QE $100g^{-1}$)	DPPH (%)	FRAP (µM $Fe^{2+}g^{-1}$)	Total Volatiles (%)	Total Non-Volatiles (%)	LOX Activity (%)
Organic solvent	16.67±0.05d	85.75±0.14d	51.30±0.11d	188.92±2.21e	1.86±0.17b	98.07±0.23a	54.52±0.87bc
Steam distillation	15.07±0.06e	78.91±0.19e	53.25±1.34cd	202.40±0.84d	1.64±0.13b	98.31±0.73a	52.02±0.73d
SCFE	20.57±0.13a	109.00±1.16a	59.55±0.78a	220.60±0.91a	2.23±0.09a	97.87±0.37a	52.66±1.21cd
3-Phase partitioning	17.42±0.09c	91.62±1.17c	54.69±1.35bc	206.99±1.67c	1.90±0.18ab	98.24±0.56a	58.33±0.36a
Cryogenic grinding	18.67±0.07b	97.96±1.67b	56.50±0.98b	213.99±2.12b	1.97±0.08ab	98.65±0.48a	55.90±1.42b

In table (TPC = Total phenolics content, TFC = Total flavonoids content, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, FRAP = Ferric reducing antioxidant power, LOX = Lipoxygenase, SCFE = Supercritical fluid extraction). Mean values with different alphabets show significant relation ($p<0.05$).

oleoresins. The results for these characterization parameters are compiled in Table 2. The data shows that different extraction methods affected the characterizing parameters. The highest values of TPC and TFC were observed in oleoresins extracted through SCF extraction followed by cryogenic grinding and lowest value were observed in extraction by steam distillation. Similarly in case of DPPH and FRAP, the highest values were observed in case of SCF extraction and the lowest were indicated by solvent extraction in both samples. The highest volatile contents were observed in SCF extracted sample and lowest in extract by steam distillation and highest non-volatiles were observed in extract by cryogenic grinding. Similarly, the highest lipoxygenase inhibition activity was observed in extract obtained by 3-phase partitioning and lowest was observed in extract obtained by steam distillation.

***FTIR Analysis of Extracted Oleoresins*:** FTIR spectroscopy of the sample was carried out for structural studies. Depending upon characterization, best sample (SCF extract) was selected for structural studies and based upon the absorbance by sample at different wave numbers, different functional groups were detected. The results of FTIR spectroscopy are depicted in Table 3. Data in the table shows that different functional groups including alkanes, alkenes, aliphatic compounds, esters, and alkyl halides etc. were detected in sample as indicated in the table against different wave numbers. The type of bond present in detected functional groups has also been depicted in the table. Stretching and bending was observed in different functional groups.

Table 3. FTIR Results of Turmeric Oleoresin Extracted by SCF Extraction

Wave #	Functional groups	Vibration types
3370.197	Aliphatic primary amine	N-H stretching
3009.576	Alkene	C-H stretching
2925.710	Alkane	C-H stretching
2847.436	Alkane	C-H stretching
1748.800	Conjugated acid halide	C=O stretching
1692.890	Conjugated acid	C=O stretching
1609.024	Conjugated alkene	C=C stretching
1444.089	Alkane	C-H bending
1231.630	Alkyl aryl ether	C-O stretching
1161.742	Ester	C-O stretching
1066.695	Sulfoxide	S=O stretching
966.057		
912.942	Alkene	C=C bending
812.303		
720.052	Alkene	C=C bending
638.982	Halo compound	C-I stretching
608.231		

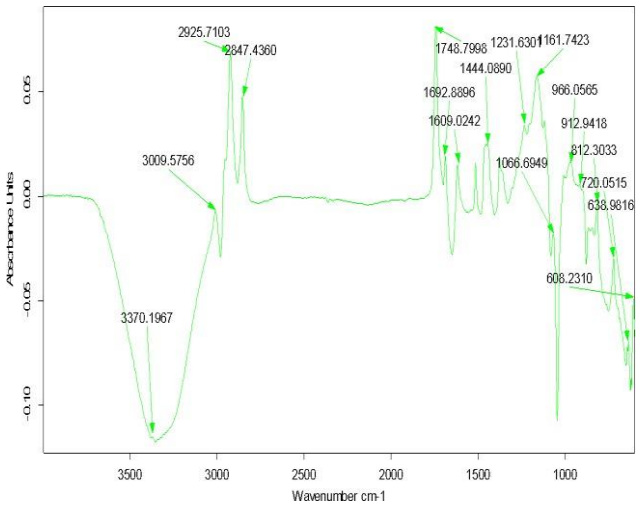

Figure 2. FTIR spectrum of turmeric oleoresins extracted by super critical fluid extraction.

***Fatty Acid Profiling of Best Selected Turmeric Oleoresin*:** Oleoresins majorly consist of oil and are a rich source of fatty acids. Fatty acids were determined in SCF extracted oleoresin which was found best based on characteristic studies. Fatty acids profile was determined using GC-FID against 36 available standards of major fatty acids and a total of 13 fatty acids were detected in the sample. The concentration of different fatty acids and their retention times are depicted in Table 4. The data in the table shows different concentrations of fatty acids ranging from 1.9-18.6%.

Table 4. Fatty Acids Profile of Turmeric oleoresin.

Fatty Acid	Retention time (min)	Concentration (%)
Solvent (methanol)	0.740	
Methyl butyrate	2.267	4.7
Pentadecane	2.900	1.9
Methyl octanoate	3.287	2.2
Methyl laurate	6.567	9.4
Methyl tridecanoate	8.653	4.5
Methyl myristate	11.727	2.4
Cis-10 pentadecanoic acid	17.200	2.6
Methyl palmitoleate	21.480	15.0
Cis-10 pentadecanoic acid	23.260	18.6
Methyl stearate	25.187	5.8
Trans-9-oleic acid	27.400	2.7
Cis-9-oleic acid	28.567	7.2
Linolelaidic acid	30.107	2.7

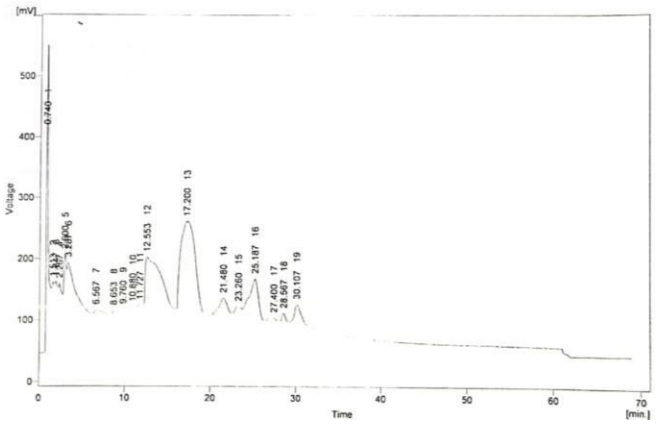

Figure 3. Graphical representation of fatty acids profile of turmeric oleoresin.

DISCUSSION

Turmeric oleoresin is composed of volatile and non-volatile contents that impart specific coloring and flavoring characteristics to the oleoresins. Oleoresins percentage in turmeric varies from varieties to varieties but while using same variety, the oleoresin extraction percentage and its characteristic profile were affected by different extraction methods. While using different organic solvents for extraction purposes, varying extraction yield of oleoresins from turmeric powder was observed. The extraction against different solvents ranged from 3.82-4.74%. The lowest extraction was observed for diethyl ether and the highest yield was obtained while using petroleum ether as extraction solvent. The other solvents indicated acetone (4.32%), hexane (4.2%) and iso-propanol (4.13%). The extract obtained in highest percentage (petroleum ether, 4.74%) was further selected for comparative and characterization studies. While comparing all extraction methods, solvent extraction followed by cryogenic grinding showed maximum oleoresins production because continuous nitrogen mixing during grinding of turmeric rhizome reduced the shear force on oleoresin and due to less heat production, their stability was maintained. Over all the extraction yield of oleoresins by different methods ranged between 4.07- 4.85%. The lowest yield was obtained for SCF extraction, but the reason may be that this technique was free of any organic solvent which in most of other cases become a part of oleoresin to some extent. CO_2 gas at specific temperature (60 ºC) and pressure (3000 psi) and flow rate (2 L per minute), was used as solvent which acted as both liquid and gas and efficiently extracted the pure oleoresins as it was also depicted while characterization. By extraction through organic solvents, Solvents are exposed to sample and being lipophilic in nature, they extract oleoresins content from sample. Then solvent is evaporated from the mixture of oleoresins by rotary or vacuum evaporation. As mentioned by previous studies and also proved in our study that during evaporating solvent, not 100% solvent is evaporated leaving some of its residues in oleoresins. While in SCFE, there is no exposure of sample to the solvent and only CO_2 as supercritical fluid is used for extraction purpose, which being gas in nature is completely removed when gas pressure is released during collecting oleoresins in attached vial. Kurmudle *et al*. (2013) also worked on turmeric oleoresin extraction and extraction % was observed within the range 4-8%.

Characterization of oleoresins was done to evaluate the most efficient method with best quality oleoresin. Total phenolic content in different samples ranged from 15.07-20.57 mg GAE/g. The lowest were found in oleoresins extracted by steam distillation. The reason for lowest TPC content in extract obtained through steam distillation could be due to more exposure to heat because in autoclave, steam was applied for extraction purpose and heat in the form of steam may affect the phytochemical profile of the sample. Our justification regarding low TPC in steam distilled extract was also strengthened by the findings of Karami *et al*. (2022) who worked on ohmic assisted hydro-distillation for oil extraction from yarrow (*Achillea millefolium*) and they also observed decreased values of TPC in hydro distilled oil than the oil extracted through no thermal extraction method. The highest amount of TPC was detected in SCF extracted oleoresin. Our findings of highest TPC content were supported by the findings of Vijayan *et al*. (2019) who worked on SCF extraction of turmeric oleoresin and based upon varying processing conditions observed the TPC content up to 32 mg GAE g-1. Extracts obtained after cryogenic grinding showed the 2nd highest value. The reason for higher TPC in extract obtained after cryogenic may be that during powder preparation by grinding for conventional extraction, heat is produced due to friction which may affect TPC content of the sample. While during cryogenic grinding prior to extraction, liquid nitrogen was used to minimize heat production during grinding process which resulted in lower decrease in TPC. Therefore, it showed 2nd highest value for TPC. It was shown that TPC content was affected by extraction conditions. Solvent adulteration while extraction may be a reason of low TPC in some methods.

Flavonoids contents are basically secondary metabolites in plants. In human diet, TFC plays an important role to hinder free radical formation in body resulting in lowering oxidation in body and providing many other health benefits (Kunyanga *et al*., 2012). After extraction, TFC was also found as a part of extracted oleoresin. The highest value of TFC was exhibited by SCF extract followed by extract obtained after cryogenic grinding. The lowest value was depicted in extract obtained by steam distillation. The reason for the high values may be due to less exposure to heat and organic solvent during SCFE and less heat production during cryogenic grinding of turmeric rhizomes. While the reason for low value

may be due to more exposure of heat in the form of steam during extraction process as it also observed in case of TPC. Antioxidants have different modes of action and due to their specific activity, they help to control oxidation process in oleoresins at different stages (Ghasemzadeh *et al*., 2010). Antioxidant activity plays an important role in deciding the stability and shelf life of oleoresin because being oil in nature, free radical formation in the samples may be a major factor to decide the shelf life of oleoresins. DPPH radical scavenging activity was used as an indication of antioxidant activity. Variation was observed in turmeric oleoresins extracted by different extraction methods. The highest DPPH radical scavenging activity was observed in extracts obtained through SCF extraction followed by extracts obtained after cryogenically grinding. The lowest value was observed in conventional organic solvents extraction. The results indicated that oleoresins of SCFE showed maximum antioxidant activity. The reason may be zero exposure to any organic solvent (except CO_2) or environmental conditions because the whole extraction process was in a controlled environment in supercritical fluid extractor and no solvent residues were observed in final extract. Overall, less variation in DPPH value of oleoresins extracted through different methods was observed indicating that variation in extraction methods affects the antioxidant activity to lesser extent. The results of this study for DPPH analysis were in compliance with Joshi *et al.* (2021) who worked on turmeric oleoresins and concluded that non-curcuminoid compounds were responsible for differences in scavenging activity.

Similarly FRAP assay was performed to evaluate antioxidant activity based upon radical scavenging activity by FRAP. Results obtained were like DPPH indicating the highest value in extract obtained through SCFE as 220.60 µM Fe^{2+}g- 1 followed by the extract obtained after cryogenic grinding (213.99 µM Fe^{2+}g-1**).** The reason for their higher values may be due to less exposure to heat as it was also observed in case of DPPH assay. The lowest value for FRAP assay was indicated in case of solvent based extraction as 188.92 µM Fe^{2+}g-1. The suggested reason for the lower band higher values was strengthened by the findings of Vijayan *et al*. (2013) who worked on comparison of turmeric oleoresins extracted through supercritical fluid extraction and by organic solvent and observed higher antioxidant activity in extract obtained through SCFE than organic solvents. Additionally, our study's results were also strengthened by their findings that increase in extraction temperature from 45 ºC to 45 ºC increased the antioxidant activity, increase in extraction temperature up to 65 ºC had non-significant effect on antioxidant activity and further increased in temperature decreased the antioxidant value as it was observed in case of solvent extraction and steam distillation but solvent based extracted oleoresins shower the lowest value may be due to more exposure to heat during extraction time and during solvent evaporation time by rotary evaporation to get concentrated oleoresins with more purity. DPPH and FRAP play a key role in shelf-life stability and post-extraction utilization of oleoresin. Both these factors determine the chances of rancidity due to oxidation in oleoresins helping to optimize storage conditions for oleoresin without damaging their stability.

An oleoresin mainly consists of volatiles and non-volatiles constituents. Volatiles consist of those components which can easily be evaporated. Volatiles contents are mainly responsible for specific flavor and aroma of any oleoresins. Volatiles and non-volatiles collectively are responsible for specific characteristics of the oleoresins. The results indicate that the highest volatiles were detected in extract obtained after SCFE. The 2nd highest value was observed in extract obtained after cryogenic grinding and the lowest volatiles were detected in extract obtained by steam distillation. In case of non-volatiles, the highest amount was detected in extract obtained after cryogenic grinding followed by extract obtained by steam distillation. SCF extract exhibited the lowest amount of total non-volatiles content. The results for total volatiles and non-volatiles indicate that volatile components were evaporated to some extent during extraction due to exposure to environment at different stages. Due to controlled conditions and less exposure to environment, the highest amount of volatiles content was observed in SCF extract. Total volatiles of turmeric oleoresins were found in small range as detected during our study (1.64-2.23%). Within this range of values, difference is significant. Non-volatiles are dependent on total volatiles content and were calculated by subtracting total volatiles from 100 to mention as %. Our findings of volatiles contents in turmeric oleoresins extracted through different extraction methods were in similarity with the findings of Kurmudle *et al*. (2011) who worked on enzyme assisted extraction of turmeric oleoresins and found the volatile contents within the range of 1.40-1.90% in different enzymes treated extracts. Total non-volatiles' values were calculated in relation to total volatiles. The results for volatiles and non-volatiles content obtained by different extraction methods were found in compliance with the findings of Kshirsagar *et al*. (2009) who worked on turmeric oleoresin extraction by three phase partitioning.

The major problem with oleoresin is rancidity due to enzymatic activity. Lipoxygenase inhibition assay was performed to analyze enzymatic activity in oleoresins. LOX assay was performed to analyze lipoxygenase activity in extracted oleoresins. The highest LOX activity was observed in extract obtained by 3-phase partitioning followed by extraction after cryogenic grinding. The lowest LOX activity was analyzed in extract obtained by steam distillation.

Oleoresins are composed of volatiles and non-volatiles content therefore they are a rich source of different functional groups. Extract obtained by SCF extraction was proceed further for FTIR analysis because it was found best during characterization studies. Different functional groups were

analyzed in turmeric oleoresin. At different wave numbers different aliphatic, aromatic compounds and other compounds were detected as mentioned in table 3. N-H, C=O, CH, S=O, C=C, C-I bonds with stretching or bending were observed in different functional groups which are responsible for the flavoring characteristics of oleoresins. The highest peak was detected at wave number 2925.710 which indicated the presence of alkane with C-H stretching followed by peak at 1748.800 indicating the presence of conjugated acid halides with C=O stretching. Alkane and alkene were detected as major part of turmeric oleoresin at different wave numbers. A similar study was conducted by Nagaveker and Singhal, (2019) who worked on turmeric oleoresin extraction at varying conditions (pressure, time and temperature) at SCFE and similar aromatic and phenolic compounds were analyzed at different wave numbers. Our findings were also favored by Sharma *et al*. (2023) who worked on oleoresins of turmeric and dry coconut shreds to further analyze them chemically and to study their bioactivity. Similar trend of peaks at different wave numbers was observed indicating the presence of structurally similar compounds to our FTIR findings of SCF extracted turmeric oleoresins.

Fatty acids consist of hydrocarbon and a carboxyl group and are mostly found in the form of esters in oils and fats. Fatty acids particularly polyunsaturated fatty acids (linoleic acid, alpha linoleic acid and arachidonic acid etc.) play a key role in maintaining different body functions and are termed as essential fatty acids (Kris-Etherton *et al*., 2004). Turmeric oleoresin is also a rich source of fatty acids. Different fatty acids were analyzed in oleoresin sample. The three major detected fatty acids were cis-10 pentadecanoic acid which was found in the highest percentage followed by methyl palmitoleate and methyl laurate. Similarly other fatty acids including Cis-9-oleic acid, Methyl stearate, Methyl butyrate, Methyl tridecanoate and Cis-10 pentadecanoic acid were found within the range of 5.8-4.5% in descending order. Other fatty acids including pentadecane, methyl octanoate, Methyl myristate, cis-10 pentadecanoic acid and linolelaidic acid were analyzed in lower amounts as mentioned in table. The results of our study regarding presence of different fatty acids in turmeric oleoresins were also favored by the research conducted by Sharma *et al*. (2023) who analyzed turmeric oleoresins and found different fatty acids in similar ranges.

***Conclusion*:** Turmeric oleoresins have been proved to be a promising ingredient for food and pharmaceutical industries due to abundance of major coloring and flavoring agents in them. Conventional extraction methods result in residual effects in obtained oleoresins affecting the quality and purity of oleoresins for intended uses. As an effort to compare and select efficient extraction method with minimally effects on oleoresins' characteristic profile, SCF extraction was found best extraction method, its extraction yield was slightly less than other extraction methods but based upon characterization (TFC, TPC, DPPH, FRAP, LOX activity and total volatiles) and purity was found free from any residual effects in extract and was found best for further industrial applications. Moving forward to industrial levels, SCFE process can be optimized to minimize extraction expenses by using efficient supercritical fluids along with optimization of other extraction conditions including temperature and pressure. SCFE was not only found as an environment friendly and economical extraction method but it was also solvent free which also resulted in more pure extract for intended applications. It can be recommended that SCFE can be used at industrial level for large scale extraction of oleoresins as functional ingredient with optimal utilization in food and pharmaceutical industries.

***Conflict of Interest*:** The authors declare that there is no conflict of interest.

***Authors' contribution statements*:** MS conducted the extraction process and other laboratory analyses, IP conceived the idea for research and supervised the research and writeup, whereas AR and AJ supervised and guided during research and reviewed writeup. MS wrote the original draft of manuscript and all the authors critically reviewed the manuscript and approved the final version.

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