ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Supercritical Fluid Extraction of *Cichorium intybus* (L) and it's Characterization

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ABSTRACT

Supercritical fluid extraction is one of the common and desirable alternative modern technique for the extraction of varity of physicochemical components. These components are separated from a solid or liquid in some cases using an extracting solvent which known as Supercritical fluid (SFC). In this study carbon dioxide has used as SFC along with methanol which acted as a modifier. The roots of *Chichorium intybus* were extracted by using this modern method as well as they extracted by traditional methods such as decoction method and soxhlet extraction. The fourth extract was produced by pure methanol due to the first step in supercritical fluid extraction which includes passing the methanol only for definite time. The physicochemical study involved measuring some physical properties like refractive index, specific gravity, viscosity and surface tension for 0.1% solution for every extract which was dissolved in the proper solvent. Additionally ash analysis was done to study whether this herb is safe for using as a drug. The present study was further conducted to characterize the bioactive compounds which present in the four types of extracts of *Chichorium intybus* roots using UV-VIS, FTIR and GC-MS analysis.

Keywords: Supercritical fluid extraction, Physical parameters, UV, FTIR, GC-MS

1. INTRODUCTION

Cichorium intybus L is one of the important herbs which belong to Acteracea family. It is existing for a long or infinite time (Uigur, 1999 ; New Medical College of Jiangsu, 1977; Wang, 2011). It is a familiar herb in Indian traditional health care system (Ayuveda) called Rasayana (Harsh, 2012) and commonly known as Chicory or Kasani. In the old ancients, Egyptians used chicory as a medicinal plant, coffee alternative, and vegetable crop and sometimes as animal forage (Munoz, 2004; Deshusses, 1961; Grieve, 1971, Plmuier, 1972; Wang, 2011). Since 4000 years Greeks and Romans started to grow it as a vegetable crop (Grieve, 1971; Plmuier, 1972; Wang, 2011). However, nowadays it is also using commercially for many purposes in Europe and North America.

Chicory is a woody herb, has a height of 30-90 cm and a fleshy tap root with length of 75 cm .It forms flowering shoots and seeds from July to October. Its flowers are often bright blue but sometimes they are of white or pink colour (Tousch, 2008) .All the parts of this herb have been studied separately and they have many applications in medicine, agriculture and industry. It was highlighted on the root of the herb in this study which consider as the important part of chicory. It is brownish yellow in color and was recorded as the preferred one for inulin extraction since it gave high yield up to 20% (Franck, 2005), it's easy cultivation (Tousch, 2005) and stable long -chain production (Toneli, 2008).Historically, this herb has a great uses in medicine for treating the liver diseases ,digestive tract and diabetes mellitus (Saly, 2015).

Extraction is the essential step to form the crude extracts from the herbs and then isolation and purification of chemical constituents can be done (Romanik, 2007; Smith, 2003; Saly, 2015).The traditional techniques of extraction depend on the good choise of solvents (Saly, 2015), heat also requires to rise the solubility of the desired components and to improve the mass transfer, they need to long time sometimes up to 24 hours or more to extract the phyto-constituents. Therefore, the need for developing of rapid and high performance extraction techniques becomes very important to save the energy and the time. Furthermore, to decrease solvents consuming, prevent pollution and degradation of thermo labile compounds (Luque, 1998; Pastot, 1997; Nyiredy, 2004). The recent extraction methods such as supercritical fluid extraction (SFE) (Huie, 2002; Sameh, 2012; Saly, 2015), microwave assisted extraction (MAE) (Taylor, 1996, Saly, 2015) and ultrasound extraction (USE) (Luque and Luque, 2003; Saly, 2015) have played important role in extraction technology and green Chemistry.

Supercritical fluid extraction is the most wide spread desirable method for the extraction of preferred compounds by using supercritical fluid (SCF) as a solvent. The supercritical fluid is defined as the substance which exists at a temperature and pressure above its critical point (Huie, 2002). At this point the density of gas and liquid become correspondent as the pressure and temperature increase. There will be no difference between gas phase and liquid phase and a supercritical fluid appear. Therefore a supercritical fluid is a single phase shows properties of both liquid phase and gas phase .It has density and solvating properties as a liquids thus it has high absorption capacity.

Furthermore it has high diffusivity and low viscosity properties as gases which allow high mass transfer rates between a solute and a supercritical fluid. Carbon dioxide ($Sc\text{-}CO_2$) is the most popular solvent used with SFE due to its low critical temperature (31.06℃) and pressure (73 atm). It has also many advantages as it is a green solvent has no harmful effects on the environment since it does not have toxicity and flammability, it is inert to majority of compounds and can be available with high purity at cheap costly (Taylor, 1996).Additionally, it can be removed

simply from the extract with no chemical residue, which may cause a problem in traditional methods of extraction. CO_2 is a linear molecule with zero dipole moment so it has no ability to extract the polar and ionic components (Luque and Luque, 2003). For this purpose , a polar co-solvent named commonly as a modifier such as methanol, ethanol and acetone is used with CO_2 to increase solubility and as a result of that numerous of polar compounds can be extracted.

2. MATERIALS AND METHODS

Commercially available dried roots of chichory were purchased from local market in Aurangabad city, India. methanol used was of HPLC grade and di ethyl ether was of analytical grade. And taken from Sigma-Aldrich Chemicals Co., India Carbon dioxide was supplied with a purity of 99.95 %, contained in a diptube cylinder.

Preparation of the sample: *Cichorium Intybus* L roots were ground in electric mixer properly and gently to avoid increasing in temperature ince it may lead to decrease of quality and quantity of the extract .All the experiments were carried out using freshly ground roots. The sample was weighed exactly before each experiment. The procedures were continued immediately after extraction to avoid the loss of the extract.

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Extraction methods: The roots of Chicrory were extracted using both conventional methods and recent method. The percentage of extractive value was given by the following equation:

$$The\ Percentage\ of\ extractive\ value = \frac{\text{wt of extract yeild}}{\text{wt of started material}}\ X\ 100 \qquad (1)$$

Decoction method: About 20 gm of dried powder was mixed with 250 ml of deionized water and heated up to boiling (100 ℃) for two hours on direct flame. The reflux condenser was used to prevent loss of the solvent by evaporating during the heating process which cooled the evaporated water and allowed it to go back into the extraction vessel. The supernatant was collected and the solvent was evaporated to dryness. The extract obtained was solid in nature, and it was weighed to obtain the extractive yield, and it was kept in air tight glass bottle.

Soxhlet extraction (SE): Dried roots of approximately 30 gm were placed in a thimble made of white cloth in a central compartment with a siphoning device and a side-arm both connected to a lower compartment. About of 300 ml of di ethyl ether was placed in a round bottom flask (RBF) (lower compartment). The reflux condenser was attached above the central sample compartment. The solvent was heated up to boiling in a water bath. And its vapour was passed through the side arm up in to the thimble containing the sample, where it was percolated. The extract was collected in the central compartment up to its height reached the top of the siphon then it flew through this and back into the RBF. This process was repeated for 6 hours or up to the solvent collected in middle compartment became color less. Then the extract obtained was concentrated and allowed to dry at room temperature. It was oily in nature and was placed in screw capped glass vials till further uses.

Supercritical fluid extraction (SFE): The air-direct plant material (6.840 gm) was introduced in a 30ml stainless steel column of 13 cm length considered as the extraction vessel. The temperature was controlled by a column oven (model HC.0.04, Jasco Corporation, Japan) and was maintained at 60℃. The CO_2 and the modifier (MeOH) were pumped by two HPLC pumps operated in constant flow (model 8210-008 and 9802 respectively, Jasco Corporation, Japan). The flow rate of MeOH pump was 0.5 min^{-1} and the flow rate of CO_2 pump was 5ml min^{-1}. The CO_2 pump was connected with a cooling jacket to cool the CO_2 supplied from the cylinder. A mixture of ethylene glycol-deionized water (50:50, v/v) was circulated through the cooling jacket using a low temperature chiller (model EX 280, Escy Corporation) in which the temperature was kept at -7℃. The pressure in the system was regulated through a back pressure regulator (model 26-1762-22-194, Tescom Corporation, USA) and was kept at 150 MPa. The extract was collected in a long and wide test tube for 10 minutes. The extract was evaporated to dryness at room temperature. The extract obtained was waxy and semi-solid in nature. It was gravimetrically weighed and stored in screw capped glass vials until further analysis.

MeOH extraction: The methanol extract was obtained when the MeOH which used as a modifier in Sc-CO2 since the process in the beginning involved passing of MeOH only through the sample with flow rate of 2 ml min-1 for 15minutes. The experiment was conducted at 60℃. The extract obtained after drying at room temperature was oily in nature and was maintained in screw capped glass vials up to its analysis.

Physicochemical Experiments: For measuring the physical parameters (0.1%) solutions of aqueous extract ,methanol extract, SE extract and SC-CO2 extracts were prepared by dissolved 0.1 gm of the dried extract in 100 ml of appropriate solvent. Water was used to dissolve the aqueous extract, methanol in case of methanol extract and acetone for SE and Sc-CO2 extracts.

Refractive index: Abbe refractometer (Guru Nanak Instruments (REGO), New Delhi, India) was placed on a table near a window to allow enough light to reach to the prism which was opened and cleaned by cotton wool and acetone,

then closed after drying. Few drops of the sample was introduced in the prism through the small hole on the prism box by dropper. So a film of liquid enclosed between the two prisms. The cross wire of the telescope was focused by rotating the eye piece. The prism box was moved backward and forward until a clear boundary between the light and the dark regions appeared, then it was rotated until the sharp boundary line was coincides with the intersection of the cross wire in the telescope. The refraction index was read directly on the scale. The procedures were repeated three times and the refractive index considered as the mean value. To achieve accuracy the apparatus first was calibrated against distilled water which has a refractive index of 1.3325 at 25℃.

Specific gravity: A clean and dry pycnometer was used and calibrated by filling it with recently boiled and cooled water at 25oC and the contents were weighed. (Assuming that the weight of 1 ml of water at 25℃ is 0.99602 gm). The pycnometer was filled with the sample and weighed, the empty weight of the pycnometer was subtracted from filled weight of the pycnometer to obtain the weight of sample. The specific gravity of the sample was calculated by dividing the weight of sample contained in the pycnometer by the weight of water contained, both determined at 25℃ as represented in the following equation:

$$S.G(of\ Sample) = \frac{\text{wt of Sample}}{\text{wt of Water}} \qquad (2)$$

Where S.G: The specific gravity of sample, wt: weight of sample and weight of water

Viscosity: The viscosity of the sample was measured by using the method of relative viscosity. Ostwald viscometer (U-shape) tube was used and calibrated with a liquid of known viscosity (e.g. water) and the viscosity of the sample was determined by comparison with that of the known viscosity. The viscometer was cleaned and dried then 10 ml of the sample was introduced in it and allowed to stand for 5-10 minutes. The sample was sucked and the time for the meniscus to pass the two specified marks was measured. The process was repeated 3 times for both the sample and water and the time required to flow the sample and flow the water were taken as the mean values. The measurements were done at room temperature (25℃).

The following equation (3) was used to calculate the viscosity of sample:

$$\eta_2 = \frac{t_2}{t_1} \times \frac{S.G_2}{S.G_1} \times \eta_1 \qquad (3)$$

Where η_2 is viscosity of sample, η_1 is viscosity of water =0.8937centipoise, $S.G_2$ is specific gravity of sample, $S.G_1$ is specific gravity of water. t_2: time to flow the sample, t1: time to flow the water

Surface tension: In this study the surface tension of the sample was measured by stalagmometer method, in which a definite volume of the sample is allowed to flow slowly out of a capillary tube and the number of drops formed was counted. The same procedure was done for a solvent of known surface tension (water) and its number of drops was counted, the process was repeated 3 times for both solvent and sample so the number of drops were taken as the mean. The measurements were done at room temperature (25℃). The surface tension of sample was calculated using the equation (4).

$$\gamma_2 = \frac{n_1}{n_2} \times \frac{S.G_2}{S.G_1} \times \gamma_1 \qquad (4)$$

Where $\gamma 2$: surface tension of sample, γ_1: surface tension of water at 25 ℃ =71.97 dynes/cm, $S.G_2$: specific gravity of sample, $S.G_1$: specific gravity of water, n2: No. of drops of sample, n1: No. of drops of water.

Ash Analysis:

Total Ash: About 2gm accurately weighed of the ground roots was incinerated in silica crucible at a temperature not exceeding 450℃ until free from carbon then cooled and weighed. The procedure was repeated to get constant weight.

Acid insoluble Ash: To the crucible containing total ash, 25 ml of dilute hydrochloric acid was added. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccators for 30 minutes and weighed without delay. The procedures were repeated to get constant weight.

Water soluble Ash: The ash obtained as described in the determination of total ash was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, and ignited for 15 minutes at temperature not more than 450℃. The procedures were repeated up to constant weight was obtained.

The weight of insoluble matter was subtracted from the weight of total ash and the difference in weight was taken as the water soluble ash.

Sulphated Ash: A silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weighed. 2gm accurately weighed of the dried roots taken into the crucible, ignited properly until the sample was charred. Cooled ,then residue was moistened with 1ml of sulphuric acid, heated perfectly until no white fumes were evolved then ignited at 800℃ ± 25℃ until all black particles had disappeared .The ignition is conducted in a place protected from air currents. The crucible was allowed to cool, the procedures were repeated as before until two successive weighing did not differ by more than 0.5 mg.

Analysis of the Extracts: Ultra violet–Visible Spectroscopy (UV-VIS): All the extracts were analyzed using UV-VIS spectrophotometer (model SL-210, Elico). They were scanned in the wavelength of the range 190-1100, and the characteristic peaks were recorded.

Fourier Transform Infra-Red Spectroscopy (FTIR): All the types of the extracts in this study were analyzed by FTIR spectrophotometer (model FT/IR- 4100, Jasco). A small quantity of each extract was applied on the cell to obtain a thin layer. KBr in powder form was used as the supporting media for the sample analysis in case of aqueous extract(solid sample)whereas NaCl cell was used with the remaining extracts(oily samples).The cell was mounted on the FTIR and scanned through a range from 400 cm^{-1} to 4000 cm^{-1}. The Infra-Red spectra was obtained and the functional groups with their corresponding peaks were recorded.

Gas chromatography - Mass spectrometry (GC-MS): The compounds involved in the concentrated extracts of chicory roots were separated and identified using a GC-MS (Model QP2010, Shimadzu) equipped with BP-20 SGE column (30 m x0.25mm id x 0.25 **µ m),** the carrier gas was helium at flow rate of 0.8 ml/min. The sample split ratio was 1:55. The column was heated from 70 ℃ to 220 ℃. The injector and detector temperatures were220℃ and 300℃ respectively. MS mode was scanned at 70 Ev and acquisition mass range of M/Z was 40-600 a.m.u (atomic mass unit). Volume injected was 2 µl.

3. RESULT AND DISCUSSION

Effect of extraction methods on the extracts yields: The methods of extraction were affected mainly on the percentage of the extracts yields. As was shown in table1 (SE) extraction gave high percentage of the extract yield 19.6667% then aqueous extract which was produced in decoction method gave percentage yield of 16.425%. The percentage of extractive value of Sc-CO_2 extract found to be 11.915% which is less than SE and aqueous extracts but by studied the variables of every extraction method it was appeared that in case of SE the started material was 30 g and the time required for completion was 6 hours, for decoction method the started material was 20 gm and the time needed was 2 hours whereas in case of Sc-CO_2 extraction the started material was only 6.840 gm and about 10 minutes only was sufficient to collect the extract. Hence it was concluded that Sc-CO_2 extraction gave a yield comparatively high with less time and less amount of solvent was consumed. Therefore it was proven that Sc-CO_2 technique can improve the extraction yield within short time at low cost.

Table.1.Percentage of extractive value of chicory roots

Type of extractive value	Percentage (w/w)
Aqueous Extract	16.425 %
(SE) Extract	19.6667 %
MeOH Extract	5.544%
(SC-CO_2) Extract	11.915 %

Physicochemical analysis: Refractive index (n) of compounds varies with the frequency of radiated light and it is affected by their colors. Hence different colored extracts showed different refractive index values. Refractive index of chicory roots extracts were measured at 25ºC and 0.1% concentration. It was found that the Sc-CO_2 extract showed the highest value(1.35883).The extracts by the traditional methods (SE, aqueous and MeOH) extracts showed (1.35673,1.33250 and 1.32687) values respectively. Both of Sc-CO_2 and MeOH extracts had nearly same color so they gave near (n) values. Refractive index increases with increasing chain length and the number of double bounds present in the oil (Nielsen, 1994) further, the molecular weight, degree of un-saturation ,fatty acid chain length and degree of conjugation affected the values of refractive index of oils (Shahidi, 2005). The values of refractive index of different extracts solutions were shown in table 2.

The specific gravity of aqueous extract (solid in nature) showed higher value (1.00109) comparing with (MeOH, Sc-CO_2 and SE) extracts (oily in nature) which gave 0.79537, 0.79305 and 0.79074 respectively. This indicated that aqueous extract has more density than water but remaining extracts solutions have less density than water so these oils are lighter than water and will form an upper layer in a water oil mixture (Table.2) described the specific gravity values of all types of chicory roots extracts.

The values of viscosity of the extracts solutions as given in table.2 demonstrated that aqueous extract has more viscosity value (0.90863 centipoise) as compare with the other extracts (0.54815,0.317552,0.31486)

centipoises for MeOH ,Sc-CO_2 and SE) extracts respectively .The viscosity values affected mainly with the density of the compounds meanly the compounds with higher density showed higher viscosity. In another words it can be said that the resistance to flow of aqueous extract solution is the highest comparing with other extracts solution and the resistance to flow of the oil extracted by MeOH was higher than the oils extracted by SE and Sc-CO_2.

Surface tension was considered as a molecular property depended on the cohesive forces among liquid molecules. These forces get balanced in the liquid bulk since every molecule is surrounded by neighbouring molecules from all direction but the molecules at the surface get deficiency of neighbouring molecule in upper direction so the cohesive forces become unbalanced therefore they are pulled inwards and the surface of liquid is forced to contract to the minimal area. The surface tension for the extracts in this study was examined and ordered from high value to low value as (47.66282,23.89747, 22.51615 and 21.27923) dynes cm^{-1} for (aqueous, MeOH, Sc-CO_2 and SE) extracts respectively. This order depended on the polarity of solvents. Since the polar solvents extract hydrophilic compounds which exhibit high surface tension values whereas the non-polar solvents extract hydrophobic compounds which exhibit low surface tension values (Properties Estimation, 2011). CO_2 is a non-polar compound but in this study it was used with MeOH as a modifier which lead to increase the polarity of CO_2 extract hence the extract by diethyl ether (SE) gave the least surface tension values as it was the least polar solvent used. The surface tension calculation of the extracts solutions explained in table.2.

Table.2.Physical Parameters of Chicory Roots Extracts Solutions at 25 °C

Type of Extract	Refractive Index	Specific gravity	Viscosity	Surface tension
Aqueous Extract	1.33287	1.00109	0.90863	47.66282
MeOH Extract	1.32687	0.79537	0.54815	23.89747
(SE) Extract	1.35673	0.79074	0.31486	21.27923
(Sc-CO_2) Extract	1.35883	0.79305	0.31756	22.51615

Recently, there is a great interest in drugs which taken from the nature since they considered as green medicines which are often expected to be safe. Ash defined as the inorganic residue which remains after removing of organic matter by heating (Vidita, 2013). Determination of the ash helps in quick, easy and significant identification of the physiological and non-physiological constituents presented in the crude drug. The mineral components of the herb itself considered as the physiological ash. However, due to contact by the soil and sand a foreign matter named as non-physiological ash may contaminate to the herbal drug. The official ash values are essential in detection of the purity of powder, herb and hence standardize it for using as a drug. Ash values for *Cichorium intybus* ground roots were determined and the results showed in table 3. It was reported that the roots of chicory had total ash of 4.8%. and the percentage of ash which was not soluble in diluted hydrochloric acid was very less and recorded as 0.2% and that were found to be within the standard ranges (not more than 15% for total ash and not more than 1.8 for acid insoluble ash) (Ghosh S, 2014) whereas the ash which dissolved in water (inorganic matter) found to be 0.6%. Furthermore, sulphated ash test was done to percentage the inorganic contents of the roots of chicory in which sulphuric acid was used to convert the inorganic metals into metal sulphates. The percentage was taken as 1.15%.

Table.3.Ash Analysis of Chicory Roots

Type of Ash	Percentage (w/w)
Total ash	4.8 %
Acid insoluble ash	0.2 %
Water soluble ash	0.6 %
Sulphated ash	1.15 %

UV –VIS spectra: The qualitative UV-VIS spectrum profile of chichory roots extracts which selected at wavelength from 190-1100 nm to obtain sharpness peaks and proper base line showed λ_{max} of 191.8 nm ,299.2 nm ,230.6nm and 258.4 nm and their correspondence absorbance of 4.3877,4.4631,4.3990 and 4.4285 for the aqueous extract,(SE) extract ,MeOH extract and Sc-CO_2 extract respectively. All the extracts absorbed in the range of 190-380 nm of near ultraviolet which cross pond to poly unsaturated and aromatic compounds. The absorption of UV visible in organic molecule is caused by the functional groups called chromophores which contain valence electrons have low excitation energys. The absorption spectra of aqueous extract felt in the region of wavelength 100-200 nm which expected transition either ($\pi \rightarrow \pi^*$) for an isolated double bond or($6 \rightarrow 6^*$) for an ordinary carbon – carbon band. The remaining extracts have absorption spectra within the range of wavelength (200-400) nm which indicated the transition ($\pi \rightarrow \pi^*$) for compounds with conjugated double bonds as well as ($n \rightarrow 6^*$) and ($n \rightarrow \pi^*$) for compounds containing atoms with lone pairs of electrons (nonbonding electrons) which may confirm the presence of mono or poly unsaturated fatty acid hence the double bond available in such compounds allow the maxima absorption ($\pi \rightarrow$

π^*) and ($n \rightarrow \pi^*$) in case a functional group existed with lone pairs of electrons as a result of that low energy is required for excited the electrons in longer wavelength.

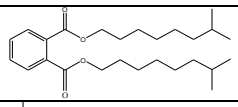
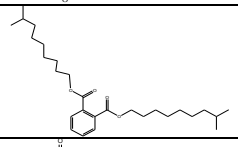
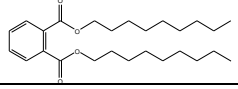
IR Analysis: The FTIR spectrum of aqueous extract was shows that the peaks occurred at 3629.37, 3517.52 cm^{-1} due to 0—H stretching and 2954.41, 2888.84 cm^{-1} due to C—H stretching indicated the presence of carbohydrates. The peaks detected at 3417.24 , 3340.1 , 3239.82 , 3143.4 , 1523.49 cm^{-1} due to N—H stretching and 1172.51 , 1068 cm^{-1} due to C = N stretching and 1523.49 cm-1 due to N—H bending indicated the presence of alkaloids and amino acids. The peaks showed at 3594.66 , 3595.95 and 3286.11 cm^{-1} due to O—H stretching ,1407 cm^{-1} due to C—C stretching and 964.233 , 844.664 , 795.816 cm^{-1} due to C—H bending indicated the presence of phenolic compounds. The FTIR profile of SC- CO2 extract illustrated the important functional groups presented and their corresponding phytochemicals compounds. The peaks appeared at 3752.8, 2333.45 cm^{-1} duo to O — H stretching, 2908.13 cm^{-1} due to C—H stretching and 1457.92 cm^{-1} due to C—H bending indicated the presence of carbohydrates. The peak appeared at 3475 cm^{-1} due to N—H stretching indicated the presence of alkaloids. The peak appeared at 2676 cm^{-1} due to O—H stretching , 1654.62 cm^{-1} due to C=C stretching , 914.093 , 721.247 cm^{-1} due to C—H bending indicated the presence of unsaturated fatty acids.

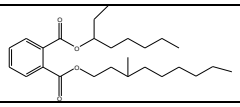
The FTIR spectrum analysis of SE extract gave the valuable details about the phytochemical presented. The peaks at 3752.8, 3513.67 cm^{-1} due to O —H stretching indicated the presence of carbohydrates. The peaks detected at 2364.3 cm^{-1} due to N—H stretching and 1176.36 cm^{-1} due to C—N stretching indicated the presence of alkaloids. The peaks showed at 2676.71cm^{-1} due to O—H stretching and 975.804, 725.104 cm^{-1} due to C—H bending indicated the presence of unsaturated fatty acids. The peaks detected at 1454.06 cm^{-1} due to C=C stretching , 1753.62 cm^{-1} due to C—O stretching and 2854.13 cm^{-1} due to C—H stretching indicated the presence of unsaturated conjugated aldehyde as it will show later in GC—MS profile of SE extract . Due to the conjugated between two double bonds and carbonyl group the absorption frequency of C=C became less than expected.

The IR spectrum profile of MeOH extract was confirmed the presence of phytochemical compounds. The peaks detected at 3756.65, 3683.37 cm^{-1} due to O —H stretching and 2857.99cm^{-1} due to C—H stretching indicated the presence of carbohydrates. The peaks showed at 3363.25 due to N—H stretching and 2672.86 cm^{-1} due to O — H stretching indicated the presence of alkaloids and amino acids .The peaks appeared at 1454.06 cm^{-1} due to C=C stretching , 952.663 , 721.247 cm^{-1} due to C—H bending and 2672.86 cm^{-1} due to O —H stretching indicated the presence of unsaturated fatty acids . The peaks detected at 1735.62 cm^{-1} due to C=O stretching and 1172.51 cm^{-1} due to C—O stretching confirmed the presence of cardiac glycosides. The peak presented at 1099.23 cm^{-1} due to C—O stretching indicated the presence of primary alcohol. It became clear that FTIR has the ability to characterize and identify the compounds presented in unknown mixture of any extract of herbal plants through the prediction of their chemical bonds hence FTIR considered as the most strong tool to detect the functional groups of the phytochemical compounds which were the reason behind the biological activities for the plants which use as drugs.

GC-MS Analysis: GC-MS analyzed results were included the amount percentage of major compounds presented in which the average peak area was compared to the total areas. National Institute of standard and Technology) NIST library data was used to detect the compounds found. The spectrum of unknown compound was compared with that of known compound available in the NIST library. The name, molecular weight, molecular formula and structure of the compounds were given. Mass spectrometry has sensitivity and selectivity for the analysis of compounds. It detect the structure of molecules as well as their molecular weight .The combination between gas chromatography and mass spectrometry gave the benefit of both0. The unknown compounds in the extract were separated by gas chromatography and identified by mass spectrometry. The gas chromatogram of aqueous extract fig. 4 showed the relative concentration of 25 compounds which getting eluted as a function of retention time. Among of them four retention times (23.385, 24.820, 27.220, 27.665) min were further studied by mass spectrum in which the fragmentation of the compounds for every retention time gave base peak at 149.00. The chemical compounds which expected by NIST library were given in table 4.The major compound with percentage of 39.54% was not detected.

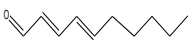
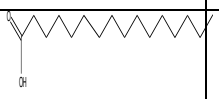
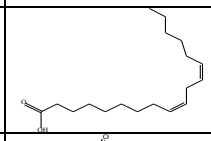
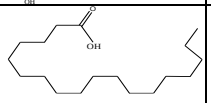
Table.4.GC-Mass Analysis of Aqueous Extract

Retention Time	Molecular Weight	Compound Formula	Compound Name	Compound strucuter	percent age
23.385	418	$C_{26}H_{42}O_4$	Phtalic acid, bis(7-methyloctyl)ester		≈ 5.16
24.820	446	$C_{28}H_{46}O_4$	Phtalic acid, bis(8-methylnonyl)ester		≈ 3.71
27.220	418	$C_{26}H_{42}O_4$	Phtalic acid, dinonyl ester		≈ 4.25

27.665	404	$C_{25}H_{40}O_4$	Phtalic acid, nonyl oct -3-yl ester		≈ 4.17

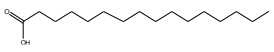
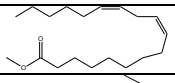
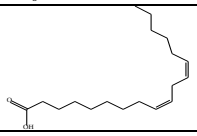
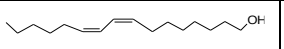
The gas chromatogram of SE extract showed the relative concentration of 37 compounds which getting eluted as a function of retention time. Among of them four retention times (7.280 , 20.695 , 23.300 , 23.535)min which were the major constituents presented were also studied by mass spectrum in which the fragmentation of the compounds for every retention time showed base peaks at (81.05 , 73.00 , 81.05 , 55.05)respectively. The chemical compounds which predicted based on NIST data were given in table 5. Linolic acid found to be the major compound in this extract with percentage of 46.74%.

Table.5.GC-Mass Analysis of SE Extract

Retention Time	Molecular Weight	Compound Formula	Compound Name	Compound structure	percentage
7.280	152	$C_{10}H_{16}O$	2,4-Decadienal		≈ 1.04
20.695	256	$C_{16}H_{32}O_2$	Plamitic acid		≈ 32.88
23.300	280	$C_{18}H_{32}O_2$	Linoleic acid		≈ 46.74
23.535	284	$C_{18}H_{36}O_2$	Stearic acid		≈ 0.54

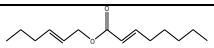
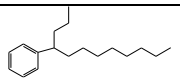
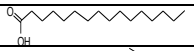
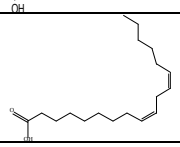
The analysis of MeOH extract gas chromatogram showed the relative concentration of 16 compounds which getting eluted as a function of retention time. Four retention times (20.600, 22. 485, 23.225, 23.450) min which represented the major components were studied by mass spectrum in which the fragmentation of the compounds for every retention time showed base peaks at (73.00, 67.05, 81.05, 55.05) respectively. The chemical compounds which got with reference of NIST library were given in table 6. The major compound considered as Linolic acid with percentage of 63.29%.

Table.6.GC-Mass Analysis of MeOH Extract

Retention Time	*Molecular Weight*	*Compound formula*	*Compound Name*	*Compound strucuter*	*percentage*
20.600	256	$C_{16}H_{32}O_2$	Palmitic acid		≈ 13.38
22.485	294	$C_{19}H_{34}O_2$	Linoleic acid, methylester		≈ 8.85
23.225	280	$C_{18}H_{32}O_2$	Linoleic acid		≈ 63.29
23.450	238	$C_{16}H_{30}O$	Z,Z-8,10-Hexadecadien-1-ol		≈ 4. 06

The studied of the gas chromatogram of Sc-CO_2 extract showed the relative concentration of 17 compounds which getting eluted as a function of retention time. Out of them four retention times (7.685, 18.570, 20.490, 22.975) min were analyzed by mass spectrum in which the fragmentation of the compounds for every retention time showed base peaks at (84.00, 18.576, 20.490, 22.975) respectively. The chemical compounds which found based on NIST library were given in table 7. The major compound with percentage of 38.77% was not detected.

Table.7.GC-Mass Analysis of Sc-CO_2 Extract

Retention Time	*Molecular Weight*	*Compound formula*	*Compound Name*	*Compound structure*	*Percentage*
7.685	224	$C_{14}H_{24}O_2$	E-2-Hexenyl E-2-Octenoate		≈ 6.67
18.576	246	$C_{18}H_{30}$	Benzene,(1-propylnonyl)		≈ 4.63
20.490	256	$C_{16}H_{32}O_2$	Palmitic acid		≈ 4.21
22.975	280	$C_{18}H_{32}O_2$	Linoleic acid		≈ 21.06

4. CONCLUSION

The presented study gave a clear view for the physical properties of the root extracts of chicory in order to enhance its uses as substituted desirable for artificial drugs in wide range of medicinal field. Ash analysis recommended the use of the chicory root as a safe medicine due to less foreign matter exhibited in it. The four extracts examined in this study were qualitatively similar to some extent due to the availability of same fatty acid like linolic acid and palmtic acid but quantitatively they were different as reported by GC-MS analysis. Linolic acid was the major component for MeOH extract with the highest percentage 63.29% and (SE) extract but with lower percentage Sc-CO_2 extract has the least percentage of linolic acid 21.06%. the percentage of palmatic acid was highest in (SE) extract(32.88%) then MeOH extract 13.38% and finally Sc-CO_2 4.63% , aqueous extract was totally different and these fatty acids was not detected in it. Further studies should be done to investigate the major component of Sc-CO_2 extract as well as aqueous extract. Spectroscopic analysis becomes a strong tool in analytical field for both qualitative and quantitative examined of biological and pharmaceutical compounds. All the results obtain by IR and UV spectroscopies were identical with GC-MS analysis and very helpful to confirm the phytochemical compounds presented in the plant extract.

Supercritical fluid extraction of chicory roots was considered as the desired alternate technology to conventional methods of extraction .Reducing the time needed for extraction, the cost of energy and solvent consumption were the main advantage to this method comparing with other traditional methods. Finally, it can be said that the phytochemical components presented in (*Cichorium intybus*) roots extracts have to find their place in the treatment of different diseases. Further studies suggested to be done to investigate the significant biological activities of the chicory roots extracted by SC-CO_2 such as anti-oxidant and anti-cancer activities, also to discover the herb potential to solve many health problems especially that occurring in our ancient literature.

ACKNOWLEDGEMENTS

The author (Ola) is thankful to Hodeidah University, Al-Hodeidah,Yemen for Supporting her to do this work and we are thankful to Dr Mohammad Mohsin, Assist Prof, Post Graduate & Research Center, Maulana Azad College, Aurangabad (MS), India for constant support and kind help .

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