Chemical Composition of Saudi Arabian *Sukkari* variety of Date Seed Oil and Extracts Obtained by Slow Pyrolysis

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The present study was designed to convert Saudi Arabian *Sukkari* waste date seeds into pyrolysis liquid char oil in a slow pyrolysis reactor and determine the phytoconstituents by gas chromatography/mass spectrometry. The date seed in particle form was pyrolysed in an externally heated pan sand bath to obtain 5.2 % v/w liquid char oil. The liquid char oil (20 ml) was dissolved in 100 ml aqueous methanol (30:70) and was four times fractionated with 25 ml of n-hexane. Small portion (1 g) of both methanol and n-hexane fraction was subjected to gas chromatography/mass spectrometry analysis. Phytochemical screening of both fractions revealed the presence of steroids, alkaloids, reducing sugars, phenolics, flavonoids, terpenoids, fatty acids and amino acids. The prevailing compounds found in n-hexane fraction were cyclolanost-24-en-3-ol (11.25 %), stigmast-5-en-3 β–ol (2.02 %), lupen-3-one (3.50 %), isopropyl myristate (0.64 %), palmitic acid, methyl ester (0.74 %), oleic acid (0.77 %), tridecane (5.66 %), and dodecane (4.42 %). Compounds found in methanol fraction were mainly stigmast-5-en-3-ol (5.77 %), propylene glycol monooleate (2.82 %), guanosine (35.92 %), DL-arabinitol (2.11 %), dodecanoic acid (8.14 %), stearic acid (4.22 %), palmitic acid (7.61 %), oleic acid (9.13 %), 1,3,5-triazine-2,4,6-triamine (1.87 %) and 2-butylbutanoic acid (1.01 %). The presence of these bioactive compounds confirmed the application of the *Sukkari* varieties of date seeds for various medicinal activities in future drug discovery system and could be analysed for antiinflammatory, antioxidant, cardiovascular, anticancer and immunosuppressant activities. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Key words: Date seed oil, pyrolysis, liquid char oil, gas chromatography/mass spectrometry (GC/MS), phytoconstituents

Date palm (*Phoenix dactylifera* L.) is an important agricultural commodity and has been cultivated extensively in the whole world especially in North Africa, Middle East, as well as some parts of Central and South America and Southern Europe[1]. Date fruit has always been considered an ideal food complement and a rich source of carbohydrate due to its high sugar, dietary fibre, macro and micro-nutrient contents for people of the Middle East[2]. Date palm is an important fruit crop in the Kingdom of Saudi Arabia and *Sukkari* variety of date palm contained the highest amount of protein and nutrients[3]. Date seeds also have potential positive health benefits and contain components with different biological actions, such as antiinflammatory, antiviral, antioxidant and some other activities but it is usually wasted[4,5]. The date seeds oil is a mixture of different types of major and minor organic compounds those belong to acids, alcohols, ketones, aldehydes, phenols, ethers, esters, sugars, furans, nitrogen compounds and multifunctional compounds[6]. The date seeds are too hard to extract out the constituents and almost unutilized. This waste seed oil can be extracted by slow pyrolysis method and used for different medicinal purpose. Pyrolysis is generally described as the thermal decomposition of the organic components into liquid products[7]. In this study, pyrolysis of *Sukkari* variety of date seeds cultivated in Saudi Arabia are carried out and after purification

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of liquid char oil products, its phytoconstituents was determined by the sophisticated analytical instruments gas chromatography/mass spectrometry (GC/MS). The underlying components in a date seeds in detail could be characterized and the valuable components would be utilized in pharmaceutical and nutraceutical industry in best possible way to minimize this waste.

Sukkari variety of date seed was directly obtained from date fruit in March 2015 in full ripe condition from Al-Madinah Al-Monwarah city markets of Saudi Arabia and was authenticated in the Department of Pharmacognosy and Phytochemistry, College of Pharmacy, Sattam Bin Abdul Aziz University, KSA (The accession no. PSA/PHAR/COG/15/04). The seeds were washed and dried at about 40° to remove the remaining moisture present and weighed.

Pyrolysis of date seeds was carried out according to the method of Islam *et al*., with some modifications[8]. At the beginning of experiment, 100 g of the seeds were milled in a heavy-duty grinder to obtain a coarse powder, which was loaded into glass funnel placed in a pan sand bath. The sand bath was heated to 400-500° with a burner for pyrolysis and the temperature was measured by means of a mercury thermometer. Simultaneously, the glass funnel containing sample was covered with another glass funnel to prevent the excess loss of sample moisture. To release the constituents and oil, distilled water was added drop-wise to the sample. During pyrolysis, the tar and oil released were collected in a container[8,9].

The products obtained from the pyrolysis of date seed were 5.2 % v/w liquid char oil. The liquid char oil (20 ml) was dissolved in 100 ml aqueous methanol (30:70) and it was four times fractionated with 25 ml of n-hexane (Merck, for analysis) by using a separating funnel. After fractionation, layer of methanol and n-hexane was separated and the solvent was removed using a Rota-vap apparatus. In the methanol fraction chocolaty brown colour extract and in n-hexane fraction, white-coloured oily substance was obtained. The physical characteristics like viscosity, refractive index and density of the both fractions of pyrolysis liquid char oil of seeds were evaluated. Viscosity was determined as such without dilution at 27° using Brookfield DV-1 Prime viscometer (Brookfield Engineering, Inc., Middleboro, MA). The refractive index was measured by using Abbe Refractometer (BESTO) by placing one drop sample on slide and the refractometer was adjusted first with distilled water. Density of the sample was determined using Puchnometer Kit (Adam Equipment co. LTD. Bond Avenue Denbigh East Estate Milton Keynes, MK, ISV-United Kingdom)[10]. Small portion (1 g) of both fractions was subjected to GC/MS analysis[11,12].

Both methanol and n-hexane fractions of pyrolyzed seeds were subjected to preliminary phytochemical investigation for the presence of various phytoconstituents like steroids, alkaloids, reducing sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids[13].

The GC/MS analysis of both fractions B and C were performed using the GC/MS-QP2010 Ultra. TR 5-MS capillary standard non-polar column, with dimensions of 30 m and 0.25 mm id, and 0.25 mm film was used and flow rate of mobile phase (He as carrier gas) was set at 1.21 ml/min. The oven temperature of GC instrument was raised from 100° to 260° at 10°/min and injection volume was 5 µl. Samples which dissolved in n-hexane and methanol were run fully at a range of 10-850 m/z and the results were compared by using Wiley spectral library search program. The mass spectra detected in 30-35 min[14]. The name, molecular weight, molecular formula and structure of the component of test materials were determined while the relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

The relative percent weight compared with the weight of the fresh fruits, crude fibre contents and total ash of date seeds were evaluated. The date seed was successfully converted into liquid char oil by slow pyrolysis system. The contiguous parameters like pH, density and kinematic viscosity of n-hexane and methanolic fractions of pyrolysis liquid char oil were presented in Table 1. Both fractions of pyrolysis oil are found to be slightly heavier than water with a density of 1052.4 and 1036.8 kg/m^3 at 27°. Preliminary phytochemical screening of pyrolysis liquid char fractions of date seeds revealed that the n-hexane fraction contains steroids, terpenoids, amino acids and fatty compounds, whereas methanolic fraction contains steroids, terpenoids, alkaloids, reducing sugars, phenolics, flavonoids, tannins and amino acids compounds.

The extraction, isolation and investigation of plant material play a vital role in the development, modernization, and quality control of herbal formulations. Hence, the present study was intended to find the bioactive compounds present in the liquid

char oil obtained by slow pyrolysis of date seeds using GC-MS. Crude liquid char oil contained both polar and non-polar phytoconstituents. Sequential fractionation with n-hexane helped in separation of polar and non-polar phytoconstituents in the respective methanol and n-hexane solvent. The results pertaining to GC/MS analysis led to the identification of a number of compounds from GC and they were identified through mass spectrometry attached with GC/MS analysis. The chromatograms obtained by n-hexane and methanol fractions of liquid char oil were shown in fig. 1A and B, respectively. The active principle, area of the peak,

TABLE 1: PHYSICOCHEMICAL CHARACTERISTICS OF DATE SEEDS AND METHANOL AND n-HEXANE FRACTIONS OF PYROLYSIS LIQUID CHAR OIL

Sample	Percentage weight (%)	Crude fibre (%)	Ash (%)	Refractive index at 27°	Density (kg/m^3)	Kinematic viscosity at 27° (cSt)
Seed	11.27	42.4	3.4	--	--	--
Methanol fraction	2.8	--	--	1.442	1052.4	7.68
n-Hexane fraction	1.36	--	--	1.468	1036.8	6.24

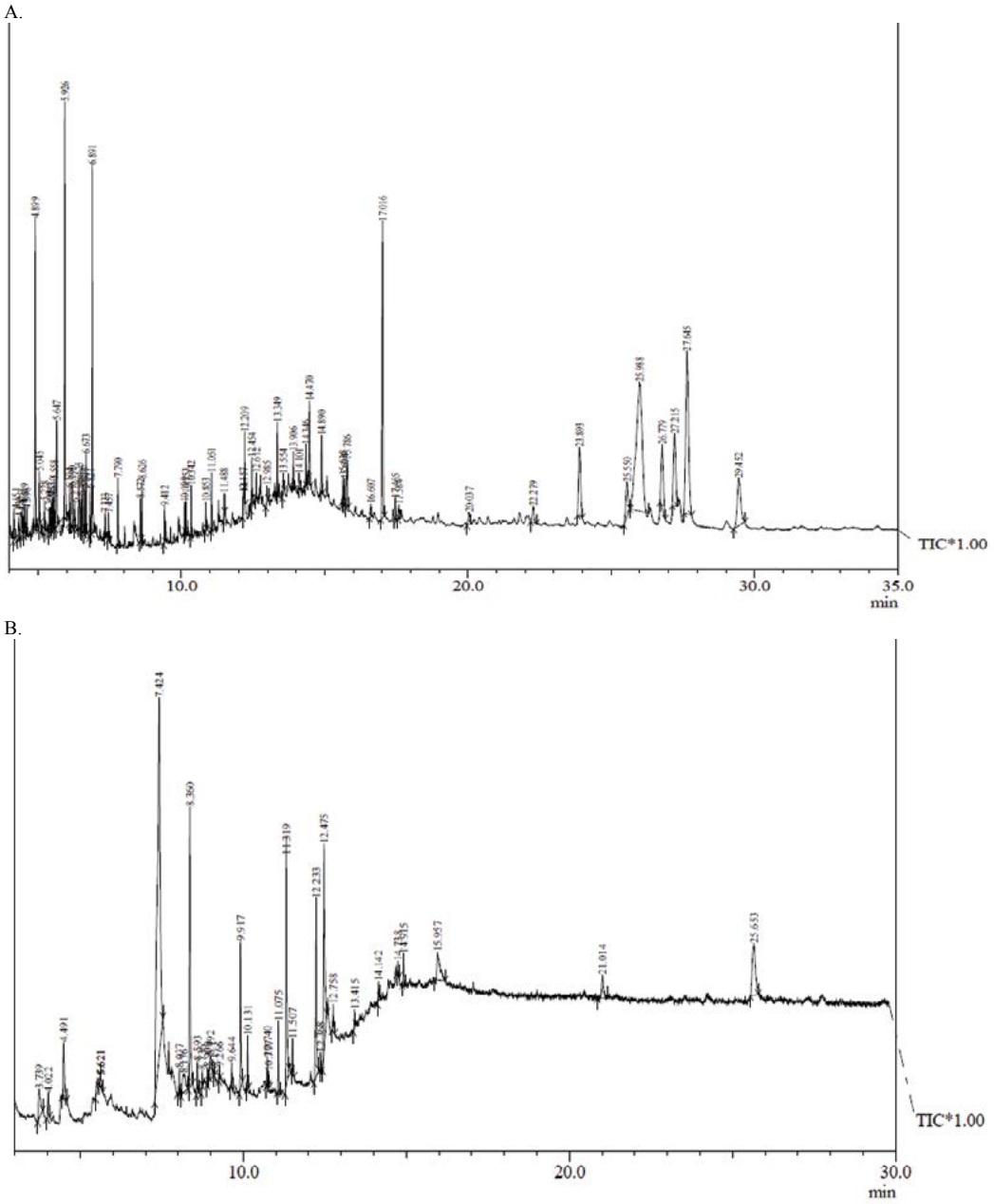

Fig. 1: GC/MS chromatograms
A: n-hexane and B: methanol fraction of pyrolysis liquid char oil of *Sukkari* date seeds

concentration (%), and retention time and details of the compounds were presented in Tables 2 and 3, respectively.

The prevailing compounds found in the n-hexane fraction were steroidal and triterpenoids (cyclolanost-24-en-3-ol, 11.25 %; stigmast-5-en-3 *β*–ol, 2.02 %;

TABLE 2: COMPOUNDS PRESENT IN THE n-HEXANE FRACTION OF PYROLYSIS LIQUID CHAR OIL OF *SUKKARI* DATE SEEDS USING GC/MS ANALYSIS

RT	Peak area, %	Name of compounds	Molecular formula	Molecular weight	Compounds nature
4.151	0.59	Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Phytol
4.330	0.44	(14Z)-14-tricosenyl formate	$C_{24}H_{46}O_2$	366	Fatty ester
4.429	0.46	5-ethyl-undecane	$C_{13}H_{28}$	184	Hydrocarbon
4.899	4.42	Dodecane	$C_{16}H_{34}$	226	Hydrocarbon
5.045	0.75	2,5-dimethyl undecane	$C_{13}H_{28}$	184	Hydrocarbon
5.228	0.38	2-ethylhexyl acrylate	$C_{11}H_{20}O_2$	184	Acrylic acid ester
5.390	0.39	Hexyl cyclohexane	$C_{12}H_{24}$	168	Hydrocarbon
5.510	0.38	4-methyl dodecane	$C_{13}H_{28}$	184	Hydrocarbon
5.558	0.70	2-methyl-6-propyl dodecane	$C_{16}H_{34}$	226	Hydrocarbon
5.647	2.12	2,6,10,14-tetramethyl pentadecane	$C_{19}H_{40}$	268	Hydrocarbon
5.926	5.66	Tridecane	$C_{13}H_{28}$	184	Hydrocarbon
6.104	0.70	3-bromodecane	$C_{10}H_{21}Br$	220	Hydrocarbon
6.190	0.74	2,4-decadienal, (E,E)	$C_{10}H_{16}O$	152	Aldehyde
6.277	0.46	1-methyl naphthalene	$C_{11}H_{10}$	142	Naphthalene
6.425	1.02	1-propyldecyl cyclohexane	$C_{19}H_{38}$	266	Hydrocarbon
6.498	0.40	4-methyl tridecane	$C_{14}H_{30}$	198	Hydrocarbon
6.617	0.56	3,8-dimethyl-decane	$C_{12}H_{26}$	170	Hydrocarbon
6.673	0.96	2,6,10,14-tetramethyl-hexadecane	$C_{20}H_{42}$	282	Hydrocarbon
6.821	0.63	1-tetradecanol	$C_{14}H_{30}O$	214	Alcohol
7.333	0.32	1,8-dimethyl-naphthalene	$C_{12}H_{12}$	156	Naphthalene
7.457	0.37	2,6,10,15-tetramethyl-heptadecane	$C_{21}H_{44}$	296	Hydrocarbon
8.572	0.54	n-pentadecanol	$C_{15}H_{32}O$	228	Alcohol
10.109	0.36	n-nonadecanol-1	$C_{19}H_{40}O$	284	Alcohol
10.342	0.64	Isopropyl myristate	$C_{17}H_{34}O_2$	270	Fatty acid ester
11.051	0.74	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid ester
11.488	0.29	1-Pentyl-2-propyl-cyclopentane	$C_{13}H_{26}$	182	Hydrocarbon
12.157	0.23	2,6,10,14-tetramethyl-(Phytan)	$C_{20}H_{42}$	282	Phytan
12.254	0.77	Oleic acid	$C_{18}H_{34}O_2$	282	Fatty acid
12.612	0.34	9-Octadecenoic acid (Z)-, ethyl ester	$C_{20}H_{38}O_2$	310	Fatty acid ester
13.349	0.84	Docosane	$C_{22}H_{46}$	310	Hydrocarbon
13.906	0.45	Tetratetracontane	$C_{44}H_{90}$	618	Hydrocarbon
14.346	0.54	Arachidic alcohol	$C_{20}H_{42}O$	298	Fatty alcohol
14.470	1.03	Hexatriacontane	$C_{36}H_{74}$	506	Hydrocarbon
14.890	0.90	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	Ester
15.784	1.21	n-tetratetracontane	$C_{44}H_{90}$	618	Alkane
16.607	0.32	2-methylhexacosane	$C_{27}H_{56}$	380	Hydrocarbon
17.016	6.71	Squalene	$C_{30}H_{50}$	410	Terpenoids
17.465	0.49	Henicosyl formate	$C_{22}H_{44}O_2$	340	Ester
17.584	0.37	Pentatriacontane	$C_{35}H_{72}$	492	Hydrocarbon
20.037	0.46	2,6,10,15,19,23-hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol	$C_{30}H_{50}O$	426	Terpenol
23.89	3.50	Lupen-3-one	$C_{30}H_{48}O$	424	Lupen
25.550	2.02	Stigmast-5-en-3 *B*-ol	$C_{29}H_{50}O$	414	Steroid
26.779	3.50	Propanoic acid, 2,2-dimethyl-, [(E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl] ester	$C_{20}H_{34}O_2$	306	Terpene ester
27.215	4.11	9,19-cyclolanost-23-ene 3 *B*,25-diol	$C_{30}H_{50}O_2$	442	Steroid
27.645	11.25	9, 19-cyclolanost-24-en-3-ol, (3.*B*)	$C_{30}H_{50}O$	426	Steroid
29.452	4.10	13,27-cycloursan-3-one	$C_{30}H_{48}O$	424	Steroid

TABLE 3: COMPOUNDS PRESENT IN THE METHANOL FRACTION OF PYROLYSIS LIQUID CHAR OIL OF *SUKKARI* DATE SEEDS USING GC/MS ANALYSIS

RT	Peak area %	Name of compounds	Molecular formula	Molecular weight	Compounds nature
3.739	1.87	1,3,5-triazine-2,4,6-triamine	$C_3H_6N_6$	126	Aza dye
4.022	1.01	2-butylbutanoic acid	$C_8H_{16}O_2$	144	Fatty acid
4.491	2.05	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	$C_6H_8O_4$	144	Flavone
5.621	0.42	Pelargonic acid	$C_9H_{18}O_2$	158	Fatty acid
5.621	0.54	(3,3,4-trimethyl-4-pentenyl) benzene	$C_{14}H_{20}$	188	Aromatic
7.424	35.92	Guanosine	$C_{10}H_{13}N_5O_5$	283	Nucleoside
8.037	0.44	Methyl laurinate	$C_{13}H_{26}O_2$	214	Fatty ester
8.176	2.11	DL-arabinitol	$C_5H_{12}O_5$	152	Sugar
8.360	8.14	Dodecanoicacid	$C_{12}H_{24}O_2$	200	Fatty acid
8.593	0.70	n-pentadecanol	$C_{15}H_{32}O$	228	Fatty alcohol
8.736	0.34	Phthalic acid, ethyl isoporpyl ester	$C_{13}H_{16}O_4$	236	Ester
8.909	0.25	1,2,4-trimethoxy-5-[(1E)-1-propenyl] benzene	$C_{12}H_{16}O_3$	208	Camphor
8.992	1.22	4,6-dimethyl-3-([(E)-(3-itrophenyl) methylidene] amino)-2(1H)-pyridinone	$C_{14}H_{13}N_3O_3$	271	Alkaloids
9.113	0.10	Mandelic acid, 3,4-dihydroxy, (4-TMS)	$C_{24}H_{34}F_5NO_3Si_3$	563	Organo-silicates
9.266	0.23	Vinyl octanoate	$C_{10}H_{18}O_2$	170	Ester
9.644	0.56	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	Ester
9.917	4.22	Stearic acid	$C_{18}H_{36}O_2$	284	Fatty acid
10.131	1.01	n-nonadecanol-1	$C_{19}H_{40}O$	284	Wax alcohol
10.740	0.52	8-octadecanone	$C_{18}H_{36}O$	268	Wax ketone
10.777	0.14	Phthalic acid, butyl tetradecyl ester	$C_{16}H_{22}O_4$	278	Aromatic ester
11.075	1.56	Methyl palmitate	$C_{17}H_{34}O_2$	270	Fatty ester
11.319	7.61	Palmitic acid	$C_{16}H_{32}O_2$	256	Fatty acid
11.507	0.77	n-Nonadecanol-1	$C_{19}H_{40}O$	284	Dehydag wax
12.233	5.83	Methyl 8-octadecenoate	$C_{19}H_{36}O_2$	296	Fatty ester
12.368	0.35	Methyl stearate	$C_{19}H_{38}O_2$	298	Fatty ester
12.475	9.13	Oleic acid	$C_{18}H_{34}O_2$	282	Fatty acid
12.758	0.76	9-tricosene, (Z)	$C_{23}H_{46}$	322	Hydrocarbon
13.415	0.50	Decanoylchloride	$C_{10}H_{19}ClO$	190	Acid salt
14.142	0.52	Myristaldehyde	$C_{16}H_{32}O$	240	Fatty aldehyde
14.738	0.21	2-azidocholestan-3-ol	$C_{27}H_{47}O$	508	Steroid
14.915	0.86	Isooctyl phthalate	$C_{24}H_{38}O_4$	390	Aromatic ester
15.957	2.82	Propylene glycol monooleate	$C_{21}H_{40}O_3$	340	Fatty ester
21.014	1.53	Stigmast-5-en-3-yl 9-octadecenoate	$C_{47}H_{82}O_2$	678	Steroidal ester
25.653	5.77	Stigmast-5-en-3-ol, (3*β*)	$C_{29}H_{50}O$	414	Steroid

lupen-3-one, 3.50 %; squalene, 6.71 %), various fatty acids (palmitic acid, methyl ester, 0.74 %; oleic acid, 0.77 %; isopropyl myristate, 0.64 %), and various hydrocarbons tridecane (5.66 %) and dodecane (4.42 %). Compounds found in methanol fraction were mainly steroids (stigmast-5-en-3-ol, 5.77 %), glycerol (propylene glycol monooleate, 2.82 %), purine base (guanosine, 35.92 %), sugar (DL-arabinitol, 2.11 %; 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, 2.05 %), fatty acids (dodecanoic acid, 8.14 %; stearic acid, 4.22 %; palmitic acid, 7.61 %; oleic acid, 9.13 %; 2-butylbutanoic acid, 1.01 %) and aza dye (1,3,5-triazine-2,4,6-triamine, 1.87 %).

The n-hexane oily fraction of liquid char oil was a semi-solid at temperatures below 10° and a viscous liquid at room temperature. The semi-solid nature of the oils is an indication of the presence of major saturated and unsaturated fatty acids in sample[15]. In the GC/MS analysis of the both fractions of pyrolysis liquid char oil were found the different types of important chemical constituents, which were the saturated and unsaturated fatty acid esters, steroids and terpenoid compounds, they were previously reported in literature[10]. Fatty acids like palmitic acid, stearic acid and oleic acid (omega-9) were mainly observed in both fractions. MS spectra of these saturated and unsaturated fatty acids

were readily identified by their high resolution masses 256, 284 and 282 with predicted molecular formulas of $C_{16}H_{32}O_2$, $C_{18}H_{36}O_2$ and $C_{18}H_{34}O_2$, respectively. Five steroids were also identified along with propylene glycol monooleate (fatty acid ester) and three terpenoids like squalene, trimethyl-2,6,10-dodecatrien-1-yl (propanoic acid terpenes ester) and lupen-3-one and a number of saturated and unsaturated long chain alcohols. *β*-Sitosterol was found to be the most abundant of the steroids followed by stigmast-5-en-3-ol, cyclolanost-23-ene-3,25-diol, cycloursan-3-one, stigmast-5-en-3-yl 9-octadecenoate and 2-azidocholestan-3-ol.

These terpenoids and steroidal compounds have several important medicinal activities in future drug discovery system. Such as lanostane triterpenoid and steroidal compounds have significant adaptogenic and anabolic activity. They enhance the general performance of organism during the stress condition by normalizing the physiological process and various functions of body[16]. On the other hand organic acids (octadecenoic acid, pelargonic acid and butylbutanoic acid), flavonoids and esters of aromatic phenolic compounds have been reported to exhibit a wide range of biological activities such as antioxidant, antimicrobial and mast cells stabilizing properties[17]. The fractionated oil could be used in cosmetics and other pharmaceutical care products due to the presence of fatty acids such as palmitic acid, stearic acid and oleic acid in the oil[18]. The stearic and oleic acid content in date seed oil could make this oil as effective percutaneous absorption enhancer by enhancing the diffusion of lipophilic non-steroidal antiinflammatory drugs, which have been widely used in conditions such as chronic rheumatic disorders treatment[19]. The presence of different components in oil such as steroids, terpenoids, organic acids, flavonoids and esters of aromatic phenolic compounds can augment antiinflammatory and analgesics potency of the pharmaceutical preparations. Oleic acid as such taken in diet has augmented the high density lipoprotein content in blood and lowered the low density lipoprotein cholesterol and lipid content showing antiatherosclerotic effect[20]. This property could prevent cardiovascular diseases. Therefore, the fractionated date seed oil could be exploited by the pharmaceutical industries to develop drug formulations for the treatment of different cardiovascular and chronic rheumatic disorders.

The *Sukkari* variety of Saudi Arabian date seed was successfully converted into liquid char oil by slow pyrolysis system. GC/MS data of both fractions of date seeds contained major compounds. These compounds may have important medicinal activities. Phytochemical and GC/MS analysis showed several bioactive compounds including steroids, flavonoids, terpenoids, fatty acids and different types of aromatic ester compounds. These constituents might demonstrate certain pharmacological activities like antiatherosclerotic, antiinflammatory, analgesic and antirheumatic activity. Therefore, the pyrolysis liquid char oil from the date seed could be used by pharmaceutical industry for the preparation of topical formulations for the treatment of chronic rheumatic disorders and its purified fatty acids could be considered as an important candidate of potential source for the treatment of cardiovascular disorder. Moreover, GC/MS profile could be used as biochemical markers in the pharmaceutical industries to identify the different components present in date seeds and in authentication of mother plants.

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