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RESEARCH ARTICLE

Phytochemical and GC-MS analysis of Saudi Arabian Ajwa variety of date seed oil and extracts obtained by the slow pyrolysis method

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Abstract The date seeds oil is a mixture of different types of major and minor organic components with potential positive health benefits. Thus, the study was designed to conversion of Ajwa verity of Saudi Arabian date seeds into pyrolysis liquid char oil by slow pyrolysis reactor and its phytoconstituents were determined by GC–MS. The date seed in particle form was pyrolysed in an externally heated pan sand bath. The sand bath was heated upto 500–600 °C by means of a burner for pyrolysis and this temperature is measured by means of a mercury thermometer. During pyrolysis, the tar and oil are released from the seeds and collected in a container. The liquid char oil was dissolved in 100 ml methanol and fractionated with 100 ml n-hexane by using separating funnel. Small portion (1 g) of both fraction was subjected to GC-MS analysis. GC-MS analysis results indicated the presence of 40 and 27 different phyto-compounds from n-hexane and methanol fraction. The presence of these bioactive compounds confirms the application of the Ajwa variety of date seeds for various medicinal activities in future drug discovery system and can be analyzed for anti-inflammatory, antioxidant, cardiovascular, anticancer and immunosuppressant activities.

Keywords Ajwa date seed · Pyrolysis · Liquid char oil · GC-MS · Phytoconstituents

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Introduction

The fruit of the date palm (*Phoenix dactylifera* L.) is globally marketed as high value commodities, which is produced in many arid and semi-arid regions of the world. It has always played an important role in economic and social life of the people of these regions. Date fruit has always been considered as a principle foods complement by the middle east peoples and a rich source of carbohydrate due to its high sugar, dietary fiber, macro and micro-nutrient contents (Al-Shahib and Marshall 2003; Myhara et al. 1999). Ajwa dates are only cultivated in Saudi Arabia/Al-Madinah Al-Monwarah and usually most of the muslim people break their fast by eating Ajwa dates. Several studies have shown that Ajwa dates are useful for lactating women because they help in enriching breast milk and protecting mothers to diseases and infections (Hasan et al. 2010). Ajwa dates are excellent source of the minerals and necessary for the protection of muscle contractions in the body. The other vast advantage of consuming Ajwa dates are that they contain of high iron content which is vital in RBC production and they may also assist to treat anemia (Ragab et al. 2013).

Date seeds also have potential health benefits and contain components with various biological actions, such as antiviral, antioxidant, anti-inflammatory and some other activities but it is ravage product that could be simply collected from the processing industries of dates or directly from the palm grove or from the gap conditioning stations (Doha and Al-Okbi 2004; Jassim and Naji 2010). The date seeds are too hard to extract out the constituents and almost unutilized. So the 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 (Ibarra et al. 2005). In this study, pyrolysis of Ajwa seeds are carried out and after purification of liquid char oil products, its phytoconstituents was

determined by the sophisticated analytical instruments Gas chromatography–mass spectrometry (GC–MS). The essential components in a date seeds in detail can be characterize and exploit the valuable components in pharmaceutical and neutraceutical industry in best possible way.

Materials and methods

Collection and authentication of seed material

Ajwa 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 authenticated by Dr. Y. T. Kamal, Assistant Professor, 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 °C to remove the remaining moisture present in them and weighed.

Chemical

The chemical and solvents used for fractionation and purification were procured from Merck Mumbai, India.

Experimental setup for pyrolysis

The pyrolysis of date seeds was carried out according to the process of (Islam and Ani 1998) with some modifications. 200 g of the seeds were pulverized in a heavy-duty grinder to obtained crude powder and loaded into glass funnel which is sited in a pan sand bath. The sand bath was heated to 500–600 °C by means of a burner for pyrolysis and this temperature is measured by way of a mercury thermometer. During pyrolysis, the tar and oil are released and collected in a container (Ayllón et al. 2006). The products obtained from the pyrolysis of date seed are liquid char oil. The liquid char oil was dissolved in 100 ml methanol and fractionated with 100 ml n-hexane (Merck, for analysis) by using separating funnel. After fractionation layer of methanol and n-hexane was separated and the solvent was evaporated. In methanolic fraction 9 g brown colour extract and in n-hexane fraction 4 g dull white coloured oily substance was obtained. Small portion (1 g) of both fractions was subjected to GC-MS analysis (Besbes et al. 2005).

Phytochemical screening

Both n-hexane and methanol fractions of seeds were subjected to preliminary phytochemical investigation for the presence of various phytoconstituents like alkaloids, saponins, steroids, reducing sugar, phenolic compounds, flavonoids, tannins, anthraquinone and amino acids. (Khandelwal 2004).

GC-MS analysis

The GC-MS analysis of both fractions were performed using a GC-MS equipment GCMS-QP2010 Ultra. TR 5-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, film: 0.25 mm was used and flow rate of mobile phase carrier gas (Helium) was set at 1.21 mL min^{-1}. The oven temperature of GC instrument was raised from 100 °C–260 °C at 10 °C min^{-1} 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 (Janakiraman et al. 2012). 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.

Results

Preliminary phytochemical screening of both fractions of date seeds revealed that the n-hexane fraction contains steroids, terpenoids, amino acids and fatty compounds, whereas methanol fraction contains steroids, terpenoids, alkaloids, reducing sugars, phenolics, flavonoids, tannins and amino acids compounds Table 1.

GC-MS data of n-hexane fraction

The results pertaining to GC-MS analysis lead to the identification of number of compounds from GC and they were identified through mass spectrometry attached with GC-MS analysis. Figure 1 reflects a typical GC chromatogram of this separated fraction and Table 2 reflects their relevant retention times, peak area and

Table 1 Preliminary phytochemical screening of n-hexane and methanol fractions of Ajwa date seeds

Test	n-Hexane	Methanol
Alkaloids	−	+
Free sugars	−	+
Flavonoids	−	+
Phenolics	−	+
Terpenoids	+	+
Steroids	+	+
Amino acids	+	+
Fatty acids	+	+

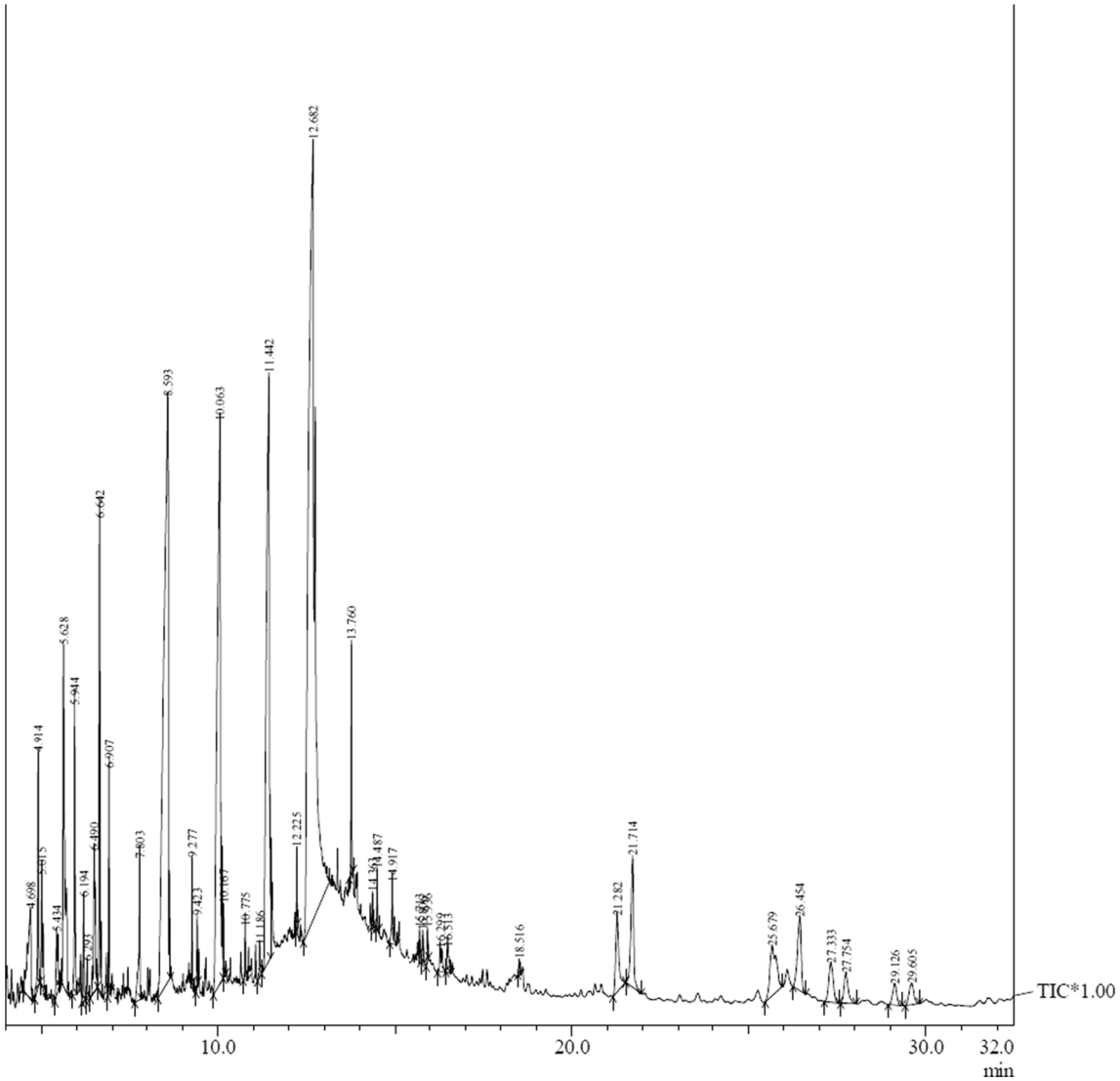

Fig. 1 GC-MS chromatogram of n-Hexane fraction of Ajwa date seeds

details of the compounds. 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 result revealed the presence of 40 different phyto-compounds with retention time 4.698, 4.914, 5.015, 5.434, 5.628, 5.944, 6.194, 6.293, 6.490, 6.642, 6.907, 7.803, 8.593, 9.277, 9.423, 10.063, 10.167, 10.775, 11.186, 11.442, 12.225, 12.682, 13.760, 14.363, 14.487, 14.917, 15.713, 15.806, 15.936, 16.299, 16.513, 18.516, 21.282, 21.714, 25.679, 26.454, 27.333, 27.754, 29.126 and 29.605, respectively. Fatty acids ester, paraffin alcohol and steroids like 4-methylenecycloartan-3-one, 9, 19-cyclolanostan-3-ol, 24-methylene-(3-β) and 9, 19-cyclolanost-23-ene-3, 25-diol, (3.β, 23E) are predominant in this fraction.

GC-MS data of methanol fraction

The compounds from GC fractions of the methanolic extracts of date seeds were identified through mass spectrometry attached with GC-MS analysis Table 3. The result revealed the presence of 27 different phyto-compounds with retention time 3.713, 4.333, 4.371, 4.472, 5.445, 5.907, 7.476, 8.010, 8.792, 8.903, 9.056, 10.129, 10.738, 11.072, 11.315, 11.507, 12.028, 12.210, 12.229, 12.472, 12.595, 12.757, 13.414, 14.663, 15.951, 21.023 and 25.651, respectively (Fig. 2). Sugar (2, 5-monomethylene-l-rhamnitol), flavon (2, 3-dihydro-3, 5-dihydroxy-6-methyl-4 h-pyran-4-one), fatty acids and esters (octadecanoic acid, pentadecanoic acid and glycerol acetate (S)-1, 2, 4-butanetriol, 2-acetate, hexadecanoic acid, methyl ester), fatty alcohols (2, 4-diethyl-1-heptanol and n-nonadecanol-1), nucleoside (cytidine), amino acids (glycyl-dl-serine), volatile oil (asarone), steroids (stigmast-5-en- β-3-ol) were the major compounds.

Table 2 Compounds present in the n-hexane fraction of Ajwa date seeds using GC-MS analysis

S. no.	RT	Peak area%	Name of the compound	MF	MW	Compound nature
1	4.698	1.71	Caprylic acid	$C_8H_{16}O_2$	144	Fatty acid
2	4.914	1.28	Dodecane	$C_{12}H_{26}$	170	Hydrocarbon
3	5.015	0.90	Decanal	$C_{10}H_{20}O$	156	Paraffin
4	5.434	0.69	Cetylic acid	$C_{16}H_{32}O_2$	256	Fatty acid
5	5.628	3.61	Trans-dec-2-enal	$C_{10}H_{18}O$	154	Paraffin
6	5.944	1.59	Tridecane	$C_{13}H_{28}$	184	Hydrocarbon
7	6.194	0.54	2,4-Decadienal	$C_{10}H_{16}O$	152	Paraffin
8	6.293	0.25	Naphthalene, 1-methyl	$C_{11}H_{10}$	142	Naphthalene
9	6.490	1.52	2-Undecenal	$C_{11}H_{20}O$	168	Paraffin
10	6.642	2.91	2-Undecenal	$C_{11}H_{20}O$	168	Paraffin
11	6.907	0.88	Tetradecane	$C_{14}H_{30}$	198	Hydrocarbon
12	7.803	1.05	Octadecane	$C_{18}H_{38}$	254	Hydrocarbon
13	8.593	15.99	Lauric acid	$C_{12}H_{24}O_2$	200	Fatty acid
14	9.277	0.44	3-Heptadecene	$C_{17}H_{34}$	238	Hydrocarbon
15	9.423	0.30	Octadecane	$C_{18}H_{38}$	254	Hydrocarbon
16	10.063	11.55	Myristic acid	$C_{14}H_{28}O_2$	228	Fatty acid
17	10.167	0.25	Nonadecane	$C_{19}H_{40}$	268	Hydrocarbon
18	10.775	0.32	Phthalic acid, butyl udecylester	$C_{23}H_{36}O_4$	376	Fatty ester
19	11.186	0.24	Delta.-undecalactone	$C_{11}H_{20}O_2$	184	Ketone
20	11.442	10.61	Palmitic acid	$C_{16}H_{32}O_2$	256	Fatty acid
21	12.225	0.31	Emery oleic acid ester	$C_{19}H_{36}O_2$	296	Fatty ester
22	12.682	29.83	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	Fatty acid
23	13.760	0.88	Oleic acid, butyl ester	$C_{22}H_{42}O_2$	338	Fatty ester
24	14.363	0.13	Arachidic alcohol	$C_{20}H_{42}O$	298	Paraffin alcohol
25	14.487	0.24	Tetracontane	$C_{40}H_{82}$	562	Hydrocarbon
26	14.917	0.58	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	Aromatic acid
27	15.713	0.10	1-Docosanol	$C_{22}H_{46}O$	326	Paraffin alcohol
28	15.806	0.20	Tetracontane	$C_{40}H_{82}$	562	Hydrocarbon
29	15.936	0.19	Glyceryl monooleate	$C_{21}H_{40}O_4$	356	Fatty ester
30	16.299	0.39	Myristyl oleate	$C_{38}H_{74}O_2$	562	Fatty ester
31	16.513	0.34	Caprylic acid triglyceride	$C_{27}H_{50}O_6$	470	Glycerol ester
32	18.516	0.10	10-Undecenyl 2-(trifluoromethyl)benzoate	$C_{19}H_{25}F_3O_2$	342	Fatty ester
33	21.282	1.47	Lauric acid, vinyl ester	$C_{27}H_{52}O_5$	456	Fatty ester
34	21.714	2.12	4-Nitrophenyl laurate	$C_{27}H_{52}O_5$	456	Fatty ester
35	25.679	1.92	Stigmast-5-en-3 β -ol	$C_{29}H_{50}O$	414	Steroid
36	26.454	1.66	Glyceryl monomyristate	$C_{17}H_{34}O_4$	302	Fatty ester
37	27.333	1.05	9,19-Cyclolanost-23-ene-3β- 25-diol	$C_{30}H_{50}O_2$	442	Sterol
38	27.754	0.76	9,19-Cyclolanost-24-ene-3β –ol	$C_{30}H_{50}O$	426	Sterol
39	29.126	0.50	24-Methylenecycloartan-3-one	$C_{31}H_{50}O$	438	Sterol
40	29.605	0.59	9,19-Cyclolanostan-3-ol, 24-methylene, 3-β- acetate.	$C_{31}H_{52}O$	440	Sterol

Discussion

The n-hexane oily fraction was a semi-solid at temperatures below 10 °C 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 (Salih et al. 2012). Both fractions of the seeds were found to contain different types of important chemical constituents which were the saturated and unsaturated fatty acid esters, fatty steroids, terpenoidal compounds, high molecular weight hydrocarbon

Table 3 Compounds present in the methanol fraction of Ajwa date seeds using GC-MS analysis

S. no.	RT	Peak area%	Name of compounds	MF	MW	Compounds nature
1	3.713	5.90	1,3,5-Triazine-2,4,6-triamine monooxalate	$C_3H_6N_6$	126	Aza dye
2	4.333	0.61	1,2,3-Propanetriol	$C_3H_8O_3$	92	Glycerol
3	4.371	0.80	Acetic acid, butyl ester	$C_6H_{12}O_2$	116	Ester
4	4.472	9.39	2,3-Dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one	$C_6H_8O_4$	144	Flavone
5	5.445	7.38	Glycerol triacetate	$C_5H_{10}O_4$	134	Ester
6	5.907	3.79	1,2,4-Butanetriol, 2-acetate	$C_6H_{12}O_4$	148	Ester
7	7.476	37.60	Guanosine	$C_9H_{13}N_3O_5$	243	Nucleoside
8	8.010	0.85	Glycyl-DL-serine	$C_5H_{10}N_2O_4$	162	Amino acid
9	8.792	4.09	1,4-Anhydro-D-mannitol	$C_5H_{10}O_3$	118	Sugar
10	8.903	0.48	Asarone	$C_{12}H_{16}O_3$	208	Volatile oil
11	9.056	0.67	2,5-Monomethylene-l-rhamnitol	$C_7H_{14}O_5$	178	Sugar
12	10.129	0.61	1-Nonadecene	$C_{19}H_{38}$	266	Hydrocarbon
13	10.738	0.87	8-Pentadecanone	$C_{15}H_{30}O$	226	Ketone
14	11.072	0.34	Methyl palmitate	$C_{17}H_{34}O_2$	270	Ester
15	11.315	6.62	Palmitic acid	$C_{16}H_{32}O_2$	256	Fatty acid
16	11.507	0.49	n-Nonadecanol-1	$C_{19}H_{40}O$	284	Fatty alcohol
17	12.028	1.09	5-Methyl-1,3-diazaadamantan-6-one	$C_{13}H_{20}N_2O_3$	252	Azo compound
18	12.210	0.28	3a, 6-Methano-3ah-inden-4(1H)-one, hexahydro-(3a.α. 6.α., 7a.α.)	$C_{10}H_{14}O$	150	Indene
19	12.229	0.27	2-Hydrazino-6-methyl-pyrimidin-4-ol	$C_5H_8N_4O$	140	Aromatic
20	12.472	6.99	Cis-9-hexadecenal	$C_{16}H_{30}O$	238	Aldehydes
21	12.595	0.29	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Fatty acid
22	12.757	0.33	(Z)-14-Tricosenyl formate	$C_{24}H_{46}O_2$	366	Fatty ester
23	13.414	0.47	2,4-Diethyl-1-heptanol	$C_{11}H_{24}O$	172	Fatty alcohol
24	14.663	0.30	Glycerol L-palmitate	$C_{19}H_{38}O_4$	330	Fatty ester
25	15.951	1.85	Propylene glycolmonoleate	$C_{21}H_{40}O_3$	340	Fatty ester
26	21.023	2.17	Stigmast-5-en- β-3-ol	$C_{29}H_{50}O$	414	Steroid
27	25.651	5.49	Stigmast-5-en- β-3-ol	$C_{29}H_{50}O$	414	Steroid

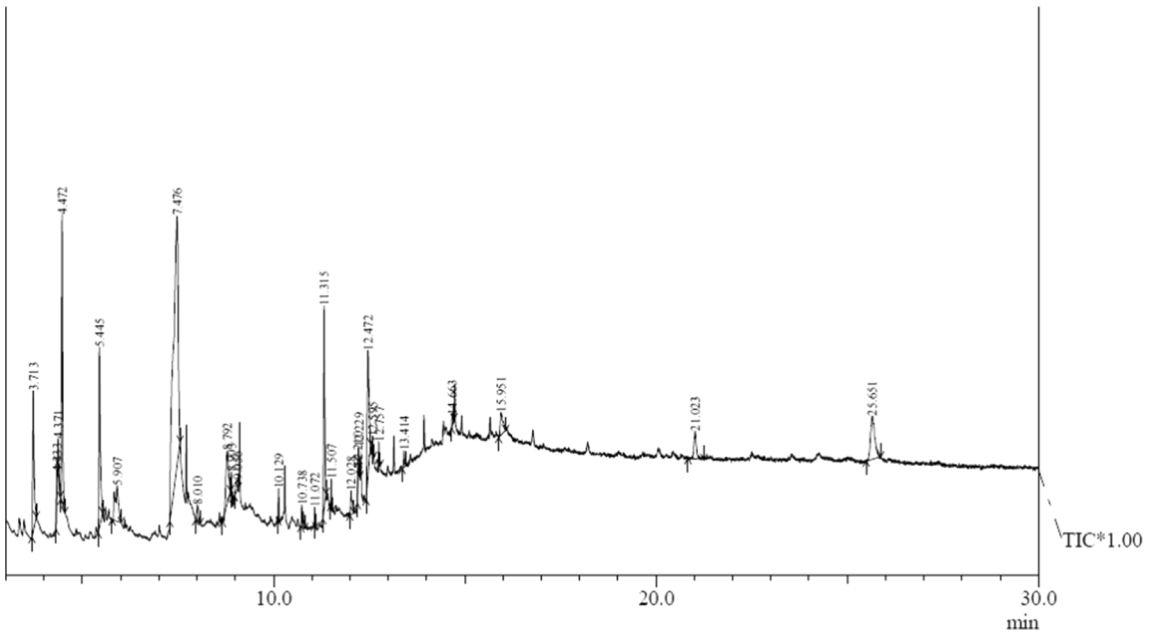

Fig. 2 GC-MS chromatogram of methanol fraction of Ajwa date seeds

and its oxygenated products. Fatty acids like palmitic acid, caprylic acid, cetylic acid, lauric acid, myristic acid and esters of oleic acid (Myristyl oleate) are mainly observed in n-hexane fractions. MS spectra of these saturated and unsaturated fatty acids were readily identified by their high resolution masses 256, 144, 256, 200, 228 and 562 and with predicted molecular formulas of $C_{16}H_{32}O_2$, $C_8H_{16}O_2$, $C_{16}H_{32}O_2$, $C_{12}H_{24}O_2$, $C_{14}H_{28}O_2$ and $C_{38}H_{74}O_2$ respectively. Five steroids were also identified along with ester compounds like Stigmast-5-en-3β-ol, 9,19-Cyclolanost-23-ene-3β-25-diol, 9,19-Cyclolanost-24-ene-3β-ol, 24-Methylenecycloartan-3-one, 9,19-Cyclolanostan-3-ol, 24-Methylene, 3-β-acetate. These steroidal compounds have various important medicinal activities in future drug discovery system. Such as Cyclolanostane 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 (Arif et al. 2013). On other hand organic acids (Benzenedicarboxylic acid and Octadecenoic acid), flavonoids (2,3-Dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one) and esters (Propylene glycolmonoleate) have been reported to exhibit a wide range of biological activity and this effect is mainly attributed to their possessing antioxidant, antimicrobial and mast cells stabilizing properties (Sachan et al. 2011). The oily fraction can used in cosmetics and other pharmaceutical care products is due to the fatty acids (Palmitic acid, Cetylic acid, Myristic acid and Oleic acid) composition of the oil (Vermaak et al. 2011). The myristic and oleic acid content in date seed oil can be a good resource of valuable percutaneous absorption enhancer by increasinging the diffusion of lipophilic non-steroidal anti-inflammatory drug (NSAID), an important anti-inflammatory and analgesics, which has been widely used in conditions such as chronic rheumatic disorders treatment (Larrucea et al. 2001). The presence of different components in the Ajwa date seed fractions such as steroids, terpenoids, organic acids, flavonoids and esters can augment anti-inflammatory and analgesics potency of the pharmaceutical preparations. Hence, the proposal of the application of these fractions of Ajwa date seeds can be use by pharmaceutical industries to develop drug formulations for cosmetic and the treatment of chronic rheumatic disorders.

Conclusion

The Ajwa variety of Saudi Arabian date seed was successfully converted into liquid char oil by slow pyrolysis system and extracted in n-hexane and methanol. Phytochemical and GC-MS analysis showed numerous bioactive compounds including steroids, flavonoids, terpenoids, fatty acids and different types of ester compounds. These components may show certain pharmacological activities like anti-atherosclerotic, anti-inflammatory, analgesic and anti-rheumatic activity. However, these fractions from the date seed may be used by pharmaceutical industries for the preparation of drug formulations for the treatment of different ailments like chronic rheumatic disorders and cardiovascular disorder. Moreover, GC-MS report can be used as biochemical markers in the pharmaceutical industries to recognize the different components present in date seeds and in validation of mother plants.

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Compliance with ethical standards

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Conflict of Interest Authors declares that he has no conflict of interest.

Ethical Statement This article does not contain any studies with human or animals.

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