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Analytical characterization of fatty acids present in seed oils by gas chromatography-Mass spectrometry and qualitative determination of various phytonutrients in dandelion plant

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Abstract

The current research was carried out to determine the phytochemicals constituents in the species of dandelion. Quantitative screenings of fatty acids was also carried out in our lab. Derivatives of methyl ester of fatty acids were analyzed. The analysis was carried out using gas chromatography coupled to mass spectrometer. The result of 19 different fatty acid components were identified and quantified. Linolenic acid was found in highest concentration (C18:2, 19.88%) among the identified analytes of interest. In addition quantification of methyl esters of Tridecanoic acid (C13:0, 1.37%), Palmitic acid (C16:0, 3.89%), linoleic acid (C18:2, 7.78%) and Behenic acid (C22:0, 1.25%) were determined. Concentrations of rest of the detected fatty acids were less than 1%. From the literature it appears that no such work has been performed for the determination of fatty acids in *Dandelion* seed oil. All these plants were collected locally.

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Introduction

Medicinal plants becoming more important with time due to the presence of potential drug compounds. Due to biologically active compounds like phytochemicals these practically play the role of medicines (Krishnaiah *et al*., 2009**)**. Phytochemicals have pronounced effect on the human body and combined with the nutrients and fibers they provide shield against certain diseases and conditions of stress (Afolabi *et al*., 2013).Phytochemicals are classified on the basis of function in plants metabolism, into two categories (i) primary phytochemicals such as carbohydrates, amino acids, proteins and chlorophylls and (ii) secondary phytochemicals such as alkaloids, terpenoids, steroids and flavonoids (Dhawale *et al*., 2013).

Although core values of large number of plants in nature have been determined and published but a vast number of them still remained undiscovered (Dhawale *et al*., 2013). Which are filled with a treasure of medicines to overcome every type of ailments (Mushtaq *et al*., 2009). Phytonutrients are one of the most important and widely distributed groups of components in plant (Shaukat *et al*., 2014).The phytochemicals impart plants a particular color, flavor and special protection against harmful agents. It is studied that a food rich in fruit and vegetables gives numerous health benefits like reducing the risk of causing various kinds of cancer (pancreas lungs, prostate, and breast as well as reducing the risk against cardiovascular diseases) (Okwu *et al*., 2007).

Dandelion "*Taraxacum* spp" used as diuretic and better digestive stimulant, preclinical research on it has revealed numerous properties, antiangiongenic and prebiotic also the leaves are rich in fibers, potassium, iron, calcium and some vitamins (Chrubasik *et al*., 2007). The plant species and herbs suggests the presence of anti-oxidative and antimicrobial constituents in their tissues. Antioxidants can be used to reverse the harmful and pathological effect of the free radicals present in phytochemicals (Clyton *et al*., 2000). In the treatment of serious gram- negative and gram-positive infections is evident and is most required because of emergence of multidrug resistance in common pathogens, and the potential for use or multidrug – resistant agents in bioweapons (Kais *et al*.,2013).

Clinically, dandelion useful in colitis (painful medical condition that affects your colon), liver diseases and also diuretic. Sesquiterpenes lactones are useful for inflammation and cancer (McGee *et al*., 2004). The dandelion did not exhibit significant antifungal activity (Bagchi *et al*., 1999). Several laboratory studies report antineoplastic chemotherapy agents used to treat this is another forms of cancer shown in properties of dandelion and other species of *Taraxacum* (Marcos *et al*., 2007; Thisbe *et al*., 2007; Hagymasi *et al*., 2000; Kim *et al*., 1999; Kim *et al*., 1998). Dandelion has anti-oxidant properties (Hudec *et al*., 2007; Greenlee *et al*., 2007; Zhi *et al*., 2007; Bohm *et al*., 1959; Maliakal *et al*., 2001; Rutherford *et al*., 1972; Bagchi *et al*., 1999). It is also useful in Endocrine effects while in a mouse study, Dandelion T-1 extracts up-regulated estrogen receptors (alpha and beta), progesterone receptor, and follicle-stimulating hormone receptor expression (Rutherford *et al*., 1972).

Dandelion has a direct effect on the gallbladder causing contraction and release of stored. Activity of hepatic-enzyme CYP1A2 in the liver microsomes of rats receiving dandelion in a green tea extract solution was decreased to 15% of a control group (which received water) (Zhao *et al*., 2006). In the same study, CYP2E activity was decreased to 48% of the control group. Following the ingestion of dandelion in a green tea extract solution, detoxifying enzyme UDP-glucoronosyl transferase activity increased to 244% of the control group enzyme activity (Bagchi *et al*., 1999). Inulin, a constituent in dandelion, may act to buffer blood glucose levels and has experimental hypoglycemic activity in animals (Marcos *et al*., 2007).

Recently, the biological importance (Willac *et al*.,

2000) of fatty acids has gained considerable importance in food nutrition evaluation (Tomaino *et al.*, 2001) and in the diagnosis of certain diseases and pharmacology (Stoddart *et al.*, 2008). Fatty acids with un-saturation, either monounsaturated or polyunsaturated, have been used in lowering the risks of heart diseases, against inflammation and in enhancing the immunity or immune system (Calder, 1999; Hamberg and Hamberg, 1996; Hargrove *et al.*, 2001; Yaqoob, 2002; Villa *et al.*, 2002; Siscovick *et al*, 1995).

Recently a variety of analytical techniques have been utilized for the identification and quantification of various types of fatty acids including enzymatic, spectrophotometric, HPLC and gas chromatography (Zhao *et al.*, 2006). Among these GC-MS is most reliable technique for the analysis of fatty acids due to various reasons like sensitivity, resolution and speed. The investigation and exploration of the composition of fatty acids from the seeds of *Taraxacum* spp (Dandelion) is the need of time in order to explore new frontiers for its pharmacological and health importance.To the best of our knowledge and literature survey it is the first report on the analysis of fatty acids extracted from the seed of *Taraxacum* spp.

Materials

Locally available dry leaves of dandelion plant. All chemicals used were of analytical grade and were purchased from Merck and sigma Aldrich.

Methods

Plants collection and identification

The plant was collected from the botanical gardens of Islamia College, Peshawar and identified by Prof. Dr. Shaukat Ali (Department of Chemistry) and by Prof Dr. Zahir Shah Department of Botany).

Pretreatment of plants

The plant collected was first dried in shade for about a month and then it was used for extraction.

Extraction

5 gram of grinded leaves was added with 80 mL of chloroform, n-hexane, ethyl acetate, distilled water, ammonia, acetone, and ethanol were extracted. The solution was filtered using Whatt- Man filter paper (size 42) and then extracted. The extracts were used for different tests.

Phytochemical screenings tests

Tannin test

If brownish color appears by adding 20 mL of chloroform to 0.5 gm of dried powder sample which was boiled and then to filtered solution a few drops of 0.1% ferric chloride were added shows the presence of tannin.

Saponin test. (Formations of Emulsion)

2 gm of the powdered sample was taken and boiled with 20mL of chloroform in water bath. 10 mL of the filtrates was taken and mix with 5ml of distilled water and shake vigorously and then mix with 3 drops of olive oil and shake well. Formation of emulsion shows saponin.

Phlobotannins test

The chloroform extract of each sample was boiled with 1% hydrochloric acid with deposition of red precipitate shows the presence of phlobotannins.

Flavonoids test

Presence of flavonoids is indicated if yellow coloration disappears on standing. For this test we add 5 mL of ammonia solution to the chloroform extract followed by addition of conc. H_2SO_4.

Steroid test

0.5 gm. of chloroform extract was added with 2 mL of H_2SO_4 followed by 2 mL of acetic anhydride, the color changes from violet to blue or green indicating the presence of steroid.

Terpenoids test

A reddish brown color shows the presence of terpenoids, if 5 mL of the sample was mixed with 2mL of chloroform and 1.0 mL of conc.H_2S0_4.

Cardiac glycosides

A brown ring of interference indicates a deoxysugar when 5 mL of each extract is treated 2 mL of glacial acetic acid, 1 drop of ferric chloride solution and 1.0 mL of conc. Sulphuric acid.

GC/MS Studies of seed oil

Extraction of oil and preparation of fames

Oil extraction and FAMEs of the seed oil were prepared according to the method described by M. Nasim ullah Qureshi *et al*(Muhammad NQ *et al.*, 2011).

Separation of FAMEs by Gas Chromatography

A Shimadzu hyphenated to a mass spectrometer gas chromatograph equipped with an auto- sampler (AOC-20S) and auto-injector (AOC-20i) was used. The carrier gas was helium. The capillary column was used and other GC-MS conditions such that ion source temperature (EI); 245 °C, interface temperature; 245 °C, pressure; 95 KPa, solvent cut time; 1.8 min. 1 µl of sample and standard were injected into the GC column. Injector was operated in a split mode with a split ratio 1:50. Injection temperature was 245 °C. The column temperature program was started at 55 °C for 1 min and changed to 150 °C at the rate of 15 °C min^{-1}. The temperature was raised to 180 °C at the rate of 2.5 °C min^{-1} and held for 5 min. the temperature was increased to 225 °C at the rate of 2.5 °C min^{-1} and kept constant for 5 min. Total elution time was 45 min. MS scanning was performed from *m/z* 85 to 380.GC.

Identification of the compounds was carried out by comparing the mass spectra obtained with those of standard mass spectra from the NIST library (NIST 05).

Results and discussions

Phytochemical screening of dandelion leaves chloroform extract test

Chloroforms extract of dandelion leaves was taken and the phytochemical test was performed. The tannins, Phlobotannins, steroids, terpenoids, and cardiac glycosides tests were - ve and the saponin and flavonoids tests were positive.

Ethanol extract test

Ethanol extract of dandelion leaves was taken and the phytochemical test was performed. The tannins, Phlobotannins, steroids, and terpenoids tests were negative and the, saponin, flavonoids and cardiac glycosides tests were positive.

Table 1. The nutritional value of dandelion per 100g can be found from the chart given below, Nutritional value per 100 gram.

Energy	188 kJ (45 kcal)	Vitamin B_6	0.251 mg (19%)
Carbohydrates	9.2 g	Folate (vit B9)	27 µg (7%)
Sugars	0.71 g	Choline	35.3 mg (7%)
Dietary fiber	3.5 g	Vitamin C	35.0 mg (42%)
Fat	0.7 g	Vitamin D	0.0 µg (0%)
saturated	0.17 g	Vitamin E	3.44 mg (23%)
Protein	2.7 g	Vitamin K	778.4 µg (741%)
Water	85.6 g	Calcium	187 mg (19%)
Vitamin A equiv.	508 µg (64%)	Iron	3.1 mg (24%)
beta-carotene	5854 µg (54%)	Magnesium	36 mg (10%)
lutein and zeaxanthin	13610 µg	Manganese	0.342 mg (16%)
Thiamine (vit. B_1)	0.19 mg (17%)	Phosphorus	66 mg (9%)
Riboflavin (vit. B_2)	0.26 mg (22%)	Potassium	397 mg (8%)
Niacin (vit. B_3)	0.806 mg (5%)	Sodium	76 mg (5%)
Pantothenic acid (B_5)	0.084 mg (2%)	Zinc	0.41 mg (4%)

Percentages are relative to US recommendations for adults.

Source: USDA Nutrient Database.

N-Hexane extract test

N-hexane extract of dandelion leaves was taken and the phytochemical test was performed. Phlobotannins, steroids, and terpenoids tests were negative and the tannin, saponin, flavonoids and cardiac glycosides tests were positive.

Distilled water extract test

Distilled water extract of dandelion leaves was taken and the phytochemical test was performed. Phlobotannins test was negative and the tannin, saponin, flavonoids, steroids, terpenoids and cardiac glycosides tests were positive.

Table 2. Various phytonutrients extracted in different solvents from dandelion**.**

Extracts	Tannin test	Saponins	Phlobotannins	Flavonoids	Steroid test	Terpenoids	Cardiac glycosides
chloroform	-	+	-	+	-	-	-
ethanol	-	+	-	+	-	-	+
n-hexane	+	+	-	+	-	-	+
Distilled water	+	+	-	+	+	+	+
Ethyl acetate	-	-	-	+	-	-	+
ammonia	+	+	-	+	+	+	-
acetone	+	+	+	+	+	+	+

Ethyl acetate extract test

Ethyl acetate extract of dandelion leaves was taken and the phytochemical test was performed. Tannin, saponin, Phlobotannins, steroids, and the terpenoids test were negative and the flavonoids and cardiac glycosides tests were positive.

Ammonia extract test

Ammonia extract of dandelion leaves was taken and the phytochemical test was performed. , Phlobotannins and cardiac glycosides tests were negative and the Tannin, saponin, flavonoids, steroids, and the terpenoids test were positive.

Table 3. Quantitative results of fatty acid methyl esters by GC-MS.

S.No.	Name	Retention Time(min)	Area	Con (%)	m/z
1	C6:0; Methyl ester of Hexanoic acid,	3.055	6903	0.0516	87.00
2	C8:0; Methyl ester of Caprylic acid	4.950	6731	0.05	87.00
3	C10:0; Methyl ester of Capric acid	6.796	8187	0.05	87.00
4	C12:0; Methyl ester of Lauric acid	8.546	21439	0.01	87.00
5	C13:0; Methyl ester of Tridecanoic acid	9.643	2617357	1.37	87.00
6	C14:0; Methyl ester of Myrestic Acid	10.989	47059	0.17	87.00
7	C15:0; Methyl ester of Pentadecanoic acid	12.654	60912	0.29	87.00
8	C16:0 Methyl ester of Palmitic Acid	14.667	1931103	3.89	87.00
9	C16:1c; Methyl ester of Palmitic acid	15.193	18125	0.37	97.00
10	C17:0; Methyl ester of Margaric acid	16.958	95076	0.50	87.00
11	C18:0; Methyl ester of Stearic acid	19.695	234709	0.61	87.00
12	C18:1c; Methyl ester of Oleic acid	20.208	169208	0.92	87.00
13	C18:1n8T; Methyl ester of Octadecanoic ac	20.448	48071	0.42	95.00
14	C18:2c; Methyl ester of Linoleic acid	21.839	911951	7.78	95.00
15	C18:3n3 Methyl ester of Linolenic acid	24.407	853201	19.88	95.00
16	C20:0; Methyl ester of Archidic Acid	29.756	22200	0.65	87.00
17	C22:0; Methyl ester of Behenic acid	34.381	211115	1.25	87.00
18	C23:0 Methyl ester of Tricosanoic acid	37.643	40207	0.26	87.00
19	C24:0; Methyl ester of Eicosadienoic acid	40.734	113359	0.68	87.00

Acetone extract test

Acetone extract of dandelion leaves was taken and the phytochemical test was performed. No test was found to be negative all the test of acetone extract were found to be positive.

Discussion

From the above work, phytochemical screening of dandelion plant was carried out and is concluded that different phytochemicals are present in dandelion plants. Some tests are positive and some are negative using different solvents such as n-hexane, chloroform, distilled water, ethanol, acetone, ethyl acetates. Qualitative phytochemical screening of dandelion plant was carried out.. Qualitative analysis of tannins, phlobotannins saponin steroids, cardiac glycosides, terpenoids and flavonoids was performed as shown in Table 2. The quantitative analyses of fatty acids present in dandelion are shown in Table **3**.The results obtained from the GC- MS analysis shows the relative concentration of individual esterified fatty acids. The results are based on the external standard method and the standard deviation values are chosen among three results in each case. Separation of (FAMEs) was performed by Gas chromatography using three points calibration curve with R2 value less than 0.99 (R2 > 0.99) in each case. Fig. 1 is the GC-MS chromatogram of dandelion seed oil with properly labeled signals of analytes detected. Both the saturated and unsaturated fatty acids were found in the sample under investigations. Total of 19 different fatty acid components were identified and quantified. Linolenic acid was found in highest concentration (C18:2, 19.88%) among the identified analytes of interest. In addition methyl esters of Tridecanoic acid (C13:0, 1.37%), Palmitic acid (C16:0, 3.89%), linoleic acid (C18:2, 7.78%) and Behenic acid (C22:0, 1.25%). Concentrations of rest of the detected fatty acids were less than 1%. Similarly the nutritional value of dandelion per 100g can be seen in Table 1 which has been taken from USDA nutritional database.

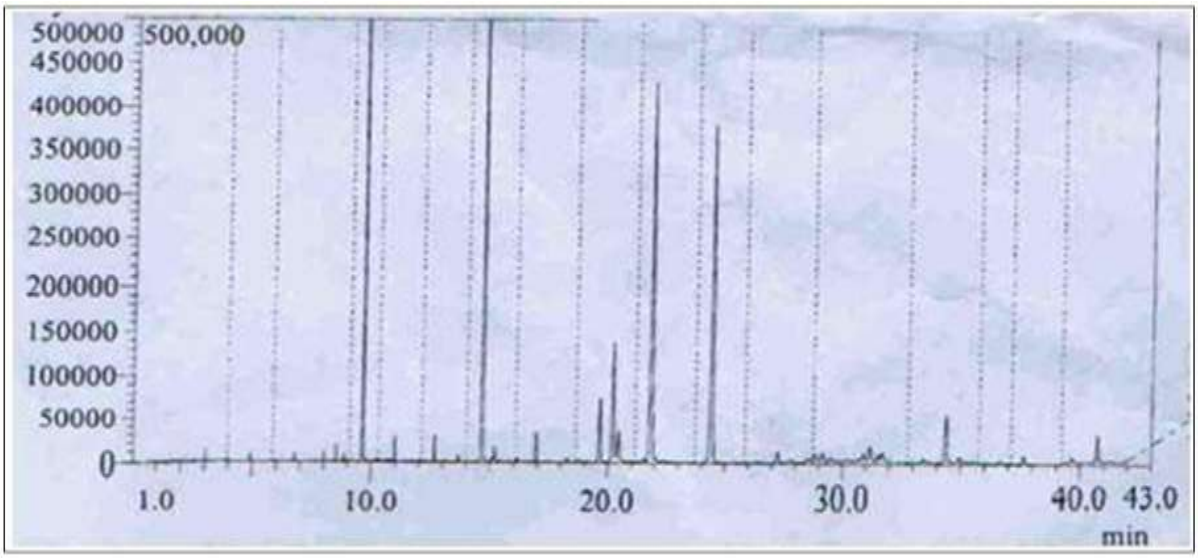

Fig. 1 . GC-MS Chromatogram of fatty acids of dandelion seeds.

Conclusion

It was concluded that the selected plant was a rich source of secondary metabolites e.g alkaloids, flavonoids, terpenoids, phlobotannins, tannin, saponin and cardiac glycosides. Medicinal plants play a major role in preventing various types of diseases. Similarly it was noted that medicinal plants are used for the discovering and screening of phytochemical constituents which are the major precursor for the synthesis of new drugs. Research institutes and pharmaceutical industries have keen interest in medicinal plants for the synthesis of new drugs for the treatment of various diseases. Similarly from the results it is clear that dandelion seeds can also be used in various pharmaceutical products as it contains different bioactive compounds like fatty

acids. The method applied is a reliable method of simultaneously analyzing many fatty acid components in a single run. Thus we are hope full that various phytochemicals and fatty acids extracted and identified by our study from local dandelion plant of Peshawar will be helpful in copping various diseases of this region.

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