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Volatile Oil Composition of *Carthamus Tinctorius L* the Flowers Grown in Kazakhstan.

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ABSTRACT

The object of this study is the flowers of Kazakhstan species of "AkMai" safflower, collected in the flowering stage in Southern Kazakhstan. Volatile oil was carry out to study the component composition of Kazakhstan "AkMai" safflower flowers**.** The composition of the volatile oil obtained from the dried flowers of *Carthamus tinctorius* L. growing in Kazakhstan. Pale yellow oily extracts were obtain by varying the process parameters. The volatile oil obtained by hydrodistillation of the petals *Carthamus tinctorius L.* was analyzed by gas chromatography/mass spectrometry (GC/MS). The yield of the oil was 0.175 % (v/w). 20 compounds representing 99.81% of the oil were characterized. Volatile oil from the flowers of Kazakhstan safflower species "AkMai" were investigated by gas chromatography/mass spectrometry GC/MS which allowed detecting 20 compounds. The volatile oil was found rich in undecanoic acid, octane, 2-nonen -1-ol, hexadecanal, dodecanal, dec-2-en-1-ol, nonanoic acid, tetradecanoic acid, 2 pentadecanone, 6,10,14-trimethyl, 1,2-benzenedicarboxylic acid, isobutyl-beta-phenylpropionate, 1.3-cyclohexadiene, myrtenoic acid, octadecanoic acid, heneicosanoic acid, 2(3H)-furanone, 4,4-dipropylheptane, hexcosane,1-eicosanol, and also heptocosane.

Keywords *Carthamus tinctorius*, Asteraceae, Safflower, phytochemistry, volatile oil.

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INTRODUCTION

It belongs to Asteraceae family in the order of Asterales that contains about 22,750 genera and more than 1,620 species. *Carthamus* species probably originate from Southern Asia and is known to have been cultivated in China, India, Iran and Egypt almost from prehistoric times. During middle ages it was cultivated in Italy, France, and Spain, and was introduced into United States in 1925 from the Mediterranean region. *C. tinctorius* has been known as "Golrang" in Iran. It is grown for the red/orange pigment in the flower petals which is used for coloring rice and bread, and for dyeing cloth. After synthetic aniline dyes took over this market in the 1800's the crop was grown as an oilseed [1]. The seeds contain 30% oil, 20% protein, and 35% crude fibre. The seeds are also a rich source of minerals (Zn, Cu, Mn, and Fe), vitamins (Thiamine and Bcarotene), and the tocopherols (alpha, beta, and gamma) [2]. Safflower leaves, petals, and seeds have tremendous medicinal and therapeutic significance, and petals are also used for extracting dye for coloring cloths and foodstuffs [3]. It contains a high amount of polyunsaturated fatty acid linoleic acid (70%) and monounsaturated oleic acid (10%) with small amounts of stearic acid [4]. The flowers of *C. tinctorius* are an important medicinal material in prescriptions used for cardiovascular, cerebrovascular and gynecological diseases. In China, the water extract of *C. tinctorius* has been developed as an intravenous injection, which is extensively applied to treat cardiovascular diseases clinically [5]. Its dye is mainly used as a coloring agent [6].

Objectives

This aim was designed to study the biological activity and chemical composition of essential oil of *C. tinctorius.* The oil was studied by GC-MS which allowed detecting 23 compounds. Biologically active complex of flower of Kazakhstan safflower species "Ak-Mai" was release for the first time by using this oil.

MATERIAL AND METHODS

Plant material and authentication

Flower of *C. tinctorius* wer collected from the Southern Kazakhstan region during the flowering stage in the summer of 2013. The plant was identified by Konyrbekov M., taxonomist of the station. A voucher specimen was deposited at the herbarium Krasnovodopadskaya Breeding Experimental Station, Ministry of Agriculture, Republic of Kazakhstan. The plant material (seeds and petals) which includes fresh green leaves which were washed, shade and dried at 18 degree Celsius for 15 days.

Isolation of volatile oil

The petals of *C. tinctorius (*350 g) were hydro-distilled for 220 minutes Clevenger apparatus. The yield of volatile oil obtained was 0.175%. The light yellowish colored volatile oil was collected in the graduate tube. The resulting volatile oil dried with anhydrous Na_2SO_4, measured band transferred to glass vials and kept at temperature of 4±2°C for further analysis.

Solvents and chemicals

Gallic acid, Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ciprofloxacin, Amphotericin-B Chloroquine, Pentamidine were brought from the Sigma Aldrich. Analytical grade solvents were used during the experiments brought from the Merck chemicals, Mumbai, India and Sigma Aldrich.

GC-MS analysis

The oil was analysed by GC-MS on a Varian CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. The GC was equipped with a DB-5 fused silica capillary column (30m x 0.25 mm, with film thickness of 0.25 um) operated using the following conditions: injector temperature, 240°C, column temperature, 60-240°C at 3°C per minute then held at 240°C for 5 min; carrier gas, He; injection volume, 1 µL (splitless). The MS mass ranged from 40 to 650 m/z, filament delay of 3 min, target TIC of 20,000, a prescan ionization time of 100 µ sec, an ion trap temperature of 150°C, manifold temperature of 60°C, and a transfer line temperature of 170°C.

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Identification

The individual peaks/constituents were identified by gas chromatography by comparison of their retention indices (R.I.) either with those of authentic compounds available in author's laboratory or with those of literature in close agreement to R.I [7-10]. After identification was make by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of libraries and published literature [11-12]. Retention indices of the components were determined relative to the retention times of a series of n-alkanes relative to C9-C20.

Biological activity

Antidiabetic assay

The *in vitro* PTPIB activity assay was conducted based on a protocol previously described by Taghibiglou et al [13]. PTPIB reaction system contained 5 mM pNNP 0.09 µM his-PTRIB 1-321 and buffer containing 20 mM HEPES, 150 mM NaCL, and 1 mM EDTA (PH 7.0). After incubation of extracts for 10 min, the reaction was initiated by addition of pNNP. The amount of produced pNP was measured by detection absorbance at 405 nm using microplate spectrophotometer (SpectaMax M5/M5e, America). IC50 value was calculated by fitting date with Origin softwar.

The *in vitro* activity experimental system was established founded by the literature methods (Burke TR, Ye Jr B, Yan X, et.al. Biochemistry 1996, 35, 15989-15996) and applied to detect inhibitory ability of the extracts NaVO3 was used as positive control. The IC50 of NaVO3 is 1.8038 ± 0.0248(µg/ml)

RESULTS

The hydro distillation of *Carthamus trinctorius L* flowers gave yellowish oil with a yield of 1.6 % (V/W), on fresh weight basis. The oil was analysed by GC/MS. 20 components were identified in the oil, which represented about 99.81% of the total detected constituents. The general chemical profiles of the tested oil, the percentage content of the individual components, retention indices and retention time are summarized in Table 1.

Table 1: Chemical composition of volatile oil *Carthamus trinctorius L*

№	Compound name	RI[b]	RI[c]	Area %
1	1.3-cyclohexadiene	617	516	1.07
2	myrtenoic acid	617	417	0.71
3	4,4-dipropylheptane	746	628	0.51
4	isobutyl-beta-phenylpropionate	633	505	1.89
5	2(3H)-furanone	747	656	0.79
6	1,2-benzenedicarboxylic acid	754	683	3.01
7	dibutyl phthalate	790	764	0.34
8	octane	837	719	1.17
9	undecanoic acid	838	812	7.79
11	heneicosanoic acid	867	789	4.29
12	tetradecanoic acid	872	820	1.52
13	octadecanoic acid	887	829	1.8
14	nonanoic acid	891	865	17.94
15	dec-2-en-1-ol	918	908	14.30
16	2 pentadecanone, 6,10,14-trimethyl	919	888	6.12
17	hexadecanal	930	917	0.20
18	2-nonen -1-ol	943	935	0.47
19	hexcosane			0.40
20	2,3,5,8-tetramythyl.	954	940	0.47
21	heptocosane	961	931	34.75
22	trifluoroacetic acid	966	946	0.12
23	1-eicosanol	966	955	0.15
25	Total			99.81

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Table 2. Inhibitory effect of extracts on PTRIB

Sample	IC_{50} (µg/ml)
Carthamus trinctorius L	13.73398±0.040706

Also it might seen from figure 1.

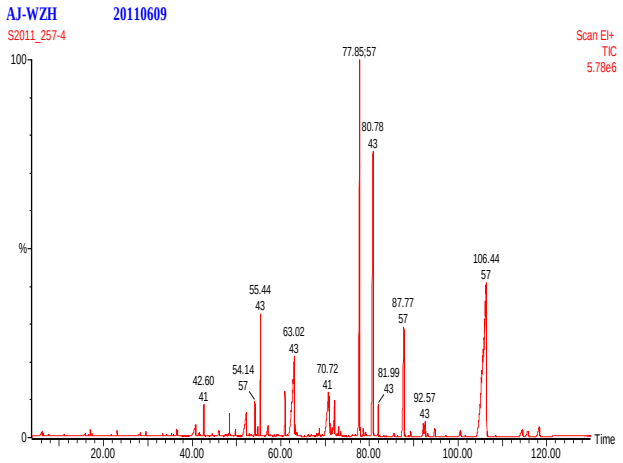

Figure 1: GC/MS spectra of volatile oil *Carthamus trinctorius L*

Pale yellow oily extracts were obtained by varying the process parameters. Volatile oil from the flowers of Kazakhstan safflower species "Ak-Mai" were investigated by GC/MS. The mass spectrum of the oily extract showed the peak at which was found 20 compounds.

From Table 1, it is evident that the major constituents of *Carthamus trinctorius L* flower oil were undecanoic acid, octane, 2-nonen -1-ol, hexadecanal, dodecanal, dec-2-en-1-ol, nonanoic acid, tetradecanoic acid, 2 pentadecanone, 6,10,14-trimethyl, 1,2-benzenedicarboxylic acid, isobutyl-beta-phenylpropionate, 1.3-cyclohexadiene, myrtenoic acid, octadecanoic acid, heneicosanoic acid, 2(3H)-furanone, 4,4-dipropylheptane, hexcosane,1-eicosanol, and also heptocosane.

DISCUSSION

Pale yellow oily extracts were obtained by varying the process parameters. Volatile oil from the flowers of Kazakhstan safflower species "Ak-Mai" were investigated by GC/MS. The mass spectrum of the oily extract showed the peak at which was found 20 compounds.

A number of chemical constituents such as flavonoids, phenylethanoid glycosides, coumarins, fatty acids and steroids have been isolated from different parts of the plant [5]. There are records that it is used for reducing ailments from the neurotropic, cardiotropic, hemopoietic, and diaphoretic systems. Many clinical and laboratory studies support the use of the medicine properties of safflower for menstrual problems, cardiovascular disease, pain, and swelling associated with trauma [14]. Safflower flowers produce red and yellow pigments and mainly used material of dye [15]. Flavonoid glycosides, carthamin, a flavonoid type dye and safflower yellow are the main constituents in the flower of *C. tinctorius* [6]. The flowers also contains carthamidin, isocarthamidin, quercetin, kaempferol, 6-hydroxykaempferol and its glycosides, chalcones including hydroxysafflor yellow A, safflor yellow A, safflamin C and safflamin A, and safflomin-A [16-17].

Application in medicine to develop a prospectively drugs mean the efficiency and prolongation of the therapeutic effect, a minor amount of adverse effects, the possibility of their wide application in the pediatric, geriatric practice and regenerative medicine applications.

Safflower is useful for treatment of diabetes and its complications. The flower can reverse the metabolic disorders occurring in alloxan induced diabetes. Considering these effects on these lipid components, it can be assumed as a potential hypolipidemic agent, which will be a great advantage both in diabetic condition as well as the associated atherosclerosis or hyperlipidemic conditions [18].

CONCLUSIONS

From the result of the study, it can be conclude that the safflower collected from the Southern region of Kazakhstan is one of the best genotype available. In conclusion, 20 chemicals in *Carthamus tinctorius L*. essential oils can be identified by GC-MS analysis. The essential oils from safflower have PTP1B antidiabetic activity. Therefore, it is suggested that further work be performed on the isolation and identification components of the extract of *Carthamus tinctorius* L. The results show that the essential oil has good antidiabetic activity.

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