CHARACTERIZATION AND EXTRACTION OF MARIGOLD (CALENDULA OFFICINALIS L.) CULTIVATED IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Chemical composition of marigold (*Calendula officinalis* L.) cultivated in Republic of Macedonia, as well as the fatty acid content in the extracts of marigold whole flower, petals and repectables, were determined.

The dry petals are characterised with the lowest content of total proteins, ash and sand. In dry receptacles the content of ash and proteins is the highest. Total sugars and sand contents in marigold dry whole flowers are higher then in dry petals and repectables.

The highest extract yield is obtained by dichloromethane extraction of dry whole flowers. The extract yield obtained from dry petals with *n*-hexane extraction is higher then those obtained from dry whole flowers and dry receptacles, under the same conditions.

The content of myristic (C14:0), palmitic (C16:0), stearic (C18:0), *cis*-oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) was determined in the *Calendula* extracts. It is determined that in all extracts linoleic acid has the highest content. *Calendula* extracts are rich in oleic and linoleic acids. The content of palmitic acid is the highest compared with other saturated acids founded in the extracts.

1. INTRODUCTION

Calendula officinalis L. (Asteraceae), a bright yellow and orange flower, commonly known as marigold or cultivated marigold, is an annual herbaceous plant, native to Mediterranean countries. The flower is used as food additive to confer both foods color and flavor. Marigold is also widely used in traditional and homeopathic medicine since its petals are used for preparing infusions and ointments. Phytopharmacological studies of different *Calendula* extracts have shown antiinflamatory, anti-viral and anti-genotoxic properties of therapeutic interest (1, 2, 3). Several studies involving marigold extracts have been performed, mainly related to the characterization of extracts obtained by extraction with organic solvents. The results indicate that such extracts have therapeutic characteristics partially due to the terpene content, however, the most important compounds are triterpenoids, flavonoids, essential oils and sesquiterpenes (4).

Characterization of dry petals, receptacles and whole flowers of *Calendula officinalis* L. cultivated in Republic of Macedonia by detemination of the content of moisture, crude proteins, ash and sand in, was the aim of this study. The influence of the organic solvents on the extracts quantity was followed by extraction of different part of marigold using Soxhlet method and *n*-hexane, dichloromethane,

benzene and diethyl ether as solvents. Determination of the fatty acid composition of the *Calendula* extracts was also objective of this work.

2. EXPERIMENTAL

Material. The orange, double-flowered cultivar (cv. "Double Orange") of *Calendula officinalis* L. (Figure 1) was grown at experimental field at Scientific Tobacco Institute in Prilep, Republic of Macedonia, under recommended agricultural practice (fertilization, irrigation, plant protection) in 2008. Seed purchased from "Dr. Josif Pancic" Institute for Medical Plan Research in Republic of Serbia, was breed in greenhouse, and seedlings were field planted in plots with randomized design in six repetitions. Rows were spaced 40 cm apart, and plants were spaced 50 cm within each row. Flowers were hand harvested at full flowering stage. Petals and receptacles were separated from fully-opened flowers. The flowers, petals and receptacles were dried on mats in the shade and at room temperature, spread into thin layers that were not mixed over the 20 days. After this interval, water loss by both drying and desiccation, according to technique by drying on 105°C to constant mass achievement, was determined (AOAC, 925.10). Plant material was dried in oven (35 °C, 5 h), then milled and sieved to a powder (0.250 mm) with grinder (Retch, Germany) immediately before the extraction.

Figure 1. Calendula officinalis L. (cv. Double Orange)

Chemical analysis. *Content of dry matter* was determined by drying on 105°C to constant mass achievement (AOAC, 925.10) and *content of ash* by burning at 900°C to constant mass achievement (AOAC, 923.03). *Proteins content* was determined from the nitrogen content by Kjedahl method using factor 6.25 (AOAC, 978.04) and calculated as N x 6.25 (5). *Total sugars* were determined by Bertrand method (6).

Extraction of the plant material. Soxhlet procedure was used for extraction of whole flowers, petals or receptacles (AOAC, 920.85). 5 g of powdered plant material (0.0001 g accurately weight, 0.25 mm particles size) was extracted in the presence of 4-5 boiling glass regulators by using pro analysis-grade solvents: *n*-hexane, diethyl ether, methylene chloride

and benzene. After 5 h extraction, the solvent was released in rotary vacuum evaporator (35°C) and solvent traces were removed by drying in vacuum drier (40°C, 105mbar) followed by cooling in a dessicator and weighting. The steps of drying, cooling and weighting were repeated until the difference between two consecutive weights was smaller than 2 mg. The yield of extract was estimated according to the dry matter weight in plant material used for extraction.

Preparation of fatty acid methyl esters (FAMEs). 100 mg of extract was transesterified with freshly prepared 0.28 mol L^{-1} solution of sodium methoxide in methanol. Reaction mixture was stirred with magnetic stirrer and heated using water bath at 75 °C, for 20 min. After the transesterification was done, saturated sodium chloride solution was added and ester layer was extracted with diethyl ether and distilled water. Prepared sample was dried with anhydrous Na_2SO_4 and then filtered. In order to remove the solvent, sample was evaporated on the rotary vacuum evaporator (35 °C). Sample was cleaned-up on the silica gel column. Clean-up column was prepared in Pasteur pipette by placing the plug of glass wool, then adding silica gel activated at 120 °C and, at the top, anhydrous sodium sulfate. It was conditioned with cyclohexane and then the sample was transferred on the top of the column. FAMEs were eluted from the column with the solution of cyclohexane/ethyl acetate mixture (2:1, v/v). Toluene was added to the sample and then solvent was evaporated on the rotary vacuum evaporator (50 °C, 150 mbar) to the volume of app. 1 mL. Sample was transferred to the 2 mL vial, evaporated in the stream of nitrogen to the dry residue and additionally dried in heating cabinet at 40 °C for 30 min.
For the GC/MS analysis, sample was diluted with *n*-hexane to obtain the concentration of sample solution of 1 mg mL^{-1}.

GC-MS analysis. The fatty acid content in extracts were determined by GC-MS, using Thermo Finnigan Trace GC unit furnished with Optima 240 capillary column: 60 m x 0.25 mm i.d. x 0.25 μm film thickness, whose working temperature was programmed as follows: 80°C at the start, 20°C/min to 120°C, 3°C/min to 240°C that held for 10 min. Helium constant flow was 1.5 mL/min. 1 μL of the sample dissolved in hexane was injected by Thermo Finnigan AS 2000 autosampler. PTV injector was used with the split ratio 10:1, at initial temperature of 60°C and heated up to 280°C. The Finnigan Trace mass selective (MS) detector, coupled to GC *via* transfer line set on 250°C, worked with ion source temperature at 220°C. The response factors were obtained using standard FAME mixture solution as external standard.

3. RESULTS

Moisture content in air-dried whole flowers, petals and receptacles were 6.80%, 6.20%, and 7.0%, respectively.
Chemical composition of whole flowers, petals and receptacles is presented in Table 1. The dry matter content was higher in petals than in receptacles. The highest content of proteins as the main nutritional relevant was determined in marigold receptacles.
In the whole flowers determined content of sugars was the highest compared with the sugars content in receptacles and petals.

Table 1. Chemical properties of Calendula officinalis L.

Characteristic	Whole flowers	Petals	Receptacles
Dry matter (%)	85.15	90.44	88.41
Total nitrogen (%*)	3.59	1.44	3.96
Proteins (%*)	22.44	9.00	24.75
Ash (%*)	9.98	8.29	11.87
Total sugars (%*)	12.44	7.02	5.36
Sand (%*)	7.05	2.16	3.79

* calculated to the corresponding dry matter

In Table 2 are presented the quantity of *Calendula officinalis* L. extracts obtained using different extraction solvents. Extract yield obtained from petals was higher than from whole flowers and receptacles, for all solvents used. The highest extract yields of 13.79%, 16.81% and 9.57% for whole flowers, petals and receptacles, respectively, were obtained using benzene. *n*-Hexane gave the lowest quantity of extracts from all plant materials, comprared with other used solvents.

Table 2. Extract yield (%*) of Calendula officinalis L.

Solvent	Whole flowers	Petals	Receptacles
n-Hexane	8.95	13.76	5.72
Methylene chloride	10.67	14.44	5.11
Benzene	13.79	16.81	9.57
Diethyl ether	10.05	14.63	7.62

* calculated to the corresponding dry matter

In the extracts of *Calendula officinalis* L. according to the determined contents, palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) fatty acids are dominant (Table 3). The content of oleic acid in extracts obtained from whole flowers, petals and receptacles, was higher than of other fatty acids content, doesn't matter which solvent was used for extraction. The highest content of oleic acid was determined in the extract obtained from whole flowers by appying methylene chloride as extraction solvent. The highest ratio of oleic to linoleic fatty acid, in the range of 0.39 - 0.93, is determined for the extracts of whole flowers, petals and receptacles obtained with *n*-hexane. Also, in this extracts the content of saturated fatty acids (SFA) was higher than in extracts obtained by methylene chlorid, diethyl ether and benzene. The ratio of mono- unsaturated fatty acids (MUFA) to poly-unsaturated fatty acids (PUFA) was the lowest for the extracts obtained with *n*-hexane.

Table 3. Fatty acid composition (% of total fatty acid content) in extract of Calendula officinalis L.

Fatty acid*	Fatty acid composition					
	n-Hexane			Methylene chloride	Diethyl ether	Benzene
	whole flowers	petals	receptacles	whole flowers	whole flowers	whole flowers
14:0	3.51	6.44	0.63	0.68	1.94	3.17
16:0	11.29	10.89	12.22	3.10	7.09	8.79
18:0	6.89	7.28	7.12	7.78	7.25	6.84
18:1 cis	38.31	42.23	35.65	53.49	46.67	42.85
18:2	23.69	16.60	33.04	14.32	18.92	16.97
18:3	11.48	11.69	6.53	15.88	13.33	16.56
C18:2/C18:1	0.62	0.39	0.93	0.27	0.41	0.40
SFA	21.69	24.61	19.97	11.56	16.28	18.80
UFA	73.48	70.52	75.22	83.39	78.92	76.38
PUFA	35.17	28.29	39.57	29.90	32.25	33.53
MUFA/PUFA	1.09	1.50	0.90	1.79	1.45	1.28

* Shorthand designations: 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid;
18:1 cis, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid;
SFA, saturated fatty acids; UFA, unsaturated fatty acids;
MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids

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