THE RELATIONSHIP BETWEEN SOIL QUALITY, SECONDARY METABOLITE CONTENT AND ANTIOXIDANT EFFICACY IN *CARTHAMUS TINCTORIUS* L. AND *CALENDULA OFFICINALIS* L.

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CARTHAMUS TINCTORIUS L. AND CALENDULA OFFICINALIS L.: NATURAL SOURCE OF ANTIAGING AGENTS FOR COSMETIC FORMULATIONS (Abstract): Asteraceae plants are commonly used for their antibacterial, anti-inflammatory and anti-aging effects. They are widely utilized in therapeutic and cosmetic formulations, while also serving decorative purposes. Safflower also serve a range of purposes, from medicine, food coloring and ornaments. *Calendula officinalis* is widely cultivated for medicinal and cosmetic purposes, especially for its therapeutic effects in topical formulations. Thus, the **aim** of our study was to investigate the influence of soil fertility on the secondary metabolites profile and its antioxidant activity. **Materials and methods:** To achieve this, *Calendula officinalis* and *Carthamus tinctorius* were cultivated in Romania under two different conditions: local soil, and enriched soil respectively. After collection, fragments of dried leaves and inflorescences were extracted with either ethanol or acetonitrile and analyzed. Semiquantitative analysis was performed using the HPLC method and he total phenolic content of each sample was quantified with Folin-Ciocalteu reagents. For the *in vitro* tests, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was used to evaluate the radical scavenger activity, and reaction with ferrozine was used to evaluate the ferrous iron chelation capacity. **Results**: The quantitative analyses showed a clear influence of soil fertility on polyphenolic profile, impacting the antioxidant activity. **Conclusions:** The timing of harvest and the soil quality should be taken into consideration in order to ensure optimal pharmacological effects. **Keywords**: *CARTHAMUS TINCTORIUS, CALENDULA OFFICINALIS,* PHENOLS, ALLERGENIC RISK, ALANTO-LACTONE.

INTRODUCTION

Secondary metabolites (SMs) identified in Asteraceae plants possess proven pharmacological effects, which are attributed to their phytochemical compounds, such as polyphenols, phenolic acids, flavonoids, acetylenes and triterpenes (1). The specific SMs profile is related to local environmental conditions and agro-techniques of cultivated plants (2). The Asteraceae are also a source of sesquiterpene lactones (SLs). Of those, alantolactone has been documented as one of the principal allergenic and sensitizing compounds in such species (3). Alantolactone (helenin, eupatal, inula camphor), an eudesmanolide derived from *Inu-*

la helenium, also has antibacterial, anti-helmintic, anti-inflammatory properties and has been studied as a novel antineoplastic agent (4-6). The plant-related allergy symptoms involve contact dermatitis, hay fever, asthma or even anaphylaxis. The typical routes of exposure include skin contact, eye contact, ingestion or inhalation (4). The evidence of cross-reactivity of Asteraceae species with other plants and anaphylactic reactions has been reported (4). Cross-reactivity can occur between plant allergens and food and pollen allergens, especially in patients sensitive to Asteraceae (4).

Species of this plant family are now widely utilized in cosmetic formulations (creams, soaps, shampoos), aromatherapy or massage, in professional care or folk medicine. This high usage leads to speculation that sensitization to Compositae will increase. Compositae containing cosmetics are thought to provoke allergic reactions in susceptible individuals because they contain SLs (4).

Carthamus tinctorius (Safflower) has been grown for various purposes: medicine, food coloring, ornamental, fabric dye, biofuel. It is adaptable to arid conditions worldwide (7). It has many beneficial effects, such as: anti-inflammatory, antioxidant, antitumor effects. Injectable formulas have been widely applied in clinics. Although this species is considered non-toxic and has negligible side effects, anaphylactic reactions to safflower injections have been reported. This annual plant is not very used in modern cosmetic products (8). Studies of extracts from seeds, stems, buds and flowers suggest the potential cosmetic of safflower has not yet been completely uncovered (9).

Calendula officinalis, usually known as common marigold, is widely cultivated and used for medicinal and decorative purposes (8, 10). Much scientific research has established that marigold has a wide spectrum of pharmacological effects (11, 12). Phenolic acids and flavonoids have been identified mainly from inflorescences (8, 13). Compounds of a sesquiterpene nature were also detected or isolated (13). Calendula cream and products have shown very few allergic and side effects (14). The European Commission accepted calendula as a safe cosmetic ingredient (15). Although sensitization to calendula and allergic contact reactions are observed, anaphylaxis incidents were only reported in the context of infusion of marigold after gargling (14). Special care must be taken by people who are allergic to species from the Asteraceae family when using or consuming preparations containing marigold extract or oil (14). Only flowers are commonly used, while the rest of the biomass is considered waste. Unfloral parts of the plant should also be taken into consideration (9).

This study aims to evaluate *Carthamus tinctorius* and *Calendula officinalis* as sources of antiaging agents for cosmetics related to their allergenic potential in cosmetic formulations. The allergenic risk factor considered consisted in assessing the presence and quantity of alantolactone in the plant. Total phenolic content was evaluated as a marker of antioxidant capacity. The two species were cultivated in local and enriched soil conditions, to evaluate their biosynthesis capacity for SLs and antiaging secondary metabolites. Aerial parts of plants were investigated, floral and unfloral, to study the potential of "waste" vegetal material as a source of natural products.

MATERIALS AND METHODS

Plant material. *Calendula officinalis*

and *Carthamus tinctorius* were cultivated in north-eastern Romania in the year 2015 under two nutritional statuses: local (V1) and enriched soil conditions (V2) with N-P-K (Mg) 15: 10: 12. The fertilizer was administered just before planting (75g/m^2). Rooted cuttings were planted at a density rate of 4 plants/m^2. Aerial parts of the plants were collected: fully bloomed inflorescences (F), leaves and stems, defined as unfloral aerial parts (L). The samples were codified as follows: V1F, V2F, V1L, V2L. Fresh aerial parts of plants were dried in a thermostatic oven at 35°C until three consecutive measurements turned out identical. Voucher specimens are deposited as DE-8425906 (marigold) and NL-62850040 (safflower) at the Department of Pharmacognosy ("Grigore T. Popa" University of Medicine and Pharmacy Iasi) for ready reference.

Extraction. Fragments (2 g) of dried leaves and inflorescences were extracted with methanol or acetonitrile R in the Laboratory of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. For polyphenol quantification, the methanolic extract was obtained (1:10, m/m) by repeated sonication for 15 minutes.

For alantolactone evaluation by HPLC, the test solutions were obtained in acetonitrile R (1.0 g powdered drug in 3 mL solvent, three times, sonicated for 15 minutes). The filtrates were reunited and brought to 10 mL in a volumetric flask. A quantum of 0.20 µL was used for HPLC analysis.

Total phenolic content determination. The total phenolic content of each sample was quantified with Folin-Ciocalteu reagent according to the Singleton and Rossi method (1965), using gallic acid as a standard (16). The measurements were performed on ABLE-JASCO V 550 UV-VIS spectrophotometer. The absorbance was measured at 765 nm versus blank samples. The blank consisted of samples and all reagents and solvents except sodium carbonate. The results were expressed as mg gallic acid equivalents (GAE)/100 g dried sample.

HPLC analysis. Semiquantitative analysis of alantolactone was performed using the HPLC method described by Ivănescu (2015) with modifications (18). The method was adapted for a Thermo-Fischer UltiMate 3000 system coupled with DAD detector used to assess the profile of sesquiterpene lactones. The working conditions were: Accucore XL-C18 column (4.6 x 150 mm, 4 Wm); column temperature: 34 °C; detection wavelength was set at 225 nm and the flow rate was 1.2 mL/min. The mobile phase consisting of two eluents as A (water) and B (methanol) used the following linear gradient elution: 0-3 min 38% B; 3-20 min 45% B; 20-30 min 45% B; 30-55 min 55% B; 55-57 min100% B; 70 min 100% B; 90 min 38% B. As standard, alantolactone was used in the amount of 20 µL of a 5 mg/mL solution. Five consecutive injections were done for repeatability purposes. Samples UV spectra registered at 225 nm were automatically compared by Chromeleon 7.2 software and the concentration was expressed as % of the standard's area/concentration.

Antioxidant activity. For the *in vitro* tests, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was used to evaluate the radical scavenger activity. The test was performed using the method of Hatanao *et al.* For each sample EC50 value (expressed in µg extract/mL) was calculated by plotting the radical scavenger activity variation and the concentration of the extract. Gallic acid

was posed as positive control (20). The scavenger activity was determined according to the formula:

$$\% \text{ activity} = (A_{DPPH}-A_P) \cdot 100/A_{DPPH}$$

where ADPPH represents the absorbance of alcoholic DPPH solution, AP represents the absorbance after 10 minutes of DPPH solution treated with methanol extract.

The ferrous iron chelation capacity was also evaluated. When reacting with ferrozine, Fe^{2+} forms a pink-colored complex, exhibiting maximum absorbance at 562 nm. The presence of a chelating agent in the reaction medium results in reduced absorbance of the complex, causing a decrease in the solution's color intensity (21).

All reagents were of analytical purity and were purchased from Sigma Aldrich (St. Louis, MO, USA). All measurements and analyses were performed in triplicates. The variants were compared with their average. Data for quantitative and semi-quantitative analytical determinations were presented as mean values ± standard deviations.

RESULTS AND DISCUSSION

Total phenolic content. Flavonoids and polyphenolic derivatives are proven natural antioxidants. Phenolic content reflects the potential of investigated Asteraceae to have important value as a source of anti-ageing compounds. Analysis of samples from different nutritional plant status pointed out the capacity of plant biosynthesis for SMs depending on local and enriched soil conditions.

The literature data confirm the polyphenolic contents in marigold flowers (11), especially in safflower oils, worldwide (10, 11, 17). As it can be seen from Table I, the amount of phenols differs between samples.

Carthamus has approximately double phenolic content in leaves and stems of unfertilized plants in comparison with flowers. Enriched soil conditions will increase total phenols in flowers, but decrease their total phenols in the aerial parts level. Total phenols in *Carthamus* are similar with quantities found in *Echinacea purpurea* cultivated in similar biotic conditions (18), but lower than the amounts of phenols found in *Tagetes erecta* and *Rudbeckia hirta* (19). The results point out the important role of biomass (leaves, stems) as a source of phenolic compounds from unfertilized cultivated safflower in circular economy.

TABLE I.

Total polyphenols determined in *Carthamus tinctorius* and *Calendula officinalis* (mg GAE/100 g dried sample)

Sample	V1F	V2F	V1L	V2L
Carthamus	145.79± 0.25	228.91± 0.52	276.07± 0.43	204.39± 0.50
Calendula	160.62± 0.35	189.41± 0.22	198.77 ± 0.40	124.36± 0.38

Similar to that of *Carthamus*, the polyphenolic content of marigold leaves and stems is greater in comparison with content determined in flowers in unfertilized plants. This emphasizes the economic importance of "vegetal waste" obtained from crops cultivated especially for flowers. The fertilization increases phenolic content in flowers but not in the aerial parts.

Alantolactone content. Proven aller-

gen, alantolactone presence is confirmed in marigold and safflower in our study (tab. II). The quantity of alantolactone detected in samples is greater in unfertilized plants, both in flower tops and in the aerial parts. On a general note, flowers have smaller quantities of alantolactone in comparison with leaves and stems in safflower and marigold. That could explain the low allergenic potential of flower tops. The risk of allergen reactions still exists in patients with known allergies.

TABLE II.

Semiquantitative determination of alantolactone in *Carthamus tinctorius* and *Calendula officinalis* (µg %)

Sample	V1F	V2F	V1L	V2L
Carthamus	0.1740	0.1561	0.6568	0.5333
Calendula	0.2414	0.0213	6.0045	0.0438

Marigold aerial parts of unfertilized plants have large amounts of alantolactone (V1L). This data suggests that the leaves and stems could be a potent source of alantolactone. The quantities found in our study are superior to data published on other ornamental Asteraceae cultivated in eastern Romania (19). On the other hand, these data could explain the low allergenic profile of marigold flower, but also point out a high allergenic risk for people who handle the crops or vegetal material.

Antioxidant capacity of the investigated samples. The obtained results for the antioxidant assays are given in third table and indicate that the nutritional status of the soil influences the biosynthetic capacity of the plant. Moreover, the accumulation pattern of secondary metabolites with antiradical potential is similar for both species, and increases in the flower tops of the fertilized group.

TABLE III.

The antioxidant potential of *Carthamus tinctorius* and *Calendula officinalis* samples against DPPH and iron radicals

Sample	V1F	V2F	V1L	V2L
DPPH inhibition (IC50, µg/mL)				
Carthamus	105.2±1.84	37.4±0.56	43.1±0.96	89.5±1.13
Calendula	147.3±0.96	100.2±1.04	128.2±0.67	213.7±0.07
Iron chelation (IC50, µg/mL)				
Carthamus	460.1±0.87	310.3±0.04	242.6±1.04	712.3±0.24
Calendula	228.2±1.02	184.2±0.16	237.1±0.82	378.6±0.09

According to our results, the investigated samples have a good effect against free radicals, but much lower if compared to gallic acid that had a value of IC50 at 1.15 µg/mL for DPPH assay, and 6.65 µg/mL for iron chelation assay. Also, the variation of the activity is directly correlated to the polyphenolic content. Interestingly, for *Carthamus* flowers the iron chelation inhibition is much lower than the capacity to

quench DPPH radicals.

Considering that the oxidative stress is a multifactorial complex process cell membrane are exposed to, the use of cosmetic applications that include natural components with antioxidant potential should prevent the degradation induced by such factors (2, 7, 22, 23). Polyphenols are compounds recognized for their antioxidative and protective activities, therefore they have been used for various preparations (22). Both marigold and safflower represent important raw materials for pharmaceutical and cosmetics industries and the quality of the plant products have a direct impact on the benefits given by the final products (2, 22). This is why the chemical composition and the antioxidant capacity of the plant material is extremely important for further development of different preparations.

Another important aspect is shown by the low quantity of alantolactone in the flower tops of the both investigated species, which indicates that harvesting should be done at the complete maturity when terpene lactones decrease and total phenols accumulate in higher amounts. This will ensure a low allergenic profile and a good protective potential of the formulations obtained from the flower material.

CONCLUSIONS

The studied Asteraceae cultivated in NE Romania are a potent source of polyphenols and could be a natural source of anti-aging agents in cosmetics. The allergenic risk of calendula and carthamus flowers extracts is low as confirmed by HPLC analysis. The allergic potential is greater in the unflower aerial parts of the plants, especially in the marigold. The use of flowers as raw materials for cosmetic formulation is generally safe. The biomass of nonflower aerial parts has very important economic value as a natural source of polyphenols (*Carthamus*) and alantolactone (*Calendula*). Our data confirm that vegetal "waste" is an important source for secondary metabolites. Other studies are needed to extend these findings.

CONFLICTS OF INTEREST AND FUNDING

All the authors declare no funding received and no conflict of interest.

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