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The Effects of Age on the Yield and Composition of the Essential Oils of *Calendula officinalis*

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Abstract: The variations in the yield and composition of the essential oils from *Calendula officinalis* L. (Asteraceae) cultivated in Alice, Eastern Cape of South Africa are reported. Essential oils of this plant were obtained by hydrodistillation using the Clevenger apparatus and analysis was performed by GC-MS. The yield in essential oil showed a maximum at the full flowering stage (0.97%) and a minimum during the pre-flowering stage (0.13%). The compositions also showed different patterns at different phases of the vegetative cycle. Sesquiterpenes (α-cadinene, α-cadinol, T-muurolol and epi-bicyclosesquiphellandrene) and monoterpenes (limonene, 1, 8-cineole and trans-β-ocimene) showed the highest correlations with the age of the plant. Aiming the use of essential oil as a food ingredient, the most interesting stage is the post-flowering period, the essential oil at this time being rich in α-cadinene, α-cadinol, t-muurolol, limonene, 1,8-cineole, with P-cymene present at lower levels. α-cadinene is an important flavouring agent in baked foods, candy and chewing gum and also used as a fragrance in cosmetics and detergents. T-muurolol and α-cadinol are important antimicrobial agents.

Key words: Essential oil, *Calendula officinalis*, oil variation, 1,8-Cineole, effect of age, α-cadinene

INTRODUCTION

Calendula officinalis L. (Asteraceae), also known as marigold, is a biennial ornamental herb (Danielski *et al*., 2007). It grows wild in the southern, eastern and central Europe (Wyk and Wink, 2004) and in the Eastern Cape Province of South Africa. It is usually multi-stemmed with a strong tap root. The vegetative parts of the plant are mid green while the stems are angular and covered in fine hairs. The composite flowers could be yellow or orange (Gilman and Howe, 1999), which blossom in the spring-summer seasons. The herb is cultivated in the fall of winter and spring. Its seeds are regarded as an important source of fatty acids with tremendous potential for use as industrial oil (Beerentrup and Robbelen, 1996). The plant has immense usefulness in the colouring and flavouring of food (Marczal, 1987). Some of the other non-food applications of the plant include its use for ornamental purposes, in paints, coatings, cosmetics and industrial nylon products (Muuse *et al*., 1992). The plant and its essential oils are highly medicinal (Janke, 2004; Wali *et al*., 2007), with several therapeutic activities, such as anti-inflammatory, anti-tumorogenic (Jimenez-Medina *et al*., 2006) and cicatrizing (Hamburger *et al*., 2003). *In vitro* studies of its oils also showed very strong antimicrobial activity. Its antiviral and immunostimulating effects have also been reported, with potential therapeutic activity against the human immunodeficiency virus (Kalvatchev *et al*., 1997). The essential oils of *C. officinalis* have been reported for their genotoxic effect (Bakkali *et al*., 2005), while extracts of the plant are known to exhibit antioxidant (Cetkovic *et al*., 2004) and wound healing properties (Lavagna *et al*., 2001).

It is a well known phenomenon in several plant species that the yield and composition of the volatile oils vary both quantitatively and qualitatively at different phases of the vegetative cycle (Dunford *et al*., 2007). This has been demonstrated for *Dracocephalum moldavica*, *Thymus capitatus*, *Artemisia judaica* and *Thymus vulgaris* (Holm *et al*., 1988; Arras and Grella, 1992; Putievsky *et al*., 1992; McGimpsey *et al*., 1994). In these reports, higher yields were observed in the flowering or post-flowering period. In *Thymus capitatus*, carvacrol (the main compound) was present at higher levels before flowering and until the post-flowering period. Some other compounds, such as p-cymene and γ-terpenene also showed seasonal variations. P-cymene showed a minimum level before flowering and a maximum after the flowering period whereas γ-terpenene showed the opposite variation. It was also reported that the content of hydrocarbons in this plant decreased with increase in the size of leaves, while the content of oxygenated hydrocarbons showed the opposite variation.

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Before the commencement of this study, there was no information in the literature where variation in the chemical composition of the essential oil of *Calendula officinalis* was reported. Yet, since the essential oil of this plant is used for flavouring, its acceptability is important. Panel test results have shown that essential oil rich in geraniol and geranyl acetate is well accepted, but not accepted or badly scored when p-cymene and γ-terpenene are present at high levels (Moldao-Martins, 1999). This study reports, the variations in the yield and composition of the essential oils of *Calendula officinalis* during the vegetative cycle of the plant in order to determine the optimum time for its harvesting for the production of good quality oils.

MATERIALS AND METHODS

Seed collection, soil preparation and cultivation of the plant: The seeds of *Calendula officinalis* were collected from wild populations within the University of Fort Hare campus. They were planted in the nursery in the greenhouse of the Botany Department in August 2006. Individual plants were grown in polythene bags. The soil was collected from the University Research Farm, dried for about 48 h, sieved through a 2 mm wire mesh (Anderson and Ingram, 1993) and homogenized before filling the polythene bags. All plants were adequately watered as required. Harvesting was not done during the first two weeks following transplanting; this was to allow the seedlings to overcome the shock of transplanting and establish themselves in the new soils. Thereafter, the leaves were harvested at weekly intervals until full flowering stage. After each harvesting, the fresh leaves were weighed and hydrodistilled for 3 h in an all-glass Clevenger apparatus in accordance with the Anonymous (1980).

Soil analysis: The sieved soil samples were digested at 360°C for 2 h using the selenium powder, lithium sulphate, hydrogen peroxides and sulphuric acid digestion mixture (Anderson and Ingram, 1993). Total phosphorus was determined from the digest using the calorimetric method without pH adjustment as described by Okalebo *et al.* (2002). Total K, Mg, Na, Ca, Fe, Cu and Mn were determined in the digest using the atomic absorption spectrometry. The soil particle size analysis was carried out using the hydrometer method while pH and electric conductivity was determined using the 1:2:5 methods described by Okalebo *et al.* (2002).

GC-MS analyses and identification of components: The GC-MS analyses were performed on Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatograph. The following column and temperature conditions were used: Initial temperature 70°C, maximum temperature 325°C, equilibration time 3 min, ramp 4°C min^{-1}, final temperature 240°C; inlet: split less, initial temperature 220°C, pressure 8.27 psi, purge flow 30 mL min^{-1}, purge time 0.20 min, gas type helium; column: Capillary, cm sec^{-1}; MS: EI method at 70 eV. The components of the oils were identified by matching their mass spectra and retention indices with those of Wiley 275 library (Wiley, New York) in the computer library and literature (Shibamoto, 1987). The yield of each component was calculated per gram of the plant material, while the percentage composition was calculated from the summation of the peak areas of the total oil composition.

RESULTS AND DISCUSSION

Soil components: The total N, P, K of the soil used for the cultivation of the plant were 0.2, 1.0 and 1.6 g kg^{-1} of soil respectively; its exchangeable cations, Ca, Mg, Na, were 2.05, 0.19 and 1.16 g kg^{-1}, respectively while Fe, Mn, Cu and Zn were 1.16, 18.26, 41.09 and 1.50 g kg^{-1}, respectively. The pH of the soil was 6.20 and the Electrical Conductivity (EC) was 115.5 μS cm^{-1}.

Oil yield: The total yields of the essential oils at the different stages of the vegetative cycle increased with the age of the plant and ranged between 0.13% (3rd week) and 0.97% (12th week) (Table 1). Increase in essential oil yields has been observed to be a mechanism that favours the pollination of the plant. According to Harborne (1991), several terpenoids have been previously reported as pollination vectors in this plant.

Oil components: The GC-MS analysis of the extracted essential oils during the growing phase of *C. officinalis* indicated the presence of 43 compounds (Table 1). These included 20 monoterpenes and 23 sesquiterpenes. No diterpene was observed in all the samples. Of all the constituents observed in the oil, the sesquiterpenes (α-cadinene, α-cadinol, t-muurolol and epi-bicyclosesquiphellandrene) and the monoterpenes (limonene, 1, 8-cineole and trans-β-ocimene) showed the highest correlations with the age of the plant (Table 2). α-cadinene is an important flavouring agent in baked foods, candy and chewing gum and also a fragrance in cosmetics and detergents. T-muurolol and α-cadinol are important antimicrobial agents (Chang *et al.*, 2003). The concentration of both compounds increased with the age of the plant, this trend was similar to that reported elsewhere (Anonymous, 2000).

Table 1: Major essential oil constituents of *Calendula officinalis* at different stages of growth

		Composition (%)										
		Week										
Compounds	KI	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Average
α-Thujene	908	-	t	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.10	0.10
α-Pinene	928	0.1	1.2	1.4	1.6	2.2	2.3	2.5	2.7	2.7	2.90	2.00
Sabinene	960	0.2	0.4	0.5	0.7	1.4	1.2	0.9	1.0	0.8	0.90	0.80
β-Pinene	969	0.1	0.3	0.5	0.8	0.9	1.3	1.1	1.5	1.3	1.40	0.90
Limonene	1020	0.2	1.0	1.2	1.4	1.5	1.7	1.6	2.0	2.4	2.60	1.60
1,8-cineole	1022	11.1	11.2	12.9	13.1	14.1	14.5	15.3	18.2	21.5	22.10	15.40
p-cymene	1026	0.1	0.1	0.3	0.5	0.1	0.1	0.1	0.1	0.1	0.10	0.20
Trans-β-ocimene	1033	0.2	0.3	0.5	0.8	1.3	1.5	1.8	1.7	1.9	2.00	1.20
γ-Terpenene	1049	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.10	0.10
δ-3-carene	1050	0.3	0.3	0.5	0.7	0.8	0.2	0.1	-	0.1	t	0.30
Nonanal	1099	0.1	2.9	2.5	2.4	4.2	-	0.4	-	-	t	1.30
Terpene-4-ol	1174	0.1	t	8.6	-	3.6	-	-	-	-	0.10	1.20
3-cyclohexene-1-ol	1175	0.1	-	-	-	2.4	-	-	0.3	-	t	0.30
α-phellandrene	1176	1.1	t	t	t	t	t	t	t	t	t	0.10
α-terpeneol	1205	0.1	1.3	0.5	0.4	0.5	0.3	0.2	t	0.1	0.10	0.40
Geraniol	1257	10.9	10.1	10.3	10.3	12.5	13.2	21.5	30.2	34.3	44.50	19.80
Carvacrol	1272	-	0.2	-	0.1	-	0.1	-	t	t	0.10	0.10
Bornyl acetate	1283	-	-	0.1	-	t	-	-	0.4	-	t	0.10
Sabinyl acetate	1288	0.1	-	-	0.1	-	-	0.1	0.1	-	t	0.00
α-cubebene	1347	-	0.3	-	0.1	0.3	-	-	0.2	-	1.70	0.30
α-copaene	1376	-	-	-	-	0.1	t	t	0.2	t	t	0.00
α-bourbonene	1385	t	0.1	-	-	t	-	-	0.1	0.1	0.20	0.10
β-cubebene	1389	-	t	-	-	2.5	-	-	1.6	-	t	0.10
α-gurjunene	1409	-	t	1.0	t	-	1.2	-	1.5	-	0.10	0.40
Aromadendrene	1410	t	-	t	-	6.1	-	-	0.1	t	t	0.60
β-caryophyllene	1420	-	t	-	t	-	-	-	0.4	-	0.90	0.10
α-Ylangene	1450	0.1	0.2	0.1	0.2	0.2	0.3	0.3	0.5	0.5	0.80	0.30
α-humulene	1454	1.0	1.2	1.3	1.3	1.2	1.4	1.4	1.4	1.5	1.70	1.30
Epi-bicyclo-Sesquiphellandrene	1463	-	0.1	0.2	0.1	0.2	0.3	0.4	0.4	0.5	0.50	0.30
Germacrene D	1481	t	0.1	0.1	0.2	1.0	1.2	1.3	1.9	1.9	11.50	1.90
Alloaromadendrene	1486	0.1	-	t	-	0.2	-	-	0.1	0.2	0.20	0.10
β-selinene	1486	0.3	-	0.2	-	-	0.9	-	1.0	-	0.30	0.30
Calarene	1494	0.2	0.2	0.1	0.4	0.5	3.3	5.0	5.5	5.7	5.70	2.70
Muurolene	1498	-	5.6	-	2.5	3.5	-	5.4	-	-	0.10	1.70
δ-Cadinene	1522	0.5	0.4	2.1	2.4	4.5	6.4	8.5	12.3	13.5	23.80	7.40
Cadina 1,4-diene	1531	0.7	-	0.8	-	0.1	-	0.2	-	-	12.20	1.40
α-cadinene	1537	1.5	1.5	1.5	1.6	3.2	7.5	8.0	8.2	9.6	10.70	5.30
Nerolidol	1559	0.6	1.4	t	1.3	1.5	1.2	1.1	1.1	1.5	1.30	1.10
Palustron	1569	1.2	1.3	-	0.2	1.4	t	-	-	-	0.70	0.50
Endobourbonene	1575	0.1	-	t	-	0.2	-	t	0.1	-	1.00	0.10
Oplopenone	1609	-	0.1	-	t	0.2	-	-	t	-	t	0.00
α-cadinol	1655	0.1	0.4	5.1	6.4	7.5	8.4	9.4	21.5	22.4	24.20	10.50
T-muurolol	1659	12.5	13.4	14.5	15.4	17.5	18.6	18.8	20.9	21.9	22.50	17.60
Oil yield (%, w/w)		0.13	0.30	0.45	0.48	0.52	0.64	0.65	0.79	0.95	0.97	0.59

Table 2: R^2 values of the main components of the essential oil of *Calendula officinalis* during its vegetative life cycle

Chemical components	R^2 value*
α-Pinene	0.8862
β-Pinene	0.8867
Limonene	0.9184
1,8-Cineole	0.9053
Trans-β-ocimene	0.9440
Geraniol	0.8042
α-Cubebene	0.5024
α-Ylangene	0.8107
α-Humulene	0.8264
Epi-bicyclo-sesquiphellandrene	0.9075
Calarene	0.8623
δ-Cadinene	0.8577
Cadina 1,4-diene	0.5432
α-Cadinene	0.9028
α-Cadinol	0.9001
T-muurolol	0.9886
Yield (%w/w)	0.9686

*: R^2 explains the relationship between concentration of chemical constituents and age of the plant

Thujones are poisonous components usually present in essential oils. In *C. officinalis*, the concentrations of these compounds (α-thujene) remained very low (0.1-0.2%) throughout the vegetative life of the plant, which is a measure of the good and safe quality of the oil. Geraniol was also a prominent component of the oil from this herb. Its concentration increased slightly from the 4th week until the 8th week after which the compound increased sharply in concentration. Geraniol is a natural antioxidant, which has been suggested to be useful in cancer prevention. According to Carnesecchi *et al.* (2001), geraniol caused a 50% increase of ornithine decarboxylase activity, which is enhanced during cancer growth. In addition, geraniol has been observed to inhibit DNA synthesis. Together with farnesol and peril alcohol, geraniol suppresses pancreatic tumor growth (Burke *et al*., 1997).

CONCLUSIONS

This study has shown that a correlation exists between the yield of *Calendula officinalis* essential oil and age of the plant and that the yield is best during the flowering stage of the plant. Also, the relative abundance of the chemical constituents of its essential oil at this stage is a veritable indicator of the appropriate period for collection and harvesting of the plant for the mining of the desired mono and sequiterpenes.

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REFERENCES

Anderson, J. and J. Ingram, 1993. Tropical Soil Biology and Fertility. A Hand Book of Methods. 2nd Edn., CABI, UK., pp: 221.

Anonymous, 1980. H.M.S. Office. London British Pharmacopoeia, 2: 109-110.

Anonymous, 2000. The European Agency for the Evaluation of Medicinal Products. EMEA/MRL/ 683/99-Final, August 1999.

Arras, G. and G.E. Grella, 1992. Wild thyme, *Thymus capitatus*, essential oil seasonal changes and antimycotic activity. J. Hortic. Sci., 67: 197-202.

Bakkali, F., S. Averbeck, D. Averbeck, A. Zhiri and M. Idaomar, 2005. Cytotoxicity and gene induction by some essential oils in the yeast *Saccharomyces cerevisiae*. Mut. Res., 585: 1-13.

Beerentrup, H.M. and G. Robbelen, 1996. *Calendula* and *Coriandrum*-new potential oil crops for industrial use. Fat. Sci. Technol., 89: 227-230.

Burke, Y.D., M.J. Stark, S.L. Roach, S.E. Sen and P.L. Crowell, 1997. Inhibition of pancreatic cancer growth by the dietary isoprenoids farnesol and geraniol. Lipids, 32: 151-156.

Carnesecchi, S., J. Schneider, J. Ceraline, B. Duranton, F. Gosse, N. Seiler and F. Raul, 2001. Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells. Pharmacology, 298: 197-200.

Chang, S.T., S.Y. Wang and Y.H. Kuo, 2003. Resources and bioactive substances from Taiwania. J. Wood Sci., 49: 1-4.

Cetkovic, G.S., S.M. Djilas, J.M. Canadanovi-Brunet and V.T. Tumbas, 2004. Antioxidant properties of marigold extracts. Food Res. Int., 37: 643-650.

Danielski, L., L.M.A.S. Campos, L.F.V. Bresciani, H. Hense, R.A. Yunes and S.R.S. Ferreira, 2007. Marigold (*Calendula officinalis* L.) oleoresin: Solubility in SC-CO_2 and composition profile. Chem. Eng. Proc., 46: 99-106.

Dunford, N.T., S. Hiziroglu and R. Holcomb, 2007. Effect of age on the distribution of oil in Eastern redcedar tree segments. Biores. Technol., 98: 2636-2640.

Gilman, E.F. and T. Howe, 1999. *Calendula officinalis*. Cooperative extension service, Institute of food and agricultural sciences, University of Florida. Fact Sheet FPS, pp: 87.

Hamburger, M., S. Adler, D. Baumann, A. Forg and B. Weinreich, 2003. Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*). Fitoterapia, 74: 328-338.

Harborne, J.B., 1991. Introduction to Ecological Biochemistry. 4th Edn., Academic Press, London.

Holm, Y., B. Galambosi and R. Hiltunen, 1988. Variation of the main terpenes of dragonhead (*Dracocephalum moldavica* L.) during growth. Flavour Fragr. J., 3: 113.

Janke, R., 2004. Farming a Few Acres of Herbs: Calendula, Kansas State University Agricultural Experiment Station and Cooperative Extension Service. MF-2610.

Jimenez-Medina, E., A. Garcia-Lora, L. Paco, I. Algarra, A. Collado and F. Garrido, 2006. A new extract of the plant *Calendula officinalis* produces a dual *in vitro* effect: Cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer, 6: 119.

Kalvatchev, Z., R. Walder and D. Garzaro, 1997. Anti-HIV activity of extracts from *Calendula officinalis* flowers. Biomed. Pharmacother., 51: 176-180.

Lavagna, S.M., D. Secci, P. Chimenti, L. Bonsignore, A. Ottaviani and B. Bizzarri, 2001. Efficacy of *Hypericum* and *Calendula* oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section. Il Farmacol., 56: 451-453.

Marczal, G., 1987. Data on the essential oil content and composition of *C. officinalis*. Herba Hung, 26: 179-89.

McGimpsey, J.A., M.H. Douglas, J.W. van Klink, D.A. Beauregard and N.B. Perry, 1994. Seasonal variation in essential oil yield and composition from naturalized. *Thymus vulgaris* L. in New Zealand. Flavour Fragrance J., 9: 347.

Muuse, B.G., F.P. Cuperus and J. Derksen, 1992. Composition and physical properties of oils from new oilseed Crops. Ind. Crops Prod., 1: 57-65.

Moldao-Martins, M., M.G. Bernardo-Gil, M.L. Beirao da Costa and M. Rouzet, 1999. Seasonal variation in yield and composition of *Thymus zygis* L. subsp. *Sylvestris* essential oil. Flavor Fragrance J., 14: 177-182.

Okalebo, J.R., K.W. Gathna and P.L. Woomer, 2002. Laboratory Methods for Soil and Plant Analysis: A Working Manual. 2nd Edn., Nairobi: Tropical Soil Fertility and Biology Program.

Putievsky, E., U. Ravid, N. Dudai, I. Katzir, D. Carmeli and A. Eshel, 1992. Variations in the essential oil of *Artemisia judaica* L. chemotypes related to phenological and environmental factors. Flavor Fragrance J., 7: 253-257.

Shibamoto, T., 1987. Retention Indices in Essential Oil Analysis. In: Capillary gas Chromatography in Essential, Sandra, P. and C. Bicchi (Eds.). Huethig A. Verlag, New York, USA., pp: 259-274.

Wali, B., M. Iqbal and Mahmooduzzafar, 2007. Anatomical and functional responses of *Calendula officinalis* L. to SO_2 stress as observed at different stages of plant development. Flora, 202: 268-280.

Wyk, B.E.V. and M. Wink, 2004. Medicinal Plants of the World. Briza Publications, Pretoria.