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Seasonal Variation in the Composition of Oil, Protein, Carbohydrate, Carbon and Nitrogen Content in Mature Leaves of *Callistemon viminalis*

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ABSTRACT

The present study deals with the seasonal variation in the composition of oil, protein, carbohydrate, carbon and nitrogen content in the mature leaves of *Callistemon viminalis*. The total amount of oil identified from different seasons was almost similar, ranging from 98.73 to 99.62%. 1, 8-cineole was identified as the major component in all the oils of different season, however, its content was maximum in winter (64%) and minimum in post-rainy (50%) season. The leaves collected in the spring season contained the maximum amount of protein and carbon content and the minimum amount of chlorophyll, carotenoids and nitrogen content. The carbohydrate content was the maximum in the rainy season and the minimum in winter season. The post-rainy season leaves contained the maximum amount of chlorophyll, carotenoid and nitrogen content and the minimum amount of carbon content. These seasonal variations may be attributed to the fluctuations in temperature and humidity in different seasons that adversely affects the volatility of the essential oil, physiological and biochemical status of the plant.

1. INTRODUCTION

Aromatic plants are reservoirs of volatile compounds/natural plant products that are involved in various ecological functions such as anti-herbivore, antifungal, antibacterial compounds, phytotoxins, as attractants of pollinators or seed dispersers and are also involved in plant-plant interactions[1,2]. In this regard, genus *Callistemon* R. Br. (Myrtaceae), represented by 50 species are mostly shrubs or small trees and bear evergreen foliage and brush-like flowering spikes that provide them the common name of "bottlebrush"[3], may hold great potential. Though a native of Australia, *Callistemon* species are cultivated worldwide because of their ornamental value. Leptospermone, natural compound obtained from roots of *C. citrinus* (Curtis) Skeels., was found to be phytotoxic and caused bleaching of grass and broadleaved weeds, while maize was found to be resistant to it[4,5]. Mesotrione, synthetically derived from leptospermone is used as a herbicide to control broadleaved weeds in maize crop. Over the years, it has been reported that *C. lanceolatus* (Sm.) DC. possess anti-inflammatory and antioxidant activity [6,7]. These reports indicate that *C. lanceolatus* possess various chemical compounds which have medicinal properties. Monoterpenes 1, 8-cineole and *α*-terpineol, the main constituents of *C. citrinus* have anthelmintic and antimicrobial activity[8].

C. viminalis (Gaertn.) G. Don., commonly known as weeping bottlebrush, is a small evergreen tree or shrub and is a native of South-eastern Australia. It is one of the very common species of *Callistemon*, extensively cultivated in the gardens, parks and road sides in different parts of the world including India owing to its ornamental value[9]. It has pendulous evergreen foliage with crimson red flowers. Besides, the tree is aromatic particularly its leaves and inflorescence[10]. According to Garg and Kasera[11], oil from *C. viminalis* exhibited anthelmintic activity against tapeworm and hookworm. The essential oil obtained from *C. viminalis* exhibited antibacterial and antioxidant [10,12], and insecticidal activities[13]. Khambay *et al.* [14] isolated two new compounds namely, viminadione A and viminadione B from hexane extracts of *C. viminalis* that exhibited insecticidal activity comparable to natural pyrethrum extracts. Owing to their selective phytotoxic effects and biodegradable nature, essential oil extracted from *C. viminalis* may offer a great deal of promise for future to serve as novel herbicides. Though the phytotoxic effect of compounds extracted from *C. citrinus* has been studied [4,5], yet studies pertaining to phytotoxic effect of *C. viminalis* essential oil are lacking. De Oliveira *et al.* [15] reported that the essential oil extracted from the flowers of *C. viminalis* possess allelopathic properties. Being an evergreen tree, the leaves remain available throughout the year. Environmental conditions like changes in climatic conditions, especially temperature and light, influence the quantity of volatile organic compounds emitted from the foliar tissue [16]. Thus, it was hypothesized that the yield and composition of the oil varies with season. A study was thus planned to determine the seasonal variation in the amount of essential oil extracted from mature leaves of *C. viminalis*. Further, the variation in protein, carbohydrate, carbon and nitrogen content in mature leaves of *C. viminalis* was also determined in different seasons.

2. MATERIALS AND METHODS

2.1 Collection of Material

The mature leaves were collected from trees of *C. viminalis*, Panjab University campus, Chandigarh, India in six seasons, namely, spring, summer, rainy, post-rainy, winter and post-winter.

2.2 Extraction of oil

The essential oil was extracted with the help of Clevenger's apparatus. The mature leaves (approximately 1 kg) were cleaned and placed in round bottom flask. The oil obtained from the nozzle was dried over anhydrous sodium sulphate and stored at 4ºC for further analysis by GC-MS. For moisture determination, 100 g of leaves for each season were kept in an oven at 80°C for 24 h. The yield of the oil was expressed in percent dry matter (v/w, volume/dry weight of tissue).

2.3 Chemical analysis of oil

The essential oil sample was analyzed by thermo GC-MS. GC was conducted on Trace 1300 Gas Chromatograph equipped with FID (Flame Ionization Detector) and TG 5 column [30m × 0.25mm (inner diameter), and 0.25µm film thickness]. The essential oil sample and leaf powder were analyzed by thermo GC-MS. GC was conducted on Trace 1300 Gas chromatograph equipped with FID (flame ionization detector) and TG 5 column [30 m × 0.25 mm (inner diameter), and 0.25 µm film thickness]. Helium was used as a carrier gas and the column flow rate was 1 ml/min. The injector temperature was set at 250°C. The oven temperature was programmed from 60°C (held isothermally for 2 min) to 250°C with a ramp of 3°C/min and held at 250°C for 5 min.

All the oil samples and leaf powder were then analyzed by GC-MS using a TSQ 8000 triple quadrapole mass spectrophotometer equipped with TG 5MS column using Helium as a carrier gas at a split ratio of 1:150. Injector temperature and ion source temperature were set at 250°C and 230°C, respectively. The oven temperature was programmed from 60°C (held isothermally for 2 min.) to 250°C with ramp of 3°C/ min and held at 250°C for 5 min. The mass spectra were in the range of *m/z* 30-400. Different components were identified on the basis of (i) comparison of their Retention Indices (RI) relative to homologous series of n-alkanes (C_7-C_{30}, Sigma-Aldrich) (ii) computer matching of mass spectra with the NIST library, and (iii) consulting libraries of NIST 98[17], pherobase and compilation by Adams[18].

2.4 Determination of protein, carbohydrate, carbon and nitrogen content

Protein and carbohydrate content was determined from dried leaf samples (freed of water, pigment, oils and fatty compounds, etc.) as per [19,20]. Water content was determined by oven drying of the leaf samples at 80 ºC for 72 h. Elemental analysis (C and N) was done on a CHN elemental analyzer using dried leaf powder.

2.5 Parameters studied

The percent yield of the oil in mature leaves of *C. viminalis* was determined in different seasons for one year (*i.e.* Jan' 2013 to Dec' 2013). Six different season were selected as follows: spring (March-April), summer (May-June), rainy (July-August), Post-rainy/Pre-winter (September-October), winter (November-January), and Post-winter (February). The content of protein, carbohydrate, carbon and nitrogen were also determined. Besides, the minimum and maximum atmospheric temperature and humidity were also noted. The quantitative analysis of the components of oil was done.

3. RESULTS

3.1 Variation in the yield and composition of Callistemon oil in different seasons

The mature leaves of *C. viminalis* yield oil in the range of ~ 4-6 ml kg^{-1} DW in all the seasons, except for post-rainy season where the yield was less, *i.e.*, 3.2 ml kg^{-1} DW. The *Callistemon* oil was found to be yellowish-brown in color in all the seasons, except in winters when the oil was pale yellow in color. The amount of oil extracted in different seasons identified by GC-MS was almost similar, ranging from 98.73 to 99.62% (Table 1). A total of

18 compounds from spring, 19 from summer and rainy, 21 from post-rainy and 22 from winter and post-winter season were identified (Table 1). The major components identified in *Callistemon* oil were 1, 8-cineole, α-pinene and α-terpineol in all the seasons. The content of 1, 8-cineole was found to be ~62% (spring season), ~55% (summer season), ~54% (rainy and post-winter season), ~50% (post-rainy season) and ~64% (winter season) and of α-pinene was found to be ~21% in spring, post-rainy and post-winter, ~24% in summer, ~25% in rainy and~17%winterseason(Table1).

Table 1 Seasonal variation in the composition of essential oil from mature leaves of *C. viminalis.*

S.No.	Seasons → / ↓ Components	Spring	Summer	Rainy	Post-rainy/ pre-winter	Winter	Post-winter
1.	4-Amino-5-hydroxy-naphthalene-1-sulfonic acid	-	-	-	-	-	4.36
2.	Vinyl crotonate	-	-	-	-	-	0.91
3.	Propanoic acid, 2-methyl-, 2-methyl propyl ester	0.15	0.12	0.18	0.21	0.14	0.74
4.	α-Pinene	20.82	24.34	25.42	20.67	16.85	20.90
5.	Camphene	0.13	-	-	-	0.05	0.71
6.	β-Pinene	0.53	0.62	0.66	0.59	0.38	0.71
7.	β-Myrcene	-	0.34	0.25	0.23	0.09	0.20
8.	α-Phellendrene	1.42	2.44	1.89	2.43	0.43	0.82
9	Propanoic acid, 2-methyl-, 3-methylbutyl ester	-	-	-	-	0.16	0.18
10.	1,8-Cineole	61.47	54.65	53.95	49.68	63.82	54.37
11.	β-(Z)-Ocimene	-	0.29	0.22	-	0.11	-
12.	γ-Terpinene	0.31	0.36	0.39	0.40	0.23	0.25

13.	*α*-Terpinolene	0.09	0.28	0.23	0.24	-	0.12
14.	Linalool	0.45	0.73	0.63	0.75	0.45	0.63
15.	3-Octanol, acetate	-	-	-	-	0.07	-
16.	L-Pinocarveol	0.29	0.11	0.09	-	0.28	0.25
17.	Santolina alcohol	0.29	0.22	0.22	0.30	0.24	0.35
18.	Terpinen-4-ol	0.34	0.43	0.36	0.53	0.32	0.38
19.	*α*-Terpineol	10.73	11.93	11.68	13.14	9.88	11.53
20.	Piperitone oxide	-	-	-	-	3.64	-
21.	Carvone	-	-	-	0.57	-	-
22.	*n*-Amyl ether	0.46	0.47	0.44	0.79	0.58	0.63
23.	Durohydroquinone	0.40	0.62	0.90	1.72	-	0.72
24.	*trans*-Ascaridole	-	-	-	-	0.50	-
25.	Caryophyllene	0.22	0.08	-	1.78	0.22	-
26.	Isocaryophyllene	-	-	-	0.22	-	-
27.	Germacrene D	-	-	-	0.65	0.11	-
28.	Ledene	-	-	-	0.62	-	-
29.	(+)-Spathulenol	0.59	0.65	0.58	1.37	0.18	0.45
30.	Viridiflorol	-	0.33	0.84	2.28	-	-
31.	τ-Cadinol	0.16	-	0.25	-	-	0.19
32.	*α*-Cadinol	-	-	-	-	-	0.22
33.	Total	**98.85**	**99.01**	**99.18**	**99.17**	**98.73**	**99.62**

The amount of *α*-terpineol in the oil was more than 10% in all the six seasons. The content of *α*-phellandrene was maximum in summer (~2.4%) and minimum in winter (~0.4%). 4-amino-5-hydroxy-naphthalene-1-sulfonic acid (~4.4%), vinyl crotonate (~0.9%) and *α*-cadinol (~0.2%) were present only in the post-winter season. Similarly, piperitone oxide (3.64%) and *trans*-ascaridole (0.50%) were exclusively present in winter season in *C. viminalis* essential oil. The oil of post-rainy/pre-winter season contained ledene (0.62%), carvone (0.57%) and isocaryophyllene (0.22%) that were absent in other seasons (Table 1).

3.2 Protein and carbohydrate content

The protein content in mature leaves of *C. viminalis* was measured to be 65.2±1.01, 24.3±0.44, 55.8±1.95, 26.0±0.77, 27.3±0.55 and 27.2±1.02 µg mg^{-1} FW in spring, summer, rainy, post-rainy, winter and post-winter season, respectively (Fig. 1). The content was maximum in spring and minimum in summer season. Similarly, the carbohydrate content ranged from 2.8-1.0 µg mg^{-1} FW in different seasons (Fig. 1). It was the maximum in rainy season (2.9±0.51 µg mg^{-1} FW) followed by spring (2.7±0.42 µg mg^{-1} FW), summer (1.7±0.56 µg mg^{-1} FW), post-rainy (1.2±0.20 µg mg^{-1} FW), post-winter (1.0±0.16µg mg^{-1} FW) and winter (1.0±0.25 µg mg^{-1} FW) season (Fig 1).

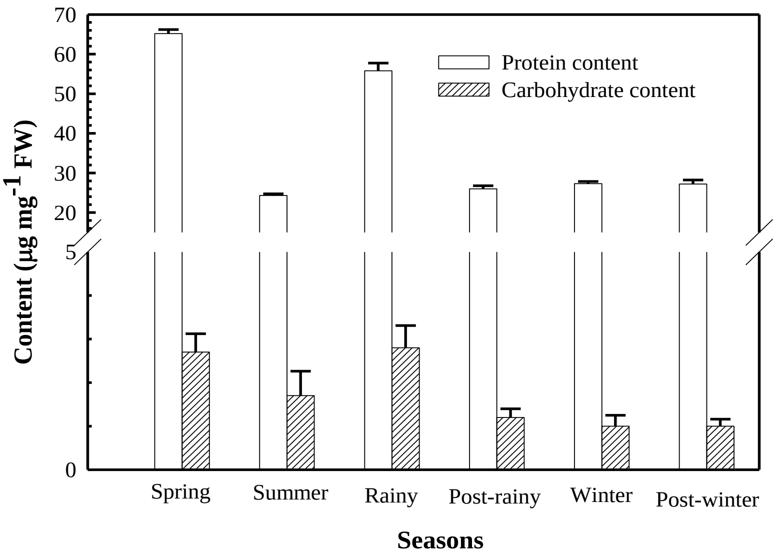

Fig. 1 Seasonal variation in protein and carbohydrate content in the mature leaves of *C. viminalis*

3.3 Carbon/Nitrogen ratio

The carbon content was maximum (~49.1%) in spring season and minimum (~47.1%) in rainy season (Table 2). However, the nitrogen content was highest in post-rainy season (~2.1%), followed by summer (~2.0%), rainy (~1.9%), post-winter (~1.7%), winter (~1.6%), and spring season (~1.3%).The C/N ratio was highest in spring season (~38.1) and minimum in post-rainy season (~23.1), respectively (Table 2).

3.4 Chlorophyll and carotenoid content

The total chlorophyll content and carotenoid content was measured in the mature leaves of *C. viminalis* in different seasons of the year (Fig 2). The results indicated the maximum chlorophyll content in the post-rainy season (2.8±0.35 µg mg^{-1} DW) and the minimum in spring season (1.4±0.13 µg mg^{-1} DW) (Fig 2). Similar results were obtained for carotenoid content in the leaves of *C. viminalis*. It was found to be highest in post-rainy (0.9±0.10 µg mg^{-1} DW) season followed by rainy, summer, post-winter, winter and spring season (Fig 2).

Table 2 Seasonal variation in carbon and nitrogen content in the mature leaves of *C. viminalis.*

Seasons	Carbon content (%)	Nitrogen content (%)	C/N ratio
Spring	49.1	1.3	38.1
Summer	48.7	2.0	24.1
Rainy	47.1	2.0	23.7
Post-rainy	47.7	2.1	23.1
Winter	48.1	1.7	29.1
Post-winter	48.6	1.7	29.1

Data presented is mean of three replicates.

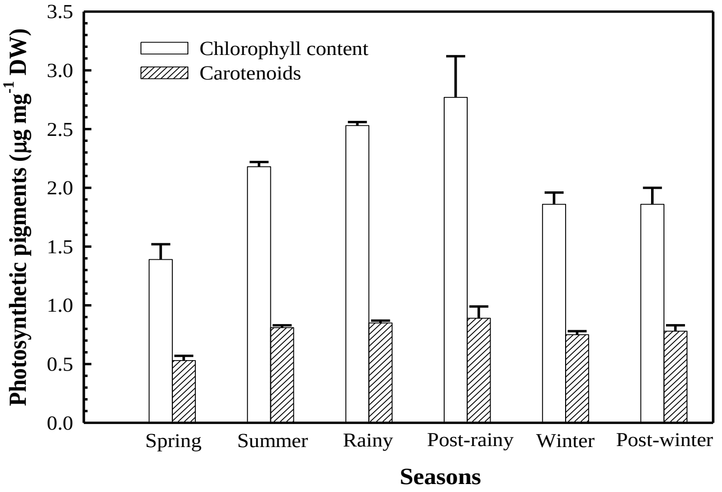

Fig 2 Seasonal variation in photosynthetic pigments (measured in terms of chlorophyll and carotenoid content) in the mature leaves of *C. viminalis*

4. DISCUSSION

Aromatic plants are reservoirs of essential oils, the content and composition of which varies with seasons. The yield of the oil from mature leaves of *C. viminalis* was different throughout the year. It was highest in the rainy season (5.90 ml/kg DW), followed by post-winter (5.70

ml/kg DW), spring (4.91 ml/kg DW), winter (4.53 ml/kg DW), summer (4.05 ml/kg DW) and post-rainy season (3.20 ml/kg DW). The variation in yield of the oil may be attributed to the seasonal changes in temperature and humidity that adversely affects the volatility of the essential oil. Sharafzadeh and Zare [21] pointed out that the environmental factors affect the biochemical and physiological processes in plant that significantly alter the metabolic activities of plant. According to Padalia *et al.* [22], the yield and composition of the essential oil largely depend on the climatic conditions and the season at the time of collection. The low yield of the oil in post-rainy season might be due to high humidity in that season. Moreover, lower yield of *Callistemon* oil in the summer may be attributed to higher temperature that leads to more volatilization of oil. Our results are in agreement to those of Da-Silva *et al.* [23] who reported highest amount of basil essential oil in the winter (January) and lowest in the summer (August). Some other reports also suggested seasonal variation in the content of essential oil in plants[24-27]. In addition to the oil content, variations in the amount of carotenoids, chlorophyll, protein, carbohydrate, carbon and nitrogen were also observed in the leaves of different seasons. This might be attributed to different environmental conditions.

5. CONCLUSION

From the above discussion, it can be concluded that environment effect the photosynthetic pigments, composition of oil and various biochemical content in the plant.

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REFERENCES

1. Langenheim JH. Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of Chemical Ecology. 1994, 20: 1223–1280.
2. Dudareva N, Negre F, Nagegowda DA, Orlova I. Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Sciences. 2006, 25: 417–440.
3. Spencer RD, Lumley PF. Callistemon In: Flora of New South Wales, eds. Harden, G. J. New South Wales University Press, Sydney, Australia. 1991, pp. 168–173
4. Gray, Reed A, Tseng, Chien K, Rusay, Ronald J. 1-Hydroxy-2-(alkylketo)-4,4,6,6- tetramethyl cyclohexene-3,5-diones. United States Patent 1980, 4,202,840.
5. Knudsen CG, Lee DL, Michaely WJ, Chin HL, Nguyen NH, Rusay RJ, Cromartie TH, Gray R, Lake BH, Fraser TE, Cartwright D. Discovery of the triketone class of HPPD inhibiting herbicides and their relationship to naturally occurring *β*-triketones. In Allelopathy in Ecological Agriculture and Forestry. eds. Narwal SS, Hoagland RE, Dilday RH, Reigosa MJ, Springer, Netherlands, 2000, pp. 101–111.

6. Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti–inflammatory activity of *Callistemon lanceolatus* leaf. Asian Pacific Journal of Tropical Biomedicine. 2011, 1: 177–181.

7. Goyal PK, Jain R, Jain S, Sharma A. A Review on biological and phytochemical investigation of plant genus *Callistimon*. Asian Pacific Journal of Tropical Biomedicine. 2012, 2: 1906–1909.

8. Krishna KVVS, Surendra G, Anjana M, Siva Nagini KSK. Phytochemical screening and antimicrobial activity of *Callistemon citrinus* (L.) leaves extracts. International Journal of PharmTech Research. 2012, 4: 700–704.

9. Anonymous. *Callistemon viminalis*. In: The wealth of India-raw materials, revised series. Vol. III (Ca-Ci). ed. Phondke GP, Council of Scientific and Industrial Research, New Delhi. 1992, pp. 63–66.

10. Oyedeji OO, Lawal OA, Shode FO, Oyedeji AO. Chemical composition and antibacterial activity of the essential oils of *Callistemon citrinus* and *Callistemon viminalis* from South Africa. Molecules. 2009, 14: 1990–1998.

11. Garg SC, Kasera HL. Anthelmintic activity of *Callistemon viminalis*. Fitoterapia. 1982, 53: 179–181.

12. Salem MZ, Ali HM, El-Shanhorey NA, Abdel-Megeed A. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian Pacific Journal of Tropical Medicine. 2013, 6: 785–791.

13. Ndomo AF, Tapondjou LA, Ngamo LT, Hance T. Insecticidal activities of essential oil of *Callistemon viminalis* applied as fumigant and powder against two bruchids. Journal of Applied Entomology. 2010, 134: 333–341.

14. Khambay BP, Beddie DG, Hooper AM, Simmonds MS, Green PW. New insecticidal tetradecahydroxanthenediones from *Callistemon viminalis*. Journal of Natural Products. 1999, 62: 1666–1667.

15. De Oliveira CM, das Graças Cardoso M, da Silva Figueiredo AC, de Carvalho MLM, de Miranda CASF, Albuquerque LRM, Nelson DL, de Souza Gomes M, Silva LF, de Andrade Santiago J, Teixeira ML. Chemical composition and allelopathic activity of the essential oil from *Callistemon viminalis* (Myrtaceae) blossoms on lettuce (*Lactuca sativa* L.) seedlings. American Journal of Plant Sciences. 2014, 5: 3551–3557.

16. Filella I, Wilkinson MJ, Llusià J, Hewitt CN, Peñuelas J. Volatile organic compounds emissions in Norway spruce (*Picea abies*) in response to temperature changes. Physiologia Plantarum. 2007, 130: 58–66.

17. Stein SE. National Institute of Standards and Technology (NIST). Mass Spectral Data Base and Software, Ver. 3.02. Gaithersburg, Maryland, USA. 1990.

18. Adams RP. Identification of essential oils components by gas chromatography and mass spectrometry. Allured Publishers Corp., Carol Stream, Illinois. 1995.

19. Lowry OH, Rosebrough NJ, Farr AL, Rendall RJ. Protein estimation with folin–phenol reagent. Journal of Biological Chemistry. 1951, 193: 265–278.

20. Loewus FA. Improvement in the anthrone method for determination of carbohydrates. Analytical Chemistry. 1952, 24: 219.

21. Sharafzadeh S, Zare M. Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family. Advances in Environmental Biology. 2011, 5: 2296–2303.

22. Padalia RC, Verma RS, Chauhan A, Chanotiya CS. Changes in aroma profiles of 11 Indian *Ocimum* taxa during plant ontogeny. Acta Physiologiae Plantarum. 2013, 35: 2567–2587.

23. Da-Silva F, Santos RHS, Diniz ER, Barbosa LCA, Casali VWD, De-Lima RR. Content and composition of basil essential oil at two different hours in the day and two seasons. Revista Brasileira de Plantas Medicinais. 2003, 6: 33–38.

24. Doran JC, Williams ER, Brophy JJ. Patterns of variation in the seedling leaf oils of *Eucalyptus urophylla, E. pellita* and *E. scias.* Australian Journal of Botany. 1995, 43: 327–336.

25. Moudachirou M, Gbenou JD, Chalchat JC, Chabard JL, Lartigue C. Chemical composition of essential oil of eucalyptus from Benin: *Eucalyptus citriodora* and *E. camaldulensis*. Influence of location, harvest time, storage of plants and time of steam distillation. Journal of Essential Oil Research 1999, 11: 109–118.

26. Chericoni S, Flamini G, Campeol E, Cioni PL, Morelli I. GC–MS analyses of the essential oil from the aerial parts of *Artemisia verlotiorum*: variability during the year. Biochemical Systematics and Ecology. 2004, 32: 423–429.

27. Van Vuuren SF, Viljoen AM, Őzek T, Demirci B, Başer KHC. Seasonal and geographical variation of *Heteropyxis natalensis* essential oil and the effect thereof on the antimicrobial activity. South African Journal of Botany. 2007, 73: 441–448.

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