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Compositional Variation of the Essential Oils of *Artemisia afra* Jacq. from three Provinces in South Africa - A Case Study of its Safety

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Safety of *Artemisia afar* has been a controversial issue due to its high thujone content. Despite the declaration of the World Health Organization in the 1970s of the plant being unsafe for consumption, it is still commonly used in folklore medication in South Africa, especially in winter. Essential oils were isolated by hydrodistillation from the twigs of *A. afra* plants from different locations in the Eastern Cape, Free State and KwaZulu-Natal. Analyses of the oils by GC and GCMS revealed compositional variations in the levels of α-and β-thujone, 1,8-cineole and camphor. α-Thujone was the major component of the essential oils of *A. afra* from Philippolis (Free State) and Keiskammahoek (Eastern Cape) (62-74%), while the camphor content was very low (≤ 0.1-0.6%). The samples from Gqumahshe, Hogsback (Eastern Cape) and Empangeni (KwaZulu Natal) had low α-thujone contents (3.7-20.0%) while 1,8-cineole (13.0-49.5%) and camphor (13.9-21.2%) were the main components of the essential oils. It was further observed that the concentration of α-thujone increased significantly in the dry leaves when compared with the fresh leaves. This implies that fresh leaves are better used for infusion than dry leaves. This study reveals that not all *A. afra* contain high concentrations of α- and β- thujone.

Keywords: *Artemisia afra*, essential oil, α- and β- thujone, 1,8-cineole, camphor.

Most *Artemisia* species, like other medicinal essential oil plants, such as *Salvia officinalis* (sage), *Tanacetum vulgare* (tansy), *Thuja occidentalis* (yellow cedar), *Juniperus* species (junipers), *Chamaecyparis* species (cypresses), and *Achillea millefolium* (yarrow), are classified as been toxic due to their high thujone content [1a]. Essential oils containing thujone have been used in traditional medicine in treating common cold (cedar leaf oil, wormwood oil), as anthelmintics (tansy, wormwood, mugwort), for digestive problems and as carminatives (sage, cedar, tansy, mugwort), and for the treatment of fever, cough, rheumatism and acne (cedar) [1a-1d,2a].

Artemisia afra Jacq. (umhlonyane), family Asteraceae, known as African woodworm, is one of the plants most widely used in herbal remedies in Southern Africa due to its availability throughout the year [2b-2c]. The herb is used to treat various types of chest infections, cough, cold, colic, heartburn, flatulence, whooping cough and gout. Most often, the leaves and stems are used in the form of either a tea (infusion) or decoction [2b,3a-3c]. Furthermore, the plant is used to treat patients with asthma (as an inhalation therapy) and respiratory infections (infusion therapy) in the Hospice Clinic within the KwaZulu-Natal region.

A. afra has been reported to contain α- and β-thujone in high concentrations [2c,3b-3d]. Thujone has been identified as a volatile organic compound emitted by vegetation to the atmosphere [3e]. Plants containing thujone, when used regularly and in large quantities, in addition to causing yellow-tinged vision, produce behavioral changes, convulsions, brain cortex lesions and renal failure [3f,3g]. Symptoms of prolonged ingestion include vomiting, restlessness, convulsion and fatty degeneration of the liver. However, the solubility of thujone in water is extremely low, hence its safety when used by traditional healers and locals [3h]. Thujones are known to be among the bioactive constituents of the essential oil [3i,4]. The LD_{50} (s.c.)

of α-thujone in mice was found to be 87.5 mg/kg, while, that of β-thujone was 42.5 mg/kg [5]. Bonkovsky *et al*. [6] reported that thujone makes up 40-90% (by weight) of the essence of woodworm. In 1979, the FAO / WHO Codex Committee on Food Additives restricted the use of α-and β- thujone in finished products to 0.5 ppm in food and 10 ppm in alcoholic drinks [7,8,9a]. This shows that despite its toxicity, thujone still has good flavoring, pharmaceutical and economical potential. Essential oils, of which *A. afra* is one [9b], are used as raw materials in the flavor and fragrance industry. Some of the components of the essential oil are bioactive while others may either increase their bioavailability or serve to aid the absorption rate of the active ingredient when used [2a]. Thus a plant containing one or two toxic constituents cannot be classified as being totally toxic or poisonous.

In South Africa, *A. afra* is a medicinal plant traded in the KwaZulu-Natal and Faraday Muti Markets. Internationally, *A. afra* is one of the marketed *Artemisia* essential oil yielding plants (Table 1). In view of the common usage of the plant, the safety of this herb as it relates to the toxic chemical components and their recommended dosage by the Food and Drug Administration, and the economic potential of the plant prompted our interest in the levels of these toxic compounds. *A. afra* from different locations within the Eastern Cape Province was the onset of this study, which later spread to the Free State and KwaZulu Natal Provinces. Also the storage of the plant by traditional healers for their ready availability prompted a comparative study of the dry and fresh plants. The oil yields (w/w) obtained by hydrodistillation, color and odor are presented in Table 2. Except for the sample from Philippolis in the Free State, all the essential oils had minty-peppermint-like odors. The chemical profiles of these essential oils are presented in Table 3.

α-Thujone formed over 70% of the total oil of both the dry and fresh leaves of *A. afra* from Philippolis; this was closely followed by the oils isolated from the Keiskammahoek samples (60-65%) and the dry leaves from the University of Fort Hare farm (43.8%). Very low concentrations were detected in both the fresh and dry oil samples from the plants collected from Gqumahshe (1.6-5.1%), University of Zululand Botanical Garden (10.5-12.2%) and Empangeni (2.3-7.9%). The fresh essential oil isolated from the Hogsback material had relatively low α-thujone content (8.1%), while the dry sample showed a remarkable increase to 16.7%. This could be due to oxidation during drying or distillation. α-Thujone was completely absent in the essential oil from Gqumahshe samples, while the concentrations in other samples were between 8.1-17.3%. Interestingly, it was observed that essential oils with a high percentage content of α-and β- thujone had low or trace amounts of camphor and 1,8-cineole.

Table 1: Traded quantities and values of some *Artemisia* essential oils on the International markets.

Essential oil	*Artemisia* species	Volume (t)	Value (US$000)	Value/ton (US$000)
Armoise	*A. herba-alba*	32	1100	34.4
Tarragon	*A. dracunculus*	10	800	80.8
Wormwood	*A. absinthum*	6	200	33.3
Artemisia maritima	*A. maritima*	1	<0.1	
Davana	*A. pallens*	1	300	300.0
Artemisia afra	*A. afra*	**0.8**	**51**	**68.0**
Artemisia annua	*A. annua*	0.6	16	26.7
Artemisia vestita	*A.vestita*	0.05	unknown	

Source: COMTRADE, UNSO, cited in [9c]

1,8-Cineole was another prominent compound in all the samples with that from Gqumahshe having the highest concentration of 47.0-49.5% in the dry and fresh samples, respectively. This compound, has a cold relieving effect with an expectorant and mucolytic properties [9d]. Essential oils of *A afra* from Hogsback and University Farm were the only specimens that had artemisia ketone and alcohol as major constituents. Not much compositional variation was noticed in the fresh and dry oil analyses. Thus the use of either dry or fresh plant material did not have significant impact on the composition of the main compounds, expect for the University farm sample. The amount of α- and β-thujone in the dry sample was twice that of the fresh sample, while this was the reverse for the camphor composition (Table 3).

The chemical composition of the oils isolated from the three Provinces shows that there are three chemotypes of *A. afra* in South Africa, namely, chemotype 1, those with high α- thujone content, and chemotype 2, those with a high 1,8-cineole content. A third chemotype could be said to contain a high content of β-thujone (fresh Hogback specimen). Thus not all South African *A. afra* essential oils have high α- thujone contents. Graven *et al*. [2c] reported that the essential oils of South African *A. afra* contain high α- and β-thujone contents (74.9-75.3%) and low concentrations of 1,8-cineole (1.3-13.7%). Further studies by Graven *et al*. [3b] of the same species from

Table 2: Physiochemical properties of the essential oils of *Artemisia afra* from seven different locations.

Location	Plant sample	Weight of sample extracted (g)	Percentage yield (w/w)	Color	Odor
Gqumahshe	Fresh	300	1.15	light blue	mint
	Dry	200	1.43	light green	mint
Keiskammahoek	Fresh	300	1.26	greenish yellow	mint
	Dry	200	1.50	light yellow	mint
Hogsback	Fresh	300	1.03	light blue	mint
	Dry	200	1.08	bluish green	peppermint
UFH Farm	Fresh	300	2.12	cloudy blue	strong mint
	Dry	200	2.73	deep blue	strong mint
Philippolis	Fresh	300	1.31	light yellow	pungent
	Dry	200	1.56	golden yellow	pungent
Empangeni	Fresh	300	2.26	light yellow	Peppermint
	Dry	200	2.85	yellow	Peppermint
UZN Botanic Garden	Fresh	300	1.90	light blue	Mint
	Dry	200	2.25	bluish green	mint

UFH University of Fort Hare; UZN – University of Zululand

Table 3: Major essential oil constituents of *A. afra* oils from seven different locations.

Compound		**yomogi alcohol**	**1,8-cineole**	**artemisia ketone**	**artemisia alcohol**	**α-thujone**	**β-thujone**	**chrysantheno ne**	**camphor**	**borneol**	**terpinen-4-ol**	**piperitone**
Gqumahshe	Fresh	1.2	**49.5**	-	-	1.6	-	-	**21.2**	18.7	1.2	1.0
	Dry	0.9	**47.0**	-	-	5.1	-	-	**23.1**	15.6	1.2	0.9
Keiskammahoek	Fresh	-	18.6	-	-	**62.2**	**17.3**	-	t	t	1.8	-
	Dry	-	17.2	-	-	**65.2**	12.6	-	0.6	t	1.6	-
Hogsback	Fresh	4.6	13.0	31.1	2.8	3.6	**16.5**	-	13.9	3.2	1.3	1.3
	Dry	12.2	**22.2**	19.5	12.6	11.4	2.7	-	10.0	0.9	1.3	1.3
UFH Farm	Fresh	1.6	**23.8**	6.7	0.2	19.3	8.1	8.6	16.4	2.9	3.0	-
	Dry	0.5	21.4	2.9	3.4	**43.8**	**16.7**	-	6.7	1.0	0.7	-
Philippolis	Fresh	-	5.2	-	-	**74.0**	**14.9**	-	0.4	-	0.9	-
	Dry	-	5.2	-	-	**74.7**	**13.6**	-	0.4	-	0.4	-
Empangeni	Fresh	2.0	**38.7**	17.0	5.8	2.3	1.4	-	15.4	8.5	12.9	1.9
	Dry	2.4	**36.7**	10.5	11.7	7.9	0.6		10.1	5.7	11.5	1.8
UZN Botanic Garden	Fresh	4.9	**35.6**	4.9	2.7	10.5	4.5	3.6	**20.5**	3.4	5.3	1.5
	Dry	3.0	**37.8**	3.0	1.9	12.2	2.6	2.1	**23.6**	2.7	4.9	1.6

other parts of South Africa revealed that the percentage composition of 1,8-cineole was higher (50.4%) than that of α- and β-thujone (27.3-60.1%). Viljoen *et al*. [9e] recently reported the percentage composition of α-thujone in the *A. afra* essential oils from Giant Castle to be 0.9-38.6%, from Qwa-qwa 0.1-5.6%, and those from Klipriversberg 15.0-55.6%. This report supports that not all *A. afra* has high thujone contents and, in some of the oils studied, α-thujone was not detected. Our findings reveal that environmental conditions play a significant role in the formation of these compounds. The results obtained in this study also show that plants with high thujone contents are still below the maximum recommended dosage for food and in alcoholic drinks, thereby making the South African material safe for export and consumption. Studies have shown that some South African *A. afra*, depending on location, have high 1,8-cineole and chrysthmone contents, both compounds being of economic value. Chrysthmone has great inhibitory potency against insects, especially weevils and beetles. Furthermore, these oils are also rich in camphor. *A afra* is economically valuable for essential oil production for the pharmaceutical industry (those with 1,8-cineole and chrysthmone content). The value of this essential oil opens the doors to encourage farmers in South Africa to cultivate the plants with low thujone and high 1,8-cineole, camphor and chrysthmone contents for local and commercial purposes.

Experimental

Plant materials: Fresh, mature, wild plants of *A. afra* was collected from the University of Fort Hare Farm, Gqumahshe, Keiskammahoek and Hogsback, all in the Eastern Cape; Philippolis in the Free State; and University of Zululand Botanical Garden (KwaDlangewza) and Anne Hutching's private garden in Empangeni, both in KwaZulu Natal. The samples were authenticated by Mr T Dold of the Selmar Schonland Herbarium, Grahamstown, and Mr MA Ngwenya of Durban Herbarium; voucher specimens were deposited at the Universities of Fort Hare and Zululand Herbaria.

Isolation and analyses of the volatile oils: Fresh mature leaves (300 g) were hydrodistilled for 3 h within 24 h of collection. The remaining plants were air-dried for a week and then 200 g was distilled in an all glass Clevenger apparatus using the British Pharmacopoeia (1980) method [10a]. GC analyses was carried out on a Hewlett Packard Gas Chromatography HP 5890 equipped with FID detector and HB-5 column (60 m x 0.25 mm id), 0.25 μm film thickness and split ratio of 1:25. The oven temperature was programmed from 50^{o}C (after 2 min) to 240^{o}C at 5^{o}C / min and the final temperature was held for 10 min. Injection and detector temperatures were 200^{o}C and 240^{o}C, respectively. The diluted oil (0.2 μL) was injected into the GC. GC-MS analyses of the oils were performed under the experimental conditions as reported earlier [10b]. The oil constituents were identified by comparison of their mass spectra and retention indices with those of standard samples and literature data [10c-10e].

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References

[1] (a) Albert-Puleo M. (**1978**) Mythobotany, pharmacology and chemistry of thujone-containing plants and derivatives. *Economic Botany*, ***32***, 65-74; (b) Kim JO, Kim YS, Lee JH, Kim MN, Rhee SH, Park KY. (**1992**) Antimutagenic effect of the major volatile compounds identified from mugwort (*Artemisia asictianakai*) leaves. *Journal of Korean Society of Food and Nutrition*, ***21***, 308-313; (c) Millet Y, Jouglard J, Steinmetz MD, Tognetti P, Joanny P, Arditti J. (**1981**) Toxicity of some essential plant oils: Clinical and experimental study. *Clinical Toxicology*, ***18***, 1485-1498; (d) Dupont P. (**1995**) Cosmetic compositions for the treatment of corns, and warts comprising plants tinctures and essential oils. (Patent No. Fr. 2710266) [Abstract: CA123-017496X]

[2] (a) Acamovic T, Brooker JD. (**2005**) Biochemistry of plant secondary metabolites and their effect in animals. *Proceeding of the Nutrition Society*, ***64***, 403-412; (b) Watt JM, Breyer-Brandwijk MG. (**1962**) *The Medicinal and Poisonous Plants of Southern Africa*, E & S Livingstone, Edinburgh, pp. 199-202; (c) Graven EH, Webber L, Gardner JB. (**1990**) The development of *Artemisia afra* Jacq. as a new essential oil crop. *Journal of Essential Oil Research*, ***2***, 215-220.

[3] (a) Robert M. (**1990**) *Indigenous Healing Plants*, Southern Book Publisher, Johannesburg, pp 226-227; (b) Graven EH, Deans SG, Svoboda K, Mavi S, Gundidza MG. (**1990**) Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq.. *Flavor and Fragrance Journal*, ***7***, 121-123; (c) van Wky BE, van Oudtshoorn B, Gericke N. (**1997**) *Medicinal Plants of South Africa*, Briza Publications, Pretoria, pp.44-45; (d) Muyima NYO, Zulu G, Bhengu T, Popplewell D. (**2002**) The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. *Flavor Fragrance Journal*, ***17***, 258-266; (e) Guenther A, Zimmerman P, Wildermuth M. (**1994**) Natural volatile compound emission rate estimates for US woodland landscape. *Atmospheric Environment*, ***28***, 1197-1210; (f) Castillo JD, Anderson M, Rubottom GM. (**1975**) Marijuana, absinthe and the central nervous system. *Nature*, ***253***, 365-366; (g) Vogt DD, Montagne M, (**1982**) Absinthe: Behind the emerald mask. *International Journal of Addiction*, ***17***, 1015-1029; (h) McGraw LJ, Jagar AK, van Staden JV. (**2000**) Antibacterial, anthelmintic and anti-amoebic activity of South African medicinal plants. *Journal of Ethnopharmacology*, ***72***, 247-263; (i) Piccaglia R, Marotti M, Giovanelli E, Deans SG, Eaglesham E. (**1993**) Antimicrobial and antioxidatant properties of Mediterranean aromatic plants. *Industrial Crops Production*, ***2***, 47-50.

[4] Dean SG, Ritchie G. (**1987**) Antibacterial properties of plant essential oil. *International Journal of Food Microbiology*, ***5***, 165-180.

[5] Anon. (**1989**) *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, USA, pp.1479-1480.

[6] Bonkovsky HL, Cable EE, Cable JW, Donohue SE, White EC, Greene YJ, Lambrecht RW, Srivastava KK, Arnold WN. (**1992**) Porphyrogenic properties of the terpenes camphor, pinene and thujone. *Biochemical Pharmacology*, ***43***, 2359-2368.

[7] Galli CL, Galli G, Tragni E, Caruso D. (**1984**) Quantitative analyses of alpha, beta-thujone, pulegone, safole, coumarin and beta-asarone in alcoholic beverages by selected-ion monitoring. *Journal of Applied Toxicology*, ***4***, 273-276.

[8] FDA (**1997**) *Flavouring Agents and Related Substance*. Title 21, Part 172.510, US Code Federal Regulation, pp. 49-52.

[9] (a) Tisserand R, Balacs T. (**1995**) *Essential Oil Safety: A guide for health care professionals*, Churchill Livingstone, Edinburgh, pp. 199-200; (b) Lawrence BM. (**1993**) *A Planning Scheme to Evaluate New Aromatic Plants for the Flavor and Fragrance Industries*, Janick J, Simon JE. (Eds.). New Crops. Wiley, New York. pp. 620-627; (c) Douglas M, Heyes J, Smallfield B. (**2005**) Herbs, spices and essential oils: post-harvest operations in developing countries. Publishing Management Service, Information Division, FAO. Viale delle Terme di Caracalla, 00100 Rome, Italy; (d) Sticher O. (**1977**) *Plant Mono-, Di-, and Sesquiterpenoids with Pharmacological or Theurapeutical Activity. In: New Natural Products and Plant Drug with Pharmacological Biological or Therapeutically Activity*, Wagner H, Wolff P. (Eds), Springer Venlag, Berlin, Heidelberg, pp.138-178; (e) Viljoen AM, van Vuuren SF, Gwebu T, Demirci B, Husnu K, Baser C. (**2006**) The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq.) essential oil. *Journal of Essential Oil Research*, ***18***, 19-25.

[10] (a) British Pharmacopoeia (**1980**) ***11***, P. A. 109, HMSO; (b) Anthony J. Afolayan and Olayinka T. Asekun (**2008**) Comparative Study of the Chemical Profiles of the Essential Oils of Ripe and Rotten Fruits of *Citrus aurantifolia* Swingle. *Natural Product Communications*, ***3***, 1133-1136; (c) Adams RP. (**1989**) *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. Academic Press, New York; (d) Joulain D, Koenig WA. (**1998**) *The Atlas of Spectra Data of Sesquiterpenes Hydrocarbons.* E.B.-Verlag: Hamburg; (e) ESO 2000. (**1999**) The complete database of essential oil. B.A.C.I.S. The Netherlands.