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The ethnobotany and major essential oil compounds of anise root (*Annesorhiza* species, Apiaceae)

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ABSTRACT

A comprehensive review of indigenous knowledge about a traditional Cape, San and Khoi food item called anise root or *anyswortel* (genus *Annesorhiza*) is presented. The first accurate record was by Thunberg in 1772 at the Berg River near Vlermuisdrift (now Bridgetown) in the Western Cape and applied to *A. grandiflora*. Four species have recorded food uses as root vegetables (*A. grandiflora*, *A. macrocarpa*, *A. nuda* and an as yet undescribed species from Namaqualand) and two species (*A. nuda* and *A. flagellifolia*) as leaf vegetables. Essential oils of this genus were studied for the first time. Hydro-distilled oil from the fleshy roots, leaves and fruits of three species recorded as edible (*A. grandiflora, A. macrocarpa* and *A. nuda*) were chemically characterized using GC–MS and NMR spectroscopy. Noteworthy differences were found between plant parts as well as species. Estragole and a small amount of anethole occur in the roots of *A. nuda* (but not in any of the other two species). The major components of *A. grandiflora* fruits were dillapiole, nothoapiole and limonene, while elemicin was identified in the roots of the same species. Fruits of *A. macrocarpa* contained methyleugenol, the leaves ethyl-benzenes and α-terpineol, whereas the roots yielded nerolidol and an unidentified sesquiterpene. In contrast, leaves and roots of *A. nuda* were quite similar with the exception of the occurrence of estragole and anethole in roots only and not in the leaves. This partially explains the reported anise flavor of the edible roots of this species. The main compound of both roots and leaves of *A. nuda* was butan-2-yl (2*Z*)-3-(methylsulfanyl) prop-2-enoate, a new trans-β-methylthioacrylate derivative. This α, β-unsaturated methylsulfanyl ester, colloquially named (−)-nudaic ester, was characterized and assigned using 2D-NMR spectroscopy. The pronounced chemical differences between the examined species require further chemosystematic studies and the differences between roots, leaves and fruits may reflect the distinct vegetative (winter) and reproductive (summer) growth phases in these summer-deciduous plants.

1. Introduction

Annesorhiza Cham. & Schltdl. (Apiaceae) is an anomalous and taxonomically poorly understood genus consisting of at least 22 species, all endemic to southern Africa (Van Wyk et al., 2013; Magee, 2015). The name *Annesorhiza* is a Latinization of the common names anise root or '*anyswortel*' (Afrikaans), which highlights the distinct anise-like aroma of the roots of *A. nuda* (Ait.) B.L.Burtt. This species is the most common and widely distributed member of the genus (Van Wyk and Gericke, 2000, 2018; Tilney and Van Wyk, 2001; Van Wyk et al., 2013). The fleshy roots of *A. nuda, A.grandiflora* (Thunb.) Hiroe and *A. macrocarpa* Eckl. & Zeyh. were formerly used as traditional food items in the Cape region of South Africa (Van Wyk, 2011; Van Wyk and Gericke, 2018) but they are currently only used to a limited extent and have not yet been commercialized.

Annesorhiza species are unusual in having hysteranthous leaves (i.e., the leaves appear after flowering). Flowering occurs in the southern hemisphere summer (mostly January, the dry period) and leaves emerge in autumn, when the winter rains start to fall. As a result, three field visits are necessary to collect complete material (i.e., roots with leaves, then flowers and later fruits). Flowering, fruiting and leaf-bearing herbarium specimens from different species are sometimes wrongly thought to be from a single species. Other unusual features include the annual replacement of the fleshy roots, the heteromericarpic fruits (in some species) and presence of scattered druses in mature fruits (Van Wyk et al., 2013). Ongoing studies of the anatomy and morphology of species of *Annesorhiza* and the closely related *Chamarea* have resulted in several taxonomic papers, including the description of new species (Tilney and Van Wyk, 2001; Magee and Manning, 2010; Magee et al., 2011; Van Wyk and Tilney, 2011; Magee, 2015). Unraveling the relationships and circumscriptions of *Annesorhiza* and *Chamarea* species is one of the remaining challenges of systematic botany in the Cape region. Many species are highly localized; herbarium collections are incomplete and often insufficient for identifying and describing new taxa. The magnitude

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Abbreviations

General abbreviations

CC	column chromatography
$CDCL_3$	chloroform
GC–MS	gas chromatography–mass spectrometry
GCT	accurate mass time-of-flight (TOF) mass spectrometer coupled to a gas chromatograph
GC-TOF MS	gas chromatography time-of-flight mass spectrometry
HR-ESI-MS	high-resolution electrospray ionization mass spectrometry
IR	infrared
J.S.Afr.Bot.	Journal of South African Botany (the predecessor of the South African Journal of Botany)
JRAU	international acronym for the Herbarium of the University of Johannesburg
MeOH	methanol
NMR	Nuclear Magnetic Resonance spectroscopy.

Abbreviations for the origins of anecdotes from Smith (1966)

Ce	Ceres
Geo	George
Hdp	Humansdorp
Mal	Malmesbury
Prl	Paarl
SE	Southeastern Cape
Suth	Sutherland
SW	Southwestern Cape
Tul	Tulbagh
VRdp	Vanrhynsdorp

of the challenge can be judged by the fact that 11 of the 22 known species of *Annesorhiza* have been described since 1991, while the number of known species in *Chamarea* increased from two in 1990 to five in 1991, with at least a further seven species yet to be described.

This study was aimed at answering three questions:

(1) Which species of *Annesorhiza* were used as traditional Khoi and San food items and what traditional knowledge about the names and uses of *Annesorhiza* species has been recorded? A comprehensive ethnobotanical review is presented.
(2) What is the chemical basis for the distinct anise-like scent and flavor of the roots of *A. nuda*, after which the genus and tribe were named? In order to answer this seemingly trivial question, we performed a preliminary study of the essential oil of roots and leaves of this species (and two others, without an anise smell) to test the assumption that anethole and/or estragole would be present.
(3) What is the feasibility of studying essential oils from a chemosystematic perspective and how do the results relate to the tribal placement, generic affinities and basally divergent position of *Annesorhiza* within the subfamily Apioideae? Our expectation was that the genus could yield interesting essential oil compounds, given its isolated phylogenetic position in the (otherwise predominantly woody) protoapioids and also its unusual morphology and phenology.

2. Materials and methods

2.1. General experimental procedures

Optical rotation was calculated on a Polartronic H532 polarimeter. The IR spectrum was recorded on a Perkin-Elmer FT-IR system (Spectrum BX). Proton and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz NMR (in $CDCl_3$), using TMS as internal standard. GC–MS analyses were performed on a GC–MS 2010 (Shimadzu) system. HR-ESI-MS spectra were done at the Mass Spectrometry Unit, Central Analytical Facility, University of Stellenbosch using a Waters Micromass GCT Premier Mass Spectrometer (GC-TOF MS).

2.2. Plant material

Fresh fruit, leaf, stem and root samples of *Annesorhiza grandiflora*, *A. macrocarpa* and *A. nuda* were collected from Western Cape Province, South Africa. A collecting permit for chemosystematic studies on Apiaceae, of which this study forms a part, was issued by CapeNature to the first author. Voucher specimens were deposited in the herbarium of the University of Johannesburg (JRAU): *Annesorhiza grandiflora – Sobiyi & Magee 8* (locality Saldanha; roots and fruits collected Nov 2015); *A. macrocarpa* – Sobiyi & Van Wyk 39 (locality Dwarskersbos, fruits collected Feb 2016, roots and leaves collected Sept 2015); *A. nuda – Sobiyi & Van Wyk 44* (locality Paradyskloof, Stellenbosch; roots and leaves collected Sept 2015, peduncles, flowers and fruits collected Feb 2017). The species were identified by the third and fourth authors, who both have many years of experience of the taxonomy of African Apiaceae and *Annesorhiza* in particular – see Van Wyk et al., 2013). Plants materials were carefully air-dried in a fume hood.

2.3. Hydro-distillation, extraction and isolation

The plant samples were subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. Essential oil components were isolated by flash chromatography using silica gel 60 (Sigma–Aldrich) as stationary phase and a mobile phase of cyclohexane for apolar constituents, followed by 5% ethyl acetate in cyclohexane to elute slightly more polar components. CC afforded 25 mg of compound **1**. Hexane extracts were also made of small fruit samples from six different individual plants of *A. nuda* (from the same population as the bulk samples).

2.4. GC–MS analysis

Routine chemical analysis of *Annesorhiza* essential oils was performed on a GC–MS 2010 (Shimadzu) system with detector interface at 250 °C; ion source 200, injector temperature at 200 °C; carrier gas helium at 1 ml min^{-1}; 1 µl injections with a split ratio (1:20) and an autosampler. Column OV-1 (WCOT), ramp: 60 °C (no hold), 5 °C min^{-1} then at 280 °C, hold for 5 min. Identification of compounds were made by comparing the mass spectra and retention indices (calculated relative to *n*-alkanes) with the NIST library and Adams (2007). Analyses of hexane extracts of the fruits from six plants of *A. nuda* (results not included in Table 1) showed very similar compositions to that of the hydro-distilled oil from the bulk fruit sample of *A. nuda*. HR-ESI-MS spectra were recorded on a Waters Micromass GCT system. Gas injector temperature at 280 °C; carrier gas helium, at 1 ml min^{-1}; 1 µl injections with a split ratio of 1:10. The column was a Restek Rxi-5sil MS Wintegra guard (15 m, 0.25 mm ID, 0.25 µm film thickness). Column ramp: 60 °C (no hold), 5 °C min^{-1} for 20 min.

2.5. (−)-Nudaic ester (**1**)

Data for (−)-nudaic ester: Butan-2-yl (2*Z*)-3-(methylsulfanyl) prop-2-enoate (**1**); Clear oil; $[\alpha]_D^{23}$ −61 (c 0.2, MeOH). IR_{Vmax} (neat): 3280, 3190, 3116, 3022, 1684, 1604, 1572, 1510, 1437, 1318, 1292, 1215, 1176, 1105, 1090 cm^{-1}; ^1H and ^{13}C NMR data (see Table 2); HREIMS *m/z* 174.0715 (GCMS-TOF, EI, M+; calcd for $C_8H_{14}O_2S$, 174.0715); *m/z* 174 (M^+ 27), 127 (2), 119 (6), 118 (44), 103 (58), 102 (9), 101 (100), 100 (33), 87 (5), 74 (11), 73 (28), 72 (19), 58 (14), 57 (11), 55 (6).

Table 1
Yields and chemical composition (%) of essential oils from *Annesorhiza* species as determined by GC/MS retention indices and NMR (*).

Species:			*A. grandiflora*		*A. macrocarpa*			*A. nuda*				
Total yield (% dry wt):			4.90	0.04	0.98	0.12	0.07	0.06	0.18	1.4	1.7	1.5
Plant part(s) used:			Fr	Ro	Fr	Le & Pe	Ro	Le	Ro	Fr	Pe	Fl
	CAI	LAI										
α-pinene	927	932	0.9	–	2.9	–	–	–	–	1.3	7.3	2.3
β-Pinene	971	974	3.6	–	11.8	–	–	–	–	1.5	18.3	4.6
β-Myrcene	980	981	0.3	–	–	–	–	–	–	9.8	24.9	18.3
p-Cymene	1022	1022	–	–	–	–	–	–	3.7	3.7	–	–
Limonene	1023	1024	22.7	–	9.2	–	–	4.9	–	41.5	7.8	3.4
Z-β-Ocimene	1030	1032	–	–	–	–	–	–	6.7	27.9	33.9	41.2
p-Diethylbenzene	1053	1053	–	–	–	8.5	–	–	–	–	–	–
γ-Terpinene	1054	1054	–	–	–	–	–	–	1.5	1.1	2.1	19.4
4-Ethyl-m-xylene	1074	1074	–	–	–	7.4	–	–	–	–	–	–
2-Ethyl-p-xylene	1080	1080	–	–	–	12.1	–	–	–	–	–	–
Linalool	1097	1100	–	–	–	2.4	–	–	–	–	–	–
Isodurene	1113	1113	–	–	–	2.1	–	–	–	–	–	–
Durene	1116	1116	–	–	–	8.4	–	–	–	–	–	–
trans-pinocarveol	1140	1140	–	–	1.4	0.8	–	–	–	–	–	–
trans-verbenol	1145	1145	–	–	0.5	1.0	–	–	–	–	–	–
Ester (C10 – n.d.)	1160	–	–	26.3	–	–	–	–	–	–	–	–
Pinocarvone	1165	1165	–	–	1.4	–	–	–	–	–	–	–
Iso-verbanol	1172	1176	–	16.9	–	–	–	–	–	–	–	–
Terpinen-4-ol	1180	1174	–	–	–	1.1	–	–	–	–	–	–
α-Terpineol	1195	1197	–	–	–	19.2	–	–	–	–	–	–
Estragole*	**1197**	**1196**	–	–	–	–	–	–	**11.5**	–	–	–
Myrtenal	1200	1196	–	–	3.3	–	–	–	–	–	–	–
E-carveol	1229	1217	–	–	0.7	–	–	–	–	–	–	–
Carvenone	1250	1255	–	–	1.1	–	–	–	–	–	–	–
E*-anethole	**1285**	**1282**	–	–	–	–	–	–	**0.9**	–	–	–
n.d	1308	–	–	10.7	–	–	–	–	–	–	–	–
(−)-Nudaic ester*	1335	2D*	–	–	–	–	–	45.7	46.5	9.5	4.2	4.5
1,8-Octanediol	1343	1339	–	8.0	–	–	–	–	–	–	–	–
α-Cubebene	1347	1345	–	–	–	1.1	–	–	–	–	–	–
Geranyl acetate	1379	1379	–	–	–	3.3	–	–	–	–	–	–
α-Bourbonene	1384	1383	–	–	–	0.9	–	–	–	–	–	–
β-Cubebene	1388	1387	–	–	–	3.8	–	–	–	–	–	–
Methyleugenol*	**1411**	**1410**	–	–	**62.9**	–	–	–	–	–	–	–
E-β-Caryophyllene	1420	1417	1.3	–	–	3.6	–	–	–	–	–	–
Bergamotene	1432	1432	–	–	–	–	0.2	–	–	–	–	–
Unknown (n.d.)	1432	–	–	1.7	–	–	–	–	–	–	–	–
β-Farnesene (*E*)	1452	1454	–	–	–	–	0.2	–	–	–	–	–
α-Caryophyllene	1456	1464	0.3	–	–	–	–	–	2.4	–	–	–
Unknown alkane (n.d.)	1456	–	–	–	–	9.3	–	–	–	–	–	–
Unknown ester (n.d.)	1475	–	–	1.7	–	–	–	–	–	–	–	–
Germacrene D	1481	1484	–	–	–	1.0	–	–	0.5	–	–	–
Phenethyl isovalerate	1489	1488	–	–	–	–	–	4.1	3.7	–	–	–
Phenyl ethyl 3-methyl butanoate	1491	1490	–	–	–	–	–	14.9	–	–	–	–
Bicyclogermacrene	1495	1500	–	–	–	1.4	–	–	2.4	–	–	–
β-Bisabolene	1507	1505	–	–	–	–	1.2	6.6	1.1	–	–	–
Durohydroquinone	1513	–	–	–	–	5.2	–	–	–	–	–	–
Citronellyl butanoate	1525	1530	–	6.8	–	–	–	–	–	–	–	–
Phenylethyl tiglate	1544	1555	–	–	–	–	–	5.5	2.2	–	–	–
Elemol	1547	1548	–	–	–	–	0.3	–	–	–	–	–
Elemicin	**1549**	**1555**	–	**8.2**	–	–	–	–	–	–	–	–
Isoelemicin	**1556**	**1568**	–	–	**3.4**	–	–	–	–	–	–	–
E-nerolidol	1559	1561	–	–	–	–	25.7	–	–	–	–	–
Spathulenol	1577	1577	–	–	0.2	1.5	–	–	1.5	–	–	–
Caryophyllene oxide	1582	1582	–	–	0.3	1.7	–	–	0.1	–	–	–
Humulene epoxide II	1610	1610	–	–	0.5	1.1	–	–	0.4	–	–	–
Dillapiole*	**1617**	**1620**	**16.5**	–	–	–	–	–	–	–	–	–
Epiglobulol	1625	1629	–	–	–	–	1.7	–	–	–	–	–
β-Eudesmol	1654	1649	–	4.4	–	–	2.2	–	–	–	–	–
Pogostol	1657	1656	–	–	–	–	0.6	–	–	–	–	–
Unknown (n.d.)	1686	–	–	–	–	–	2.7	–	–	–	–	–
Nothoapiole*	**1760**	**1759**	**52.9**	–	–	–	–	–	–	–	–	–
Nerolidyl isobutyrate	1783	1783	–	1.2	–	–	–	–	–	–	–	–
Butyl dodecanoate	1786	1786	–	–	–	–	5.1	–	–	–	–	–
Phenylethyl octanoate	1840	1846	–	–	–	–	–	–	1.2	–	–	–
Unknown (n.d.)	1887	1913	–	1.7	–	–	–	–	9.8	–	–	–
Unknown (n.d.)	1904	–	–	1.7	–	–	–	–	–	–	–	–
Totarene	1927	1922	–	–	–	–	1.0	–	–	–	–	–
Cyclohexadecanolide	1930	1933	–	–	–	–	2.8	–	–	–	–	–
Sesquiterpene (n.d.)	1956	–	–	–	–	–	22.7	–	–	–	–	–
Falcarinol	2033	2035	–	–	–	–	1.1	–	–	–	–	–
n-Octadecanol	2078	2077	–	–	–	–	12.7	–	–	–	–	–
Total			**98.5**	**78.6**	**99.6**	**96.9**	**80.2**	**81.7**	**96.1**	**96.3**	**98.5**	**93.7**

Notes: Phenylpropanoids are shown in bold. Abbreviations: n.d. = not determined (unidentified, due to sample limitations); CAI = calculated experimental retention indices relative to C9–C30 alkanes on the HP-5MS; LAI = Literature retention indices (Linstrom and Mallard, 2005; Adams, 2007); (*) = identity confirmed by NMR; plant parts abbreviated as Fr = fruits, Ro = roots, Le = leaves and Le & Pe = leaves and peduncles, Pe = peduncles, Fl = flowers.

3. Results and discussion

3.1. Ethnobotany

According to Smith (1966), the first record that probably refers to an *Annesorhiza* species can be ascribed to the Dutch Governor Jan van Riebeeck. The following paragraph from "Common Names of South African Plants" by Smith (1966), p. 63, provides a good summary of the recorded ethnobotanical history of *Annesorhiza* species, which is further elaborated below.

"**anyswortel.** Species of *Annesorrhiza* (sic!), the tubers of which have a pleasant taste suggestive of anise (Afr.: anys). Critical interpretation of olden-day records shows that the roots of at least *A. montana* and probably also *A. macrocarpa* entered into the domestic or culinary economy of the Hottentots before the advent of the European and that they took the practice over from the Bushmen, together with the tubers of *Chamarea capensis* (VINKELWORTEL). In 1652, van Riebeeck found a local Hottentot tribe looking for "worteljens" which he later described as of a somewhat aromatic flavour, tasting something like aniseed and parsnip. The probabilities are that the name ANYSWORTEL gained currency for one or more species of *Annesorrhiza* species during van Riebeeck's time, though the name was only mentioned some 45 years later. Harvey (1862) refers to herbs known by the colonial name ANYSWORTEL, *A. altiscapa* (Suth), the roots of which were known to the early Hottentots, as Burchell noted in 1811. He also pointed out that the ANYSWORTEL of the Roggeveld is entirely different from the ANYSWORTEL of the Zwartland (Malmesbury). *A. capensis** (Geo to SE), see SOETANYSWORTEL; *A. hirsuta* (Cape to Pik), see SUURANYSWORTEL. Thunberg (1772) recorded that "The anyswortel was eaten roasted on the embers, or boiled in milk, or else stewed with meat and tasted well". Lichtenstein (1803) also mentioned edible quantities of the "Anijswortelen". *A. macrocarpa* (Cape to Hdp), see WILDE-ANYSWORTEL; *A. montana** (Cape to Geo), see BERGANYSWORTEL; A. villosa (Tul. to Ce)." "*These two species, according to R. S. Adamson, J. S. Afr. Bot. IV: 61-63 are conspecific."

Smith (1966), p.426: "**soetanyswortel**. *Annesorrhiza capensis* (Geo). See anyswortel." The reference to George as the source of the name/anecdote is curious, as the distribution of the species extend to Still Bay near Mossel Bay but not as far east as George (Tilney and Van Wyk, 2001).

Smith (1966), p.446: "**suuranyswortel**. *Annesorrhiza hirsuta* (Cal to Bdp). See anyswortel, berganyswortel."

Smith (1966), p.85: "**berganyswortel**. *Annesorrhiza hirsuta* (SW), see suuranyswortel. *A. montana* (Cape to Prl to Mal), see wilde-anyswortel. … E. & Z. Cape to M. C. 1830. "Incolis; Berg Anyswortel" Enum. 344. Harvey, Cape c. 1838 "Root … called by the Colonists Berg-Anyswortel" Fl. Cap. II: 545.

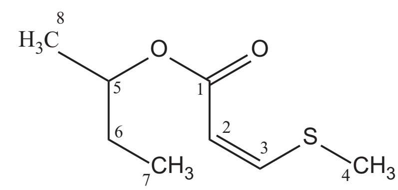
The recording of the common name *berganyswortel* for *A. grandiflora* (syn. *A. hirsuta*) therefore dates back to 1830 (Ecklon and Zeyher) and 1838 (Harvey).

Smith (1966), p.503: "**wilde-anys (wortel)**. *Annesorrhiza montana* (VRdp), see berganyswortel."

Both *A. altiscapa* Schltr. and *A. grandiflora* occur in the Sutherland region (Roggeveld), but Burchell in 1811 probably referred to the former species, which does not occur in the Swartland and which is quite different from the latter (Burchell, 1822-24). There are no known use-records for *A. altiscapa* as a food item but it has been used in traditional medicine in the Vanrhynsdorp and Nieuwoudtville districts (where it is known as *boklamvinkel*). Archer (1994) recorded the names "*anyswortel*, *boklamvinkel* and *bokvingel*" for this species and reported that the roots are used as a source of yeast or ferment when brewing honey beer (mead): "Roots are pounded and dried. Water is poured on the dried powder and replaced every two days until the fluid is no longer so bitter. Then honey and water are added to make a very potent beer. All beers are regarded as medicinal…".

Table 2
Proton and carbon shifts for butan-2-yl-(2*Z*)-3-(methylsulfanyl)-prop-2-enoate **(1)** ($CDCl_3$).

C No.	13C	1H	COESY
1	166.4	–	–
2	113.6	1Hd 8.82 (10.2)	3
3	151.3	1Hd 7.02 (10.2)	2
4	19.2	3Hs 2.39	–
5	72.1	1Hhex 4.91 (6.32)	6, 8
6	28.9	2Hdt (1.5–1.7)	5, 7
7	9.7	3Ht 0.9 (7.49)	6
8	19.6	3Hd 1.24 (6.3)	5



(**1**)

The earliest accurate record of anise root as a food item or vegetable is that of Thunberg in 1772 (Forbes, 1986). With the help and companionship of Claus Riding (a land surveyor of Ceres in the Western Cape with an interest in, and extensive knowledge of old roads and routes), one of us (B-EvW) retraced the exact route that Thunberg followed on his journey to the Hantam and Roggeveld in 1772. The identity of the species reported by Thunberg on 25 September 1772, when he crossed the Berg River via the ferry at "Vliermuys Drift" (Vledermuisdrift, now Bridgetown) was correctly identified by Dr. John Rourke (who wrote the following footnote): "The fleshy parsnip-like edible root of various species of *Annesorrhiza* (Apiaceae), in this instance probably *Annesorrhiza hirsuta*." *Annesorhiza hirsuta* is now a synonym of *A. grandiflora* (see Tilney and Van Wyk, 2001). The roots of this species is shown in Fig. 1 (and also in Van Wyk and Gericke, 2018, p. 93). The photographs were taken at Bridgetown, where a relatively large population still grows in close proximity to the site of the original ferry crossing. According to Thunberg, "The root of the anise (anyswortel) was eaten here roasted, and tasted well… The farmers sometimes make their slaves dig up a large quantity of them which they sell in town." (see Skead, 2009, p. 33).

In 1774, at Daunis [Downes], 22 km southeast of Calvinia, Thunberg reported that "Moorwortel [moerwortel], an umbelliferous plant, was also spoken of here as a root from which, with water and honey, the Hottentots prepare an intoxicating liquor." (Skead, 2009, p. 167). The editor of Skead (2009) suggested that this may have been a species of *Annesorhiza* because the well-known moerwortel (*Glia prolifera* Thunb.) does not occur further north than Tulbagh. Two facts indicate that *Glia prolifera* was indeed the plant referred to. Firstly, Thunberg specifically stated that the plant was only spoken about (i.e., he did not see it locally); secondly, the town of Tulbagh was the administrative and religious centre for the newly settled settlers of the Calvinia region at that time, so that there were regular visits to Tulbagh (e.g., for communion, baptisms, weddings and funerals).

Lichtenstein (1928-30) also reported the use of an *Annesorhiza* species (probably *A. macrocarpa*, but it may also have been *A. hirsuta* – the former is common in sandy places, while the latter is common on granite outcrops where is soil is typically clayey). At Teefontein (ca. 50 km NW of Malmesbury), Lichtenstein reported the following on 12 October 1803: "We here regaled with a genuine African dish, the anis root (footnote: Probably *Sium filifolium* of Thunberg [added by the editor: more probably *Annesorhiza macrocarpa*, which is common here] which has a

Fig. 1. The edible, fleshy roots (anise root, "*anyswortel*") of three *Annesorhiza* species for which ethnobotanical information have been recorded. A, *Annesorhiza nuda* (near Stellenbosch); B. *A. grandiflora* (near Vlermuisdrift, the type locality); C, *A. macrocarpa* (near Leipoldtville). Photographs by B.-E. van Wyk.

strong spicy taste and when cooked seems extremely nourishing. It is perfection at this time of the year." (Skead, 2009, p. 38). Lichtenstein was correct in his interpretation of the phenology – at the end of spring and early summer, the young roots of *Annesorhiza* species have already sufficiently developed to be harvested; later in summer they become fibrous and much less suitable for food use. This characteristic shrinking of the old root(s) from the previous season and the development of the new root is characteristic for the genera *Annesorhiza* and *Chamarea*. Referring to local *Chamarea* species, Mr. Willem ("Blikkies") Steenkamp of Nieuwoudtville explained this "root replacement" in a curious and humorous way: "the roots are edible in November, when the female has separated from (or left behind) the male" ("*wanneer die wyfie die mannetjie gelos het*").

Pappe (1862) listed anise root, as *Annesorhiza capensis* Cham. & Schltdl. (now *A. nuda*) amongst several food plants that were added to the second edition of the "Silva Capensis". According to him, "The turnip-like root of this umbelliferous plant is very nutritious, and has been used for many years past by the natives and colonists, who call it *Anise-root* (Anys-wortel). It is much improved with by cultivation, loses its acrid taste, and becomes a very good vegetable."

The well-known South African poet and culinary expert, C. Louis Leipoldt, included anise root in his books on Cape cookery, the one dating from 1933 (Leipoldt, 1978) and the other from 1947 (Leipoldt, 1976). In the first book (from 1933), he stated that "*Die groter wortelsoorte, soos kameroo* [*Fockea* species] *en baroe* [*Cyphia* species] *en finkel* [*Chamarea* species], *aard na patats en kan op dieselfde manier berei word. Dit is besonder geskik om konfyt van te maak, veral finkel en wilde anyswortel* [*Annesorhiza* species]. [The larger root types such as kameroo and baroe and finkel, resemble sweet potatoes and can be prepared in the same way. It is especially suited for making preserve, especially finkel and wilde anyswortel.]. In the second book, the following details were recorded: "The turgid underground rootstocks of *wild fennel* [*Chamarea capensis* and other species] and *wild anise* [*Annesorhiza nuda* and other species] can be eaten raw for they are fairly sweet, with a pungent, aromatic flavour. By themselves they make an indifferent dish when boiled or stewed, for their flavour is far too intense and does not seem to be influenced by spicing. They make an excellent preserve which, like that made from *baroe* [*Cyphia* species], is characterized by a delicate crispness, a tinge of acidity, and an agreeable aromatic flavour."

In their book on southern African edible plants, Fox and Norwood Young (1982) recorded the following: "Thunberg reported that the root of the anyswortel [*Annesorhiza capensis* (sic!)] was eaten by the early colonists, roasted in the embers or boiled in milk or else stewed in milk. Pappe (1862) states that this turnip- or parsnip-like root has been used for many years as food by whites and blacks. It is much improved by cultivation, loses its acrid taste and becomes a very good vegetable."

Under the names "*Annesorrhiza*-spp. (sic!) (*anyswortel, soetanyswortel, vlakte-anys*)" Rood (1994) in her book "Kos uit die veldkombuis" ["Food from the veld kitchen"] reported that "… *Die wortels het 'n aangename smaak soos anys. …*"*Thunberg het in 1772 aangeteken dat die anyswortel geëet kan word. Dit kan oor die kole gebraai, in melk gekook, of saam met vleis gestowe en geëet word. Mev. Laubscher van Cloeteskraal, Vredenburg, stem hiermee saam.*" [The roots have a pleasant taste similar to anise. Thunberg recorded in 1772 that the anise root can be eaten. It can be roasted over the coals, boiled in milk or braised with meat and eaten. Mrs. Laubscher of Cloeteskraal, Vredenburg agrees with this.]

Van Wyk and Van Wyk and Gericke (2000) included the following entry under *A. macrocarpa*: "*Annesorhiza macrocarpa* has numerous fleshy roots that have a distinctive anise-like flavor, hence the common name *anyswortel*. These roots were likely to have been used as a vegetable by the Khoi and San from the earliest times, although the earliest documented use may date back to 1652. In 1772, Thunberg recorded that "The anyswortel was eaten roasted in the embers and boiled in milk, or else stewed with meat and tasted well (Smith, 1966). Pappe also reported it to be used as a food by people in the Cape, and that on

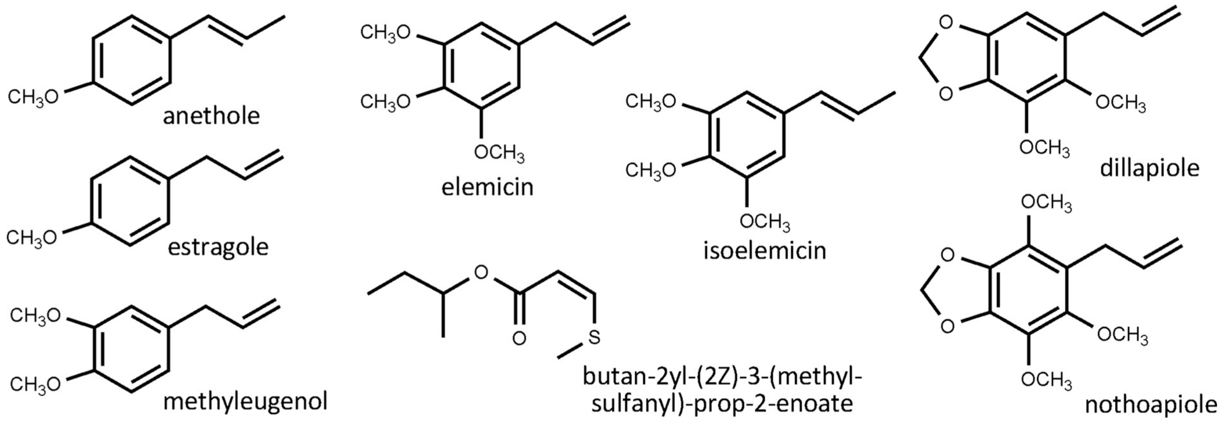


Fig. 2. Chemical structures of phenylpropanoids and a novel α,β-unsaturated methylsulfanyl ester, here called (−)-nudaic ester, identified in essential oils of *Annesorhiza grandiflora*, *A. macrocarpa* and *A. nuda*.

cultivation it loses its acrid taste and becomes a palatable vegetable. *Annesorhiza nuda* (previously known as *A. capensis*) was used in the same way. The authors tried *A. macrocarpa* cooked in milk, but the taste was bitter and resinous, although the texture was soft and pleasing. There may be a commercial opportunity to domesticate *A. macrocarpa* again for speciality restaurants catering for the tourist trade." In the revised edition of the book, Van Wyk and Van Wyk and Gericke (2018) added the following note after the Thunberg citation: "The anecdote of Thunberg applies to *Annesorhiza grandiflora*, which produces a bunch of thick white roots resembling medium-sized carrots in shape."

The whole plant, as well as the shoots and leaves of *Annesorhiza flagellifolia* Burtt Davy are used as spinach in eSwatini (Swaziland) (Dlamini, 1981; Ogle and Grivetti, 1985; Peters et al. (1992). The Swati common name for this species is *sibhadze*.

De Vynck et al. (2016) recorded the use of both the roots and leaves of *A. nuda* as food items in the Still Bay area of the southern Cape. The roots were reported to be eaten as a vegetable, while the leaf is chewed fresh for its liquorice taste.

The most recent addition to the ethnobotanical record for *Annesorhiza* species is that of an as yet undescribed species that is a popular food source in the Steinkopf district in Namaqualand (Nortje and Van Wyk, 2019). This means that four species (*A. grandiflora, A. macrocarpa, A. nuda* and *A. sp. nov.*) have been recorded as root vegetables (Fig. 1), two species (*A. nuda* and *A. flagellifolia*) as leaf vegetables and one (*A. altiscapa*) as yeast or ferment for making honey beer (and as traditional medicine). Given the current interest in foraging and indigenous foods, it may be worthwhile to investigate *Annesorhiza* species as potential new crops and indigenous vegetables for niche markets.

3.2. Volatile compounds

Ten samples of roots, leaves, peduncles, flowers and fruits of *A. nuda*, *A. grandiflora* and *A. macrocarpa* (Table 1) were hydro-distilled in a Clevenger-type apparatus for 3 h. The yields of essential oils obtained (% dry wt) were generally low: 0.04–0.18% in roots and 0.06–0.12% in leaves but considerably higher (0.98–4.9%) in fruits. A summary of the compounds identified in the essential oils from different plant parts is shown in Table 1. The oils generally contained phenylpropanoids as main constituents, together with some monoterpenoids. The identities of anethole, dillapiole, estragole, methyleugenol and nothoapiole were confirmed by matching ^{13}C NMR spectra to published values (Okunade and Olaifa, 1987; Benevides et al., 1999; Choudhary et al., 1999).

The presence of estragole (11.5%) and a small amount of anethole (in the roots of *A. nuda*) seems to clarify the origin of both the vernacular and scientific names of this species (i.e., anise root and *Annesorhiza*). However, the low levels of these compounds are unexpected and hard to explain (estragole gives a basil-like, rather than an anise-like flavor). It was also surprising to find that estragole and anethole are restricted to the roots of *A. nuda* and that they are apparently absent from all other plant parts (including solvent extracts of *A. nuda* fruit samples from six different individual plants), as well as from all the other *Annesorhiza* species investigated.

The main compounds in fruits of *A. grandiflora* were nothoapiole (52.9%), limonene (22.7%), and dillapiole (16.5%). In the roots of the same species, an unknown C10 ester (26.3%), iso-verbanol (16.9%) and epidolichodial (10.7%) were the major constituents, with elemicin (8.2%) as the only phenylpropanoid. Several minor compounds were also present to make up a total of 98.5% of fruit oil and 89.3% of root oil. Nothoapiole is a relatively uncommon essential oil component but nevertheless has been reported in *Carum montanum* (Coss. & Durieu) Benth. & Hook.f. ex Arcang., *Bunium bulbocastanum* L [as *Carum bulbocastanum* (L.) Koch.], *Pimpinella saxifraga* L. [as *Carum nigrum* (Willd.) Baill.] and *Pleurospermum angelicoides* (Wall. ex DC.) Benth. ex C.B.Clarke (Singh et al., 2006; Laouer et al., 2009; Kapoor et al., 2010; Mathela et al., 2015). Dillapiole has been reported as one of the major components in *Astydamia* and *Molopospermum* (Pérez-Alonso et al., 1999; Andreu et al., 2015).

The fruit oil of *A. macrocarpa* was dominated by methyleugenol (62.9%), β-pinene (11.8%) and limonene (9.2%) as main compounds. In the leaf and stem oil, the major components were α-terpineol (19.2%), 2-ethyl-*p*-xylene (12.1%), *p*-diethyl-benzene (8.5%), an unknown alkene (9.3%) and durene (8.4%). In the roots of this species, nerolidol (25.7%), an unknown sesquiterpene (22.7%) and *n*-octadecanol (12.7%) were the main compounds.

Annesorhiza nuda leaf and root oils yielded a new compound as the major constituent, assigned as butan-2-yl (2*Z*)-3-(methylsulfanyl) prop-2-enoate (Fig. 2, Table 2); a trans-β-methylthioacrylate which required elucidation by NMR. The chemical shifts of "(−)-nudaic ester" are provided in Table 2. In brief, the accurate mass of 174.0715 indicated a molecular formula of $C_8H_{14}O_2S$. The ^{13}C spectra indicated two olefinic carbons at 113.6 and 151.3 ppm and a carbonyl group at 166.4 ppm, which is further upfield than a typical carbonyl group, suggesting an adjacent oxygen.

The apolar mobile phase used in silica gel chromatography to isolate **1** indicated that it was a relatively apolar molecule. The even number of protons integrated on the ^{1}H NMR confirmed this by indicating the absence of an OH group and that this compound was therefore an ester. The absence of an IR max in the 1700–1800 band indicated that it was an atypical α,β-unsaturated ester (vmax 1684), which is probably a consequence of the placement of the methyl sulfanyl group to the downfield olefinic carbon at 151.3 ppm. The 3H methyl peak at 2.39 ppm is less electronegative than a methoxy bond and is evidently part of this methylsulfanyl (methylthio) group. The ^{13}C shift of carbon 5 at 72.1 ppm indicated that it is at the ester bond. Lastly, the coupling constant between the two olefinic protons (10.2 Hz) indicated a *cis* configuration. The 1H–1H COESY spectra indicated two separate spin systems, which clearly elucidated the connectivity on either side of the ester bond.

A search of the literature revealed that **1** has not previously been reported as a natural product, but can be made available commercially by synthesis. Synthetic analogs that differ from **1** by the ester group, such as a butyl ester substituent, have been developed as flavors and perfumes (Williams and Bend, 2008). Furthermore, similar trans-β-methylthioacrylates demonstrate antifungal and insect antifeedant activity (Labbé et al., 2005), which provides evidence of a possible ecological role for **1** against insect feeding.

The essential oil of *A. nuda* contains 45.7% of **1** out of the 81.7% of the total leaf oil identified, while in the root, it was found to be 43.5% of the total 96.3% of the total root compounds identified (Table 1). Other major compounds that were present include phenylethyl 3-methyl butanoate (14.9%), estragole (11.5%), β-bisabolene (1.1–6.6%) and limonene (4.9–6.8%). The peduncles, flowers and fruits differed markedly from the roots and leaves – they were totally devoid of phenylpropanoids but dominated by limonene, β-myrcene, *Z*-β-ocimene and other monoterpenoids (Table 1). Solvent extracts of fruits from six different individual plants (not reported in Table 1) showed the same pattern of main compounds that was found in the steam-distilled bulk fruit sample.

The three species of *Annesorhiza* analyzed showed distinct and intriguing differences. Fruits of *A. grandiflora* had nothoapiole as major compound (52.9%), together with dillapiole (16.5%) but neither were detected (not even in trace amounts) in the fruit oil of *A. macrocarpa*, which was dominated by methyleugenol. A comparison of oils from roots and leaves of *A. grandiflora* and *A. macrocarpa* showed almost no shared compounds (i.e., neither between the species nor between the plant parts extracted). In contrast, roots and leaves of *A. nuda* had almost equal percentages of (−)-nudaic ester, with a relatively high concentration of estragole only in roots. These discontinuities, preliminary as they may be, indicate that a detailed chemosystematic study may yield interesting results, given the current poor understanding of the relationships within and between the genera *Annesorhiza* and *Chamarea*. A few monoterpenes were shared between species (e.g. α- and β-pinene, limonene), but in general the differences (between species and between plant parts) were far more striking than the similarities.

Molecular systematic studies (Magee et al., 2008; Magee et al., 2010) showed that *Annesorhia* belongs to one of the basally divergent lineages within the subfamily Apioideae, together with the genera *Astydamia* DC., *Chamarea* Eckl. & Zeyh., *Ezosciadium* B.L.Burtt, *Itasina* Raf. and *Molopospermum* W.D.J.Koch. From the DNA result it was apparent that this group of genera represents a distinct new tribe of Apioideae, for which the name Annesorhizeae has been proposed. Only two of the genera are not endemic to southern Africa; *Astydamia*, with a single species, *A. latifolia* (L.f.) Kuntze, on the seashores of the Canary Islands and Morocco, and *Molopospermum*, with a single species, *M. peloponnesiacum* (L.) W.D.J. Koch, from the mountains of southern, western and central Europe. *Astydamia latifolia* and *Molopospermum peloponnesiacum* are the only two species previously studied for their essential oil characters (Kubeczka and Ullman, 1981; Pérez-Alonso et al., 1999; Andreu et al., 2015). It was found that dillapiole, 3-carene and myristicin were the major constituents in both genera. There appears to be no published information on any chemical compounds from the southern African genera (*Annesorhiza*, *Chamarea*, *Ezosciadium* and *Itasina*).

The presence of phenylpropanoids as major compounds in *Annesorhiza* suggested that these compounds may also be present in other genera of the tribe. This is indeed the case. *Astydamia* fruits had myristicin (ca. 40%) and dillapiole (ca. 17%) as major compounds (Pérez-Alonso et al., 1999), while *Molopospermum* was reported to have dillapiole and nothoapiole as main compounds in one of two chemotypes (Kubeczka and Ullman, 1981; Kubeczka and Ullman, 1983). The dominance of dillapiole (60%) in stems was confirmed by Andreu et al. (2015). *Molopospermum* shares with three *Annesorhiza* species (*A. nuda, A. macrocarpa* and *A. thunbergii*) the unusual feature of heteromericarpic fruits. Elsewhere in the Apiaceae, heteromericarpic fruits are found in another protoapioid genus, *Heteromorpha* Cham. & Schltdl., from the predominantly woody tribe Heteromorpheae (Van Wyk et al., 2013). The accumulation of phenylpropanoids in the tribe Annesorhizeae and the apparent absence of coumarins are of considerable chemosystematic interest at the generic and especially tribal level. It appears that phenylpropanoids, which are common in many euapioids, first evolved in the protoapioids, and particularly in the predominantly herbaceous tribe Annesorhizeae (Magee et al., 2010; Van Wyk et al., 2013; Plunkett et al., 2018). No records of the occurrence of phenylpropanoids in other protoapioids could be found, and they also appear to be absent from all members of the subfamilies Mackinlayoideae and Azorelloideae. Phenylpropanoids are the precursors of coumarins, and it appears that the pathway to the latter may have been blocked (or have not yet evolved) in the tribe Annesorhizeae. A wider consideration of the homology of phenylpropanoid accumulation (Matern, 1991), and the associated absence of coumarins, in other members of the subfamily Apioideae may lead to a better understanding of the evolution and role of chemical characters.

The lack of chemical uniformity, not only within a single individual plant but also amongst species of *Annesorhiza,* may be related to the very distinct seasonal growth phases of these plants. The leaves are hysteranthous; they appear at the onset of the wet winter (southern hemisphere) period in April–May and last until the onset of summer drought in November–December. At this point, the roots of the previous year have shriveled up and died, having been replaced by a corresponding number of newly formed roots. November is therefore the traditional time at which anise root is harvested as food. It is also at this time that inflorescences start to emerge. The fruits are formed during the hot and dry summer months, relying on the reserves stored in the roots. These pronounced seasonal growth phases may possibly explain the differences observed in the major volatile compounds. A detailed chemosystematic study of all the species of *Annesorhiza* and other genera of the tribe Annesorhizeae (with consistent sampling of plant parts in different seasons) is called for to unravel the interesting preliminary observations made in this study. However, collecting a representative sample of these evasive and poorly recorded plants presents a major challenge.

4. Conclusions

The ethnobotany of *Annesorhiza* species has remained poorly recorded despite their apparent popularity as an indigenous food item since the seventeenth and eighteenth centuries. At least four species (*A. grandiflora*, *A. macrocarpa*, *A. nuda* and an as yet undescribed species) have been recorded as sources of root vegetables. *Annesorhiza nuda* and especially *A. flagellifolia* are used as leaf vegetables (spinach), while *A. altiscapa* is a traditional source of yeast when making honey beer. It may be worthwhile to evaluate these plants as potential new crops and indigenous vegetables.

A consequence of this study was the identification of a novel major compound in *A. nuda*, which we named colloquially as (−)-nudaic ester, and which is yet to be discovered in other members of the genus or related genera.

Available data show that phenylpropanoids occur as major components of the essential oils of all species of the tribe Annesorhizeae studied thus far, supporting the idea that the phenylpropanoid pathway first evolved in this protoapioid tribe, and that the sporadic but widespread presence of phenylpropanoids in the euapioid tribes of the Apioideae may be interpreted as independent convergences. This hypothesis requires further research.

The presence of anethole and estragole (only in roots, and only in *A. nuda*) partly explains the reported anise-like scent. However, a more detailed study is called for to explore possible geographical and seasonal variation in the presence and concentration of anethole and other phenylpropanoids.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sajb.2019.07.014.

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