

Volatile Constituents of Rooibos Tea (*Aspalathus linearis*) As Affected by Extraction Process

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Volatile components of Rooibos tea produced in South Africa were analyzed by gas chromatography and gas chromatography/mass spectrometry. The GC pattern of brewed tea rudimentarily extracted by dichloromethane (brewed extract) is extremely different from the GC pattern of that prepared by simultaneous steam distillation and extraction (SDE). The former consisted of 50 components such as 2-phenylethanol, 2-methoxy-2-buten-4-olide, guaiacol, dihydroactinidiolide, 4-butanolide, methyl-ethylmaleimide, and hexanoic acid. The latter consisted of 123 components such as guaiacol, acetic acid, 2-phenylethanol, geranylacetone, β -damascenone, hexanoic acid, 3-methylbutanoic acid, and 6,10,14-trimethylpentadecanone. A total of 42 components were newly identified as Rooibos tea volatiles, including 5 hydrocarbons, 7 alcohols, 7 aldehydes, 5 ketones, 4 acids, 1 ester, 8 lactones, 2 anhydrides, 1 imide, 1 phenol, and 1 furan. The aroma of brewed extract was found to be characterized by many kinds of lactone compounds. Model experiments using the two extraction methods were carried out on standard mixtures of the Rooibos tea volatiles. While the GC pattern of the standard mixture was accurately reproduced by the brewed extract, the SDE extract was found lacking in lactone compounds.

INTRODUCTION

Rooibos tea is a local tea consumed in South Africa. The material from the legume shrub, *Aspalathus linearis*, grows in the Cederberg Mountains near Cape Town (Dahlgren, 1968; Komaitis, 1992). Rooibos tea is manufactured as follows: cutting of the leaves into 5-mm length, rolling, fermenting by leaf enzymes, and solar-drying, similar in process to that for black tea or oolong tea. In recent studies, it was revealed that Rooibos tea has antioxidative activities by superoxide dismutase mimetic substances (Yoshikawa et al., 1990; Ito et al., 1991) and has effects on dermatological diseases such as Behcet's disease, Sweet disease, and photosensitive dermatitis (Shindo et al., 1991).

Rooibos tea has a characteristic sweet flavor. Ninety-nine and 218 components, respectively, were previously reported in the vacuum steam volatile and headspace vapor (Habu et al., 1985). In this work, an analysis of the volatile components of brewed extract was compared with an SDE analysis, and model experiments using standard mixtures were carried out.

EXPERIMENTAL PROCEDURES

Sample Preparations. A sample of Rooibos tea was imported from South Africa. One hundred grams of sample was brewed by 700 mL of deionized boiling water for 10 min. After filtration, the filtrate was saturated with sodium chloride and was extracted by using 200 mL of dichloromethane. Another 100 g of sample was placed in a 1000-mL flask with 500 mL of deionized boiling water. The steam distillate was simultaneously extracted with 50 mL of dichloromethane for 1 h by using a modified Likens-Nickerson apparatus (SDE method; Schultz et al., 1977). Both extracts were dried over anhydrous sodium sulfate for 12 h. After sodium sulfate and solvent were removed, both concentrates were obtained. Then they were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Instrumental Methods. A Hitachi Model G-3000 gas chromatograph equipped with an FID and a 50 m \times 0.25 mm Carbowax 20M fused silica capillary column was used. The peak area was integrated by using a Hitachi D-2500 integrator. The oven temperature was held at 60 °C for 4 min and then programmed to 180 °C at 2 °C/min. The helium carrier gas flow rate was 30 cm/s. The injector and detector temperatures were 200 and 210 °C, respectively. A JEOL JMS-DS 300 mass spectrometer interfaced to a Hewlett-Packard 5790 gas chromatograph was used for MS identification. The GC conditions were the same as above; the ion source temperature was 200 °C, and electron energy was 70 eV. The MS data were analyzed by a JEOL DA-5000 data processing system. The GC Kovats index (KI) and MS fragmentation patterns of each component were compared to those of the authentic compound as reported in the literature.

Model Experiment. The eight principal components of Rooibos tea volatiles were picked as the standard mixture (see Table II). The standard mixture, 30 μ L of each compound dissolved in dichloromethane, was prepared. Twenty microliters of standard mixture was added to 200 mL of boiling water. After the standard mixture solution was stirred for 10 min, it was saturated with sodium chloride and was extracted by dichloromethane three times. Another 20 μ L of standard mixture in 200 mL of deionized boiling water was simultaneously steam distilled and extracted with 50 mL of dichloromethane for 1 h by using a modified Likens-Nickerson apparatus (SDE method). Both extracts were dried over anhydrous sodium sulfate for 12 h. After sodium sulfate and solvent were removed, both concentrates were obtained. Then they were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

RESULTS AND DISCUSSION

The aroma obtained from the brewed extract bore stronger sensory resemblance to Rooibos tea itself than the aroma of the SDE extract. The amount of brewed extract was about $1/10$ that of SDE extract. Gas chromatograms of each extract are shown in Figure 1. They are extremely different from each other in GC pattern. The identified compounds and relative quantities of each compound, as calculated by the peak area, are listed in Table I. Fifty compounds were identified from the brewed extract and 123 compounds from the SDE extract. In

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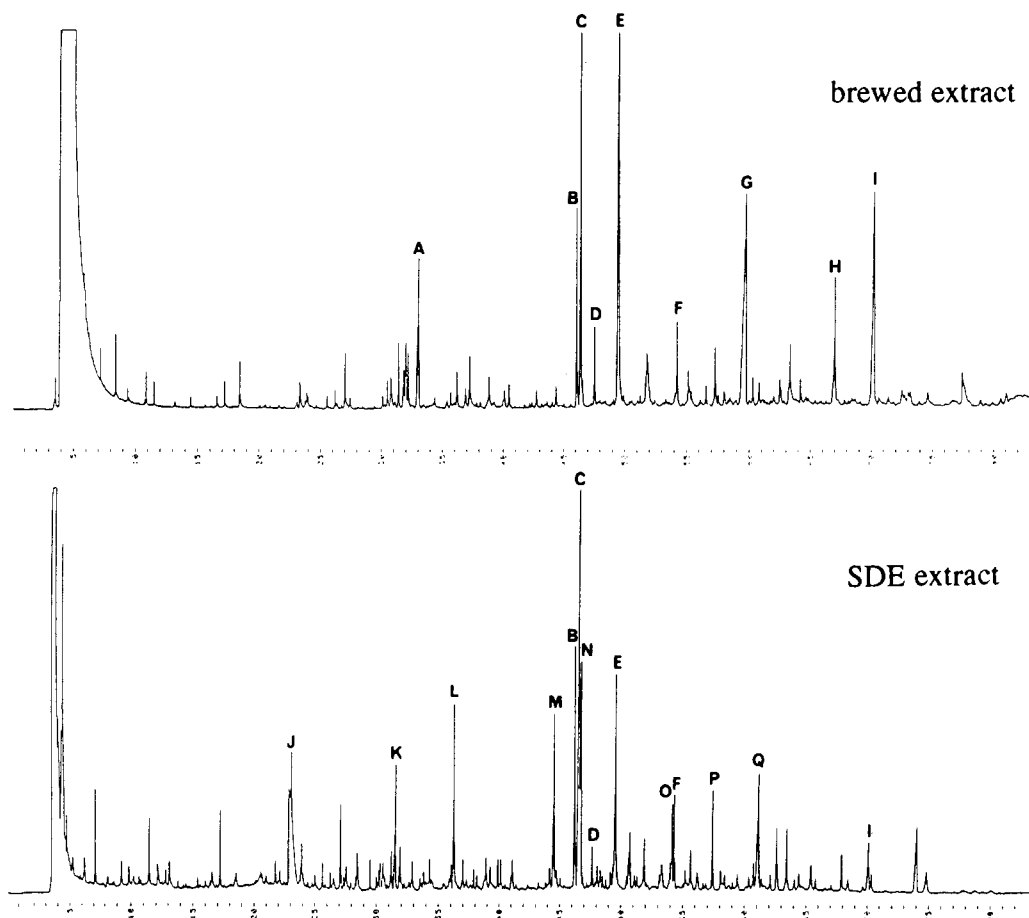


Figure 1. Gas chromatograms of the Rooibos tea extracts. Peaks: (A) 4-butanolide; (B) hexanoic acid; (C) guaiacol; (D) benzyl alcohol; (E) 2-phenylethanol; (F) phenol; (G) 2-methoxy-2-buten-4-olide; (H) methylethylmaleimide; (I) dihydroactinidiolide; (J) acetic acid; (K) 6-methyl-(*E*)-3,5-heptadien-2-one; (L) 3-methylbutanoic acid; (M) β -damascenone; (N) geranylacetone; (O) epoxy- β -ionone; (P) octanoic acid; (Q) 6,10,14-trimethyl-2-pentadecanone.

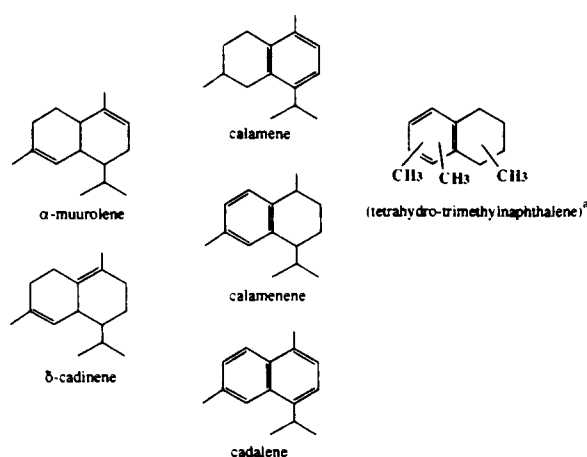


Figure 2. Characteristic fused bicyclic hydrocarbons in the SDE extract. ^aFive isomers were isolated by GC.

these, 38 components are shared in both concentrates, and 104 components are shared with *Camellia sinensis* tea (Straten et al., 1989). A total of 42 components were newly identified as Rooibos tea volatiles, including 5 hydrocarbons (α -muurolene, δ -cadinene, calamene, calamenene, cadalene), 7 alcohols [3-methyl-1-penten-3-ol, 5-hexenol, (*E*)-3-nonen-2-ol, (*E*)-2-octenol, decanol, tridecanol, nerolidol], 7 aldehydes [(*Z*)-2-heptenal (*E*)-6-nonenal, (*E*)-2-decenal, neral, geranial, (*E*)-2-undecenal, (*E*)-2, (*E*)-4-decadienal], 5 ketones (2,6,6-trimethyl-2-hydroxycyclohexanone, 2,6,6-trimethylcyclohex-2-ene-1,4-dione, 6,10-dimethyl-2-undecanone, 6,10,14-trimethyl-2-pentadecanone, farnesylacetone), 4 acids (3-methylbutanoic acid, heptanoic acid, octanoic acid, dodecanoic acid), 1

ester (2-phenylethyl formate), 8 lactones (4-pentanolide, 3-methyl-4-butanolide, 2-buten-4-olide, 2-hexen-4-olide, 5-octanolide, 2-methoxy-2-buten-4-olide, boviolide, dihydroactinidiolide), 2 anhydrides (acetic anhydride, dimethylmaleic anhydride), 1 imide (methylethylmaleimide), 1 phenol (4-vinylguaiacol), and 1 furan (4-methyl-2,3-dihydrofuran).

The concentrate of brewed extract consists of high levels of 2-phenylethanol, guaiacol, methylethylmaleimide, and three kinds of lactones such as 2-methoxy-2-buten-4-olide, dihydroactinidiolide, and 4-butanolide. Other important components in brewed extract were also lactones such as 4-pentanolide, 3-methyl-4-butanolide, 2-methyl-4-butanolide, 2-methyl-2-buten-4-olide, 4-hexanolide, 2-hexen-4-olide, 2-buten-4-olide, 5-pentanolide, 5-octanolide, and 4-nonanolide. These lactones were not identified in the SDE extract except low levels of 4-nonanolide, dihydroactinidiolide, and 2-hexen-4-olide.

The main components in the SDE extract were guaiacol, 2-phenylethanol, ketones such as geranylacetone, β -damascenone, and 6,10,14-trimethyl-2-pentadecanone, and acids such as acetic acid, 3-methylbutanoic acid, hexanoic acid, and octanoic acid. Fused bicyclic hydrocarbons, probably derived from terpenes, were also identified in the SDE extract as shown in Figure 2. The difference in these two compositions is primarily due to differences in the analytical methods. The lactone skeleton is known to be fragile in aqueous medium heating, and therefore the lactone ring is easily opened during SDE. Ketones are also considered to be degradation products from carotene by SDE (Kawakami, 1982; Onyewu et al., 1989). Phenolic compounds such as guaiacol, phenol, 4-vinylguaiacol, and

Table I. Composition of the Volatiles of the SDE Extract and the Brewed Extract from Rooibos Tea

KI ^a	compound	GC peak area, %		KI ^a	compound	GC peak area, %	
		SDE	brewed			SDE	brewed
888	2-butanone	0.56	c, d	1595	phenylacetaldehyde	0.13	c, d
912	3-methylbutanal	0.12	c, d	1596	(E)-2-decenal ^b	0.33	c, d
925	ethanol	0.24	c, d	1596	safranal	0.54	c, d
960	pentanal	0.09	c, d	1596	4-butanolide	c	4.30, d
972	2-methyl-3-buten-2-ol	0.12	c, d	1609	acetophenone	0.33	0.39, d
1017	toluene	0.33	c, d	1622	C ₁₃ H ₁₈ ^b	0.40	c
1064	hexanal	1.33	0.30, d	1631	nonanol	0.33	c, d
1104	(E)-2-pentenal	0.17	c, d	1631	3-methylbutanoic acid ^b	3.30	c, d
1104	(E)-3-penten-2-one	0.17	c, d	1649	neral ^b	0.07	c, d
1118	butanol	0.11	c, d	1650	C ₁₃ H ₁₈ ^b	0.54	c
1139	1-penten-3-ol	0.46	0.21, d	1655	2,6,6-trimethylcyclohex-2-ene-1,4-dione ^b	0.58	0.57, d
1160	heptanal	0.20	c, d	1660	4-hexanolide	c	1.50, d
1170	4-methyl-2,3-dihydrofuran ^b	0.13	c	1660	6,10-dimethyl-2-undecanone ^b	0.46	c, d
1158	2-heptanone	0.08	c, d	1662	α-terpineol	0.33	c, d
1184	limonene	0.03	c, d	1671	α-murolene ^b	0.28	c, d
1192	(E)-2-hexenal	1.05	0.36, d	1682	dodecanal	1.00	0.08, d
1215	2-pentylfuran	0.33	c, d	1682	2-methyl-2-buten-4-olide	c	0.18
1218	(Z)-4-heptenal	0.07	c	1683	C ₁₃ H ₁₈ ^b	0.49	c
1222	3-methyl-1-penten-3-ol ^b	0.07	c	1684	δ-cadinene ^b	0.46	c, d
1232	pentanol	0.40	0.16, d	1685	dimethylmaleic anhydride ^b	0.81	1.12
1235	o-methylanisole	1.44	c	1692	2-buten-4-olide ^b	c	0.73
1240	acetic anhydride ^b	0.51	c	1693	geranial ^b	0.13	c, d
1240	styrene	0.10	c, d	1698	pentanoic acid	0.40	c, d
1266	acetoin	0.13	0.18, d	1698	naphthalene	0.33	c, d
1278	octanal	0.07	c, d	1720	(E)-2-undecenal ^b	0.13	c, d
1280	α-terpinolene	0.19	c, d	1725	2-hexen-4-olide ^b	0.15	0.53, d
1294	4-pentenol	0.27	0.08	1726	methyl phenylacetate	0.15	c, d
1296	(Z)-2-pentenol	0.46	0.29, d	1728	(Z)-β-damascenone	0.21	c
1298	(Z)-2-heptenal ^b	0.20	c	1730	decanol ^b	0.15	c, d
1308	2,6,6-trimethylcyclohexanone	0.46	c, d	1738	o-methylacetophenone	0.11	c
1320	6-methyl-5-hepten-2-one	1.30	0.48, d	1741	5-pentanolide	c	0.50
1332	hexanol	0.50	0.97, d	1750	2-phenylethyl formate ^b	0.10	c, d
1361	(Z)-3-hexenol	0.17	c, d	1776	calamene ^b	0.54	c
1368	nonanal	0.23	c, d	1777	(E)-2,(E)-4-decadienal ^b	0.36	c, d
1376	2,6,6-trimethylcyclohex-2-en-1-one	0.63	c, d	1787	2-tridecanone	0.10	c
1392	(E)-3-octen-2-one	0.33	c, d	1788	2-phenylethyl acetate	0.43	c
1394	5-hexenol ^b	0.17	0.10	1792	(E)-β-damascenone	3.96	0.59, d
1395	C ₁₃ H ₁₈ ^b	0.71	c	1807	hexanoic acid	3.78	3.50, d
1400	(E)-2-octenal	0.33	c, d	1815	guaiacol	9.22	9.81, d
1401	C ₁₃ H ₁₈ ^b	1.40	c	1820	geranylacetone	4.63	0.21, d
1405	acetic acid	5.39	0.71, d	1833	benzyl alcohol	0.74	2.23, d
1415	linalool oxide I (cis, furanoid)	0.43	c, d	1863	2-phenylethanol	4.88	15.50, d
1418	1-octen-3-ol	0.40	c, d	1887	5-octanolide ^b	c	1.02, d
1425	heptanol	0.20	c, d	1887	heptanoic acid ^b	1.03	c, d
1427	furfural	0.70	0.14, d	1889	β-ionone	1.33	c, d
1430	(E)-2,(Z)-4-heptadienal	0.36	0.32, d	1913	calamenene ^b	0.44	c, d
1440	linalool oxide II (trans furanoid)	0.13	c, d	1954	5,6-epoxy-β-ionone	0.93	0.38, d
1453	2-ethylhexan-1-ol	0.93	0.17, d	1957	phenol	1.58	2.02, d
1456	(E)-2,(E)-4-heptadienal	0.39	0.24, d	1981	4-nonanolide	0.50	1.55, d
1458	C ₁₃ H ₁₈ ^b	0.18	c	1991	nerolidol ^b	0.50	c, d
1463	2-acetylfuran	0.28	0.31, d	1995	(E),(Z)-pseudoionone	0.82	c
1467	decanal	0.10	c, d	2013	octanoic acid ^b	2.45	c, d
1469	(E)-6-nonenal ^b	0.80	0.26	2041	tridecanol ^b	0.10	c
1482	benzaldehyde	1.27	1.07, d	2043	2-methoxy-2-buten-4-olide ^b	c	11.67
1482	(E)-3,(Z)-5-octadien-2-one	0.22	c, d	2069	6,10,14-trimethyl-2-pentadecanone ^b	3.27	c, d
1491	propionic acid	0.56	c, d	2073	(E),(E)-pseudoionone	1.17	0.76
1509	(E)-2-nonenal	0.26	c, d	2087	bovolide ^b	0.35	c, d
1522	linalool	0.76	c, d	2096	nonanoic acid	1.34	c, d
1534	octanol	0.66	c, d	2099	eugenol	c	2.50
1535	2-methylpropionic acid	0.41	c, d	2115	4-ethylphenol	0.50	c, d
1537	C ₁₃ H ₁₈ ^b	0.29	c	2131	dihydrobovolide ^b	0.11	c, d
1539	5-methylfurfural	0.20	c, d	2131	C ₁₆ H ₁₈	0.10	c
1539	(E)-3,(E)-5-octadien-2-one	0.39	0.22, d	2136	4-vinylguaiacol ^b	0.93	2.71, d
1548	6-methyl-(Z)-3,5-heptadien-2-one	0.56	c, d	2149	cadalene ^b	0.47	c
1555	(E)-2,(Z)-6-nonadienal	0.19	c, d	2149	methyl palmitate	0.93	c, d
1557	2-methyl-4-butanolide	c	0.83	2159	methylethylmaleimide ^b	c	3.93
1561	6-methyl-(E)-3,5-heptadien-2-one	0.56	1.18, d	2163	decanoic acid	0.78	c, d
1570	2,6,6-trimethyl-2-hydroxycyclohexanone ^b	0.93	0.93, d	2260	dihydroactinidiolide	0.93	9.96, d
1574	4-pentanolide ^b	c	1.52, d	2270	farnesylacetone ^b	1.19	c
1579	3-methyl-4-butanolide ^b	c	1.21		dodecanoic acid ^b	1.91	c, d
1582	(E)-3-nonen-2-ol ^b	0.60	c				
1587	(E)-2-octenol ^b	0.27	c, d		total	99.22	87.98
1594	β-cyclocitral	0.60	c, d				

^a Kovats index on Carbowax 20M. ^b Newly identified components in Rooibos tea aroma. ^c Not detected. ^d Components of *C. sinensis* tea (Straten et al., 1989).

Table II. Comparison of the GC Patterns^a between the Brewed Extract and the SDE Extract by Model Experiment

compound	standard mixture	brewed extract	SDE extract	(remains) ^b
linalool	14.42	13.94	19.53	(17.61)
4-butanolide	14.06	9.80	1.57	(1.45)
hexanoic acid	10.11	9.39	7.99	(7.84)
guaiacol	16.22	15.23	20.48	(19.31)
geranylacetone	5.65	7.02	9.63	(10.10)
2-phenylethanol	17.87	18.29	16.45	(15.65)
β -ionone	11.39	14.30	19.30	(22.01)
dihydroactinidiolide	10.29	12.03	5.05	(6.02)

^a GC peak area %. ^b Remains (standard mixture boiling solution of SDE), extracted by dichloromethane.

eugenol, which are important high-level components of Rooibos tea with the effect against bacteria, were well extracted by both preparations.

To clarify the cause of the differences in composition, a model analysis using standard mixture was carried out. Table II shows the comparison of the GC patterns between the brewed extract and the SDE extract. While the brewed extract accurately reproduced the GC pattern of the standard mixture, the SDE extract lacked lactones, i.e., 4-butanolide and dihydroactinidiolide. Lactones were easily decomposed, probably hydrolyzed to hydroxy acid compounds by SDE. Therefore, the SDE method is not suitable for the analysis of samples containing high amounts of lactones. Usually, Rooibos tea is prepared by either of two methods: soaking in boiling water for 10 min or boiling for 15–30 min. The different preparations change the quality of aroma, especially the amount of lactones.

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LITERATURE CITED

- Dahlgren, R. Revision of the Genus *Aspalathus*. II. *Bot. Notiser* 1968, 121, 165–208.
- Habu, T.; Flath, R. A.; Mon, T. R.; Morton, J. F. Volatile Components of Rooibos Tea (*Aspalathus linearis*). *J. Agric. Food Chem.* 1985, 33, 249–254.
- Ito, A.; Shinohara, K.; Kator, K. Protective Action of Rooibos Tea (*Aspalathus linearis*) Extract against Inactivation of L5178Y Cells by H₂O₂. In *Proceedings of the International Symposium on Tea Science*; The Organizing Committee of ISTS: Shizuoka, 1991; pp 381–384.
- Kawakami, M. Ionone Series Compounds from β -Carotene by Thermal Degradation in Aqueous Medium. *Nippon Nogei Kagaku Kaishi* 1982, 56, 917–921.
- Komaitis, M. E. Rooibos Tea (*Aspalathus linearis*). In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, 1992; pp 417–418.
- Onyewu, P. N.; Daun, H.; Ho, C-T. Volatile Thermal Decomposition Products of β -Carotene. In *Thermal Generation of Aromas*; Parliment, T. H., McGorin, R. J., Ho, C-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC 1989; pp 247–256.
- Schultz, T. H.; Flath, R. A.; Mon, T. R.; Egging, S. B.; Teranishi, R. Isolation of Volatile Compounds from a Model System. *J. Agric. Food Chem.* 1977, 25, 446–449.
- Shindo, Y.; Kator, K. Effect of Rooibos Tea on Some Dermatological Diseases. In *Proceedings of the International Symposium on Tea Science*; The Organizing Committee of ISTS: Shizuoka, 1991; pp 385–389.
- Straten, S. V.; Maarse, H. *Volatile Components in Food*; Institute CIVO-analysis TNO: Utrechtseweg, 1989; pp 680–710.
- Yoshikawa, S.; Naito, Y.; Oyamada, H.; Ueda, S.; Tanigawa, T.; Takemura, T.; Sugino, S.; Kondo, M. Scavenging Effects of *Aspalathus linealis* (Rooibos tea) on Active Oxygen Species. In *Antioxidants in Therapy and Preventive Medicine*; Emerit, I., Ed.; Plenum Press: New York, 1990; pp 171–174.

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