Research Journal of Pharmacognosy (RJP) 1(3), 2014: 27-33
Received: Feb 2014
Accepted: Apr 2014

Original article

Cytotoxic activity of the essential oil of *Salvia verticillata* L.

N. Khosravi Dehaghi[1], S. N. Ostad[2], N. Maafi[3], S. Pedram[3], Y. Ajani[4], A. Hadjiakhoondi[3], M. Khanavi[3*]

[1] *Department of Physiology and Pharmacology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran.*
[2]*Department of Toxicology & Pharmacology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[3]*Department of Pharmacognosy, Faculty of Pharmacy and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
[4]*Institute of Medicinal Plants, ACECR, Karaj, Iran.*

Abstract

Salvia is one of the largest genera of Lamiaceae family. Several species of this genus are perfumed and wealthy in essential oils. Some of them are used in industry, pharmacy and aromatherapy. They have shown different biological effects such as antibacterial and antioxidant activity. For the present study, *Salvia verticillata* L. was collected from Shahrestanak, Mazandaran, Iran. Hydrodistilled essential oil from the aerial parts of this plant was obtained with a Clevenger type apparatus and was analyzed by GC and GC/MS. Moreover, the cytotoxic activity of the essential oil was investigated against HT-29 (colon adenocarcinoma), Caco-2 (colorectal adenocarcinoma), T-47D (breast ductal carcinoma) and NIH-3T3 (Swiss mouse embryo fibroblast) cell lines by MTT test. 59 components were characterized from the oil with *trans*-caryophyllene (24.40%), β-phellandrene (9.08%), α-humulene (8.61%), bicyclogermacrene (6.32%), spathulenol (5.89%) and β-pinene (5.00%) as the major constituents. These compounds represented 97.67% of the essential oil and included monoterpenes (34.83%) and sesquiterpens (61.84%). The results of the cytotoxicity assay demonstrated that the essential oil of *S. verticillata* showed higher cytotoxic effect on Caco-2 cell line.

Keywords*:* cytotoxic activity, essential oil, MTT, *Salvia verticillata*

Introduction

Genus *Salvia* (Lamiaceae) includes many species and varieties. It is distributed all over the world with about 900 species, among which, 56 are represented in Iran flora [1]. This genus has been used in traditional medicine of China, South Africa and many other countries against various infectious and inflammatory diseases, malaria, hard swellings, abscesses, warts and cancer [2-4]. Furthermore, there are some reports about *Salvia* species effects against various diseases such as respiratory and gastrointestinal disorders, hepatitis, cardiovascular diseases, infections, cancer, inflammations, loss of memory, menstrual disorders, miscarriage and insomnia

 Copy right© 2014 by the Iranian Society of Pharmacognosy
Corresponding author: khanavim@tums.ac.ir, Tel/Fax: +9821-66954706

[5]. Other researches have demonstrated biological properties of the essential oil of *Salvia* species such as antibacterial, anti-inflammatory and antimalarial activities [6-9]. *Salvia miltiorrhiza* has been used for treatment of insomnia, arthritis, menostasis, menstrual disorder, menorrhagia and coronary heart diseases, particularly angina pectoris and myocardial infarction [10-12]. Tanshinones (diterpenoids) have been isolated from *S. miltiorrhiza* [13,14] with antimicrobial [15,16] and antitumor [12,17,18] activities. *Salvia verticillata* has been introduced as an antioxidant plant [19,20] which contains polyphenols and diterpenoids [21]. It has radical scavenging effect [22] which is the skill to protect cells from different kinds of oxidative tensions. There are some reports about biological activity of this plant such as antibacterial and anti-diabetic effects [23] and in the present study, the constituents of the essential oil of *S. verticillata* were identified and their cytotoxic effect was evaluated against four cell lines by MTT assay.

Experimental

Plant material

The sample (aerial parts) was collected from Shahrestanak in Chaloos road, Mazandaran, Iran, at an altitude of 1100 m, in July 2009 during the flowering stage. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Isolation procedure

200 g of the flowering aerial parts were coarsely minced and placed in a flask containing 1 L of water and were hydrodistilled in a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulfate and kept at 4 °C in a sealed vial until required.

Gas chromatography-mass spectroscopy

Analytical gas chromatography was carried out using a Termoquest 2000 GC with capillary column DB-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness); carrier gas, He; split ratio, 1:25. A flame ionization detector (FID) was used. The column temperature was programmed at 50 °C for 1 minute and then heated up to 265 °C at a rate of 2.5 °C/min and kept constant at 265 °C for 20 min afterwards. GC-MS was performed on a Thermoquest 2000 with a qudrupole detector, on capillary column DB-5 (GC); carrier gas, He; flow rate, 1.5 mL/min. The temperature was held at 50 °C for 1 minute and was programmed to increase up to 265 °C at a rate of 2.5 °C/min, and then the temperature was kept constant at 256° C for 20 minutes. The MS was operated at 70 eV ionization energy. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil were identified by comparison of their mass spectra and the retention indices with Wiley library and those published in the literature.

Cell culture

The colon carcinoma (HT-29) colorectal adenocarcinoma (Caco-2), the breast ductal carcinoma (T-47D) and the Swiss mouse embryo fibroblast (NIH-3T3) were cultured in T-25 mm^2 culture flasks (NUNC) at 37 °C, in a 5% CO_2 incubator. HT-29, T-47D and NIH-3T3 were maintained as exponentially growing cultures in RPMI 1640 cell culture medium (Bio sera) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and Caco-2 was maintained in 50% RPMI 1640 and 35% DMEM-F12 cell culture medium (Bio sera) supplemented with 15% FBS. 100 IU/mL penicillin and 100 μg/mL streptomycin (Bio sera) were added to the media.

Determination of cell viability by MTT assay

The essential oil was dissolved in dimethyl sulfoxide (DMSO, Merck) followed by dissolving in the medium to prepare the final concentrations of 50, 100, 250, 500 and 1000 μg/mL. The control, received equally normal saline. 1×10^4 cells/wells for HT-29, T-47D and

NIH-3T3 cell lines and 1×10^5 cells/wells for Caco-2 cells were seeded into 24 well plates (NUNC) and were incubated for 24 hours before treatment. After 72 hours, the medium was removed and the cells were then washed with phosphate buffered saline (PBS). A 200 µL of 3-(4, 5-dimethylthiazol -2-yl) -2,5 -diphenyltetrazolium bromide (MTT 5 mg/mL in PBS) was added to each well. The plates were incubated at 37 °C for 4 h. At the end of incubation, 200 mL DMSO was added to each well. The solubilized formazan crystals were quantified by reading the absorbance at 570 nm using a microplate reader. Percentage of dead cells was calculated in comparison to control. The concentration of the essential oil that inhibited 50% cells growth (IC_{50}) was determined from the graph plotted by the concentration *vs* percentage of dead cells.

Results and Discussion

The results of the present study are in accordance with what has been published before about the major constituents of the essential oil extracted from *S. verticillata* L [23-25]. The hydrodistillation of the flowering aerial parts of *S. verticillata* L. presented a yellow oil with a distinct sharp odor (yield 0.15% W/W, based on dry weights). 59 components were identified in the oil of *S. verticillata* L. representing 97.67% of the total oil. The identified components and their percentages have been presented in table 1. The oil of *S. verticillata* L. was characterized by a high content of *trans*-caryophyllene (24.40%), β -phellandrene (9.08%), α-hummulene (8.61%), bicyclogermacrene (6.32%), spathulenol (5.89%), and β-pinene (5.00%). The oil was characterized by high amount of sesquiterpene hydrocarbons (52.92%) and monoterpene hydrocarbons (34.10%) (table1). Previous investigations on the oil of some species of *Salvia* have shown various results. In a study on *S. verticillata* was collected from Mazandaran, Iran, hydrodistilled essential oils from the leaves and flowers of this plant was

Table 1. Chemical composition of essential oil of *Salvia verticillata* (%)

No.	Compounds	KI	Percentage %
1	α-Thujene	925	0.34
2	α-Pinene	939	3.03
3	Camphene	950	0.18
4	Sabinene	970	4.44
5	β-Pinene	979	5.00
6	Myrcene	987	1.92
7	ρ-mentha-1(7),8-diene	1004	0.24
8	α-Phelandrene	1004	0.32
9	δ-3-Carene	1030	1.78
10	α-Terpinene	1035	0.10
11	ρ-Cymene	1039	0.22
12	β-Phellandrene	1039	9.08
13	Limonene	1042	3.80
14	*(Z)*-β-Ocimene	1044	1.65
15	(*E*)-β-Ocimene	1047	1.68
16	γ-Terpinene	1050	0.21
17	α-Terpinolene	1058	0.11
18	Nonanal	1075	0.15
19	Linalool	1090	0.05
20	Borneol	1099	0.20
21	Terpinen-4-ol	1170	0.28
22	Bornyl acetate	1200	0.05
23	α-Cubebene	1310	0.23
24	β-Damascenone	1317	0.11
25	α-Copaene	1322	0.25
26	β-Borbonene	1325	0.17
27	β-Cubebene	1388	0.23
28	α-Gurjunene	1400	3.32
29	*trans*-Caryophyllene	1410	24.40
30	*cis*-Thujopsene	1425	0.21
31	Aromadendrene	1430	0.05
32	Gernylacetone	1438	0.06
33	α-Humulene	1440	8.61
34	*trans*-Prenyl limonene	1458	1.35
35	α-Amorphene	1468	4.89
36	γ-Gurjunene	1477	0.21
37	δ-Selinene	1482	0.12
38	Germacrene –D	1487	0.21
39	*cis*-Cadina-1,4-diene	1496	0.33
40	Bicyclogermacrene	1500	6.32
41	β-Bisabolene	1506	0.58
42	*trans*-Calamenene	1510	0.17
43	*cis*-Calamenene	1520	0.53
44	β-Sesquiphellandrene	1523	0.21
45	*trans*-Cadina –1(2),4-diene	1535	0.09
46	*trans*-γ-Bisabolene	1537	0.27
47	Spathulenol	1565	5.89
48	Caryophyllene oxide	1569	0.89
49	Globulol	1585	0.22
50	Viridiflorol	1593	0.12
51	Juniperol	1599	0.30
52	Cedranone	1630	0.37
53	α-Cadinol	1640	0.17
54	t-Cadinol	1640	0.21
55	Valeranone	1675	0.38
56	hexahydrofarnsylacetone	1700	0.37
57	1-Octadecene	1750	0.10
58	Phytol	1896	0.84
59	Pentacosane	2275	0.06
	Monoterpenes hydrocarbons		34.10
	Monoterpenes oxygenated		0.73
	Sesquiterpenes hydrocarbons		52.92
	Sesquiterpenes oxygenated		8.92
	Diterpenoids		0.84
	Nonterpenes		0.16
	Unknown		2.33
	Total identified		97.67

Table 2. In vitro cytotoxicity of essential oil of *salvia verticillata*

Cell line	IC_{50} (µg/mL)
Caco-2	125.12 ± 27.59
HT-29	90.90 ± 14.88
NIH-3T3	81.81 ± 3.47
T47-D	80.20 ± 8.91

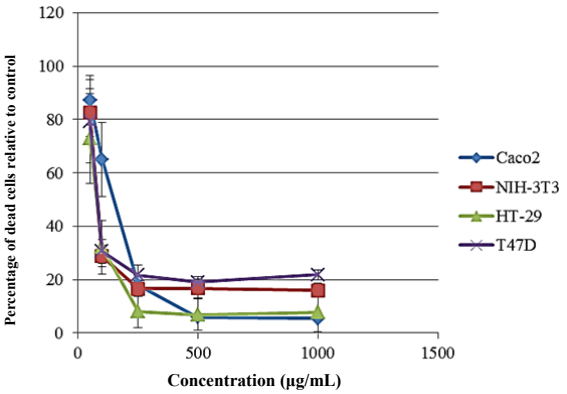

Figure 1. Percentage of dead cells relative to control: the cells were treated with *Salvia verticillata* essential oil as described in experimental

analyzed by GC and GC/MS and 54 and 36 compounds were identified respectively. β-caryophyllene (13.6%), β-phellandrene (12.9%), germacrene-D (11.5%), β-pinene (7.5%), α-humulene (5.6%), were premier components of the leaves oil. Spathulenol (23.6%), β-caryophyllene (17.2%), caryophyllene oxide (16.4%) and sabinene (8.4%) were the main compounds of flowers oil [24]. One research has previously compared the constituents of the essential oil of *S. verticillata* grown wild (Ghazvin) and cultivated (Fars). The essential oil was obtained by hydrodistillation of the dried aerial parts and was analyzed by GC and GC/MS. 51 components were characterized for cultivated plants including (E)-caryophyllene (17.8%), β-phellandrene (14.2%), *α*-humulene (10.2%), α-pinene (5.7%), germacrene D (5.2%) and 64 constituents were identified for the wild plants containing (E)-caryophyllene (14.7%), α-gurjunene (12.8%), germacrene D (8.7%), α -humulene (7.7%), β-phellandrene (6.6%), β-pinene (6.5%) and bicyclogermacrene (6.4) as the major constituents [23]. Other studies about the oil of this species that were collected from Tehran suburbs (Fasham-Garmabedar) have demonstrated that β-caryophyllene (24.7%), γ-murolene (22.8%), limonene (8.9%) and α-hummulene (7.8%) were the major components [25]. Another research in Greece has introduced the essential oil of *Salvia verticillata* with β-pinene (30.7%), *p*-cymene (23.0%) and isopropyl ester of lauric acid (16.8%) as the dominating constituents [26]. There is also a report from Lebanon introducing caryophyllene oxide (16.6%) as the major compound in the oil of *Salvia bracteata* [27] whereas in another research on the species gathered from different zones of Iran, the main constituents of the oil have been reported to be β-caryopyllene (10.7-41.6%) and γ-muurolene (27.1–36.3%) [28]. Previous studies have shown that, the essential oil obtained from *Salvia* species have shown antioxidant activity and *S. verticillata* has presented the most antioxidant activity so it could be used as a natural antioxidant in the food industry [29]. Cytotoxic effects on tumor cell lines has been also reported for *Salvia* genus [27] and in the present study, *S. verticillata* essential oil has demonstrated cytotoxic activity in HT-29, T-47D, Caco-2 and NIH 3T3 cell lines. Considering our results, it is confirmed that different cell lines showed different sensitivity to the agent (figure 1 and table 2). Other studies have shown that, *α*-humulene and β-caryophyllene have been described as cytotoxic compounds against A-549, DLD-1, M4BEU, HeLa, Bel-7402 and CT-26 cells [30-33]. Another study has verified that both linalool and *α*-pinene presented cytotoxic activity against murine B16 melanoma and human HL-60 leukemia cells [34]. Limonene is one of the constituents that was found in the oil of *S. verticillata* (3.80%) and has chemoprotective effects against rodent and human tumor [35]. Regarding that bioactive cytotoxic compounds have been found in the essential oil of *S. verticillata*, it is expectable to observe cytotoxic activity against the examined cell lines. The synergtic effects of the constituents of the oil and the presence of *trans*-

caryophyllene, β-phellandrene, α–humulene, bicyclogermacrene, spathulenol , β-pinene , α-amorphene , sabinene and limonene as the major compounds could explain the observed effects of the essential oil of *S. verticillata.*

Acknowledgments
This research has been supported by Tehran University of Medical Sciences and health Services grant (Grant Number 8923-33-02-88)

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Zargari A. *Medicinal Plants*. Vol 4. Tehran: Tehran University Publications, 1990.
[2] Watt JM, Breyer-Brandwijk MG. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed. Edinburgh: Livingstone, 1962.
[3] Ulubelen A, Topcu G, Chai HB, Pezzuto JM. Cytotoxic activity of diterpenoids isolated from *Salvia hypargeia*. *Pharm Biol*. 1999; 37: 148–151.
[4] Shoemaker M, Hamilton B, Dairkee SH, Cohen I, Campbell MJ. *In vitro* anticancer activity of twelve Chinese medicinal herbs. *Phytother Res*. 2005; 19: 649–651.
[5] Imanshahidi M, Hosseinzadeh H. The pharmacological effects of *Salvia* species on the central nervous system. *Phytother Res*. 2006; 20: 427-437.
[6] Kamatou GPP, Viljoen AM, Gono-Bwalya AB, Van Zyl RL, Van Vuuren SF, Lourens ACU, Baser KHC, Demirci B, Lindsey KL, Van Staden J, Steenkamp P. The *in vitro* pharmacological activities and a chemical investigation of three South African *Salvia* species. *J Ethnopharmacol*. 2005; 102: 382–390.
[7] Kamatou GPP, Van Zyl RL, Van Vuuren SF, Viljoen AM, Figueiredo AC, Pedro LG, Barroso JG, Tilney PM. Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* species indigenous to southern Africa. *J Essent Oil Res*. 2006; 18: 72–79.
[8] Kamatou GPP, Van Zyl RL, Van Vuuren SF, Viljoen AM, Figueiredo AC, Pedro SF, Barroso L.G. Biological activities and composition of *Salvia muirii* L. Bol. essential oil. *J Essent Oil Res*. 2006; 18: 48–51.
[9] Kamatou GPP, Van Zyl RL, Davids H, Van Heerden FR, Lourens ACU, Viljoen AM. Antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S. radula*. *S Afr J Bot*. 2008; 74: 238–243.
[10] Jiangsu New Medical College. *Dictionary of Chinese Materia Medica*. Shanghai: Shanghai Scientific and Technological Publishers, 1988.
[11] Chen WZ. Pharmacology of *Salvia miltiorrhiza*. *Acta Pharm Sinica*, 1984; 19: 876–880.
[12] Chang HM, But P. *Pharmacology and Applications of Chinese Materia Medica.* Singapore: World Scientific, 2001.
[13] Akisawa H, Hayashi T, Yamazaki T. Structures of isotanshinones. *Tetrahedron Lett*. 1969; 5: 301–304.
[14] Chang HM, Cheng KP, Choang TF, Chow HF, Chui KY, Hon PM, Lau Tan FW, Yang Y, Zhong ZP, Lee CM, Sham HL, Chan CF, Cui YX, Wong HNC. Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* Bunge (Danshen). *J Org Chem*. 1990; 55: 3537–3354.
[15] Honda G, Koezuka Y, Tabata M. Isolation of an antidermatophytic substance from the root of *Salvia miltiorrhiza*. *Chem Pharm Bull.* 1988; 36: 408–411.
[16] Gao YG, Song YM, Yang YY, Liu WF, Tang JX. Pharmacology of tanshinone. *Acta Pharm Sinica.* 1979; 14: 75–82.

[17] Ryu SY, Lee CO, Choi SU. *In vitro* cytotoxicity of tanshinones from *Salvia miltiorrhiza*. *Planta Med*. 1997; 63: 339–342.
[18] Wu WL, Chang WL, Chen CF. Cytotoxic activities of tanshinones against human carcinoma cell lines. *Am J Chin Med*. 1991; 19: 207–216.
[19] Orhan I, Kartal M, Naz Q, Ejaz A, Yilmaz G, Kan Y, Konuklugil B, Sener B, Iqbal Choudhary M. Antioxidant and anticholinesterase evaluation of selected Turkish *Salvia* species. *Food Chem*. 2007; 103: 1247-1254.
[20] Tepe B, Eminagaoglu O, Akpulat HA, Aydin E. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *Amasiaca* (Freyn and Bornm.) Bornm. *Food Chem*. 2007; 100: 985-989.
[21] Nagy G, Gunther G, Mathe I, Blunden G, Yang MH, Crabb TA. Diterpenoids from *Salvia glutinosa*, *S. austriaca*, *S. tomentosa* and *S. verticillata* roots. *Phytochemistry.* 1999; 52: 1105-1109.
[22] Nickavar B, Kamalinejad M, Izadpanah H. *In vitro* free radical scavenging activity of five *Salvia* species. *Pak J Pharm Sci*. 2007; 20: 291-294.
[23] Nasermoadeli S, Rowshan V, Abotalebi A, Nasermoadeli L, Charkhchian MM. Comparison of *Salvia verticillata* essential oil components in wild andcultivated population. *Ann Biological Res*. 2013; 4 (5): 252-255.
[24] Sarebanha S, Yasa N, Hadjiakhoondi A. Chemical composition and antioxidant activity of essential oil of flowers and leave of *Salvia verticillata* and *Salvia virgata*. Pharm. D. Thesis. Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, 2009.
[25] Sefidkon F, Khajavi MS. Chemical composition of the essential oils of two *Salvia* species from Iran: *Salvia verticillata* L. and *Salvia santolinifolia* Boiss. *Flavour Frag J*. 1999; 14: 77-78.
[26] Pitarokili D, Tzakou O, Loukis A. Essential oil composition of *Salvia verticillata, S. verbenaca*, *S. glutinosa* and *S. candidissima* growing wild in Greece. *Flavour Frag J*. 2006; 21: 670–673.
[27] Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA, Piozzi F. Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *J Ethnopharmacol*. 2009; 126: 265–272.
[28] Sefidkon F, Hooshidary F, Jamzad Z. Chemical variation in the essential oil of *Salvia bracteata* Banks & Soland from Iran. *J Essent Oil Bear Pl.* 2007; 10: 265–272.
[29] Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol Res*. 2009; 42(2): 175-81.
[30] Legault J, Dahl W, Debiton E, Pichette A, Madelmont JC. Antitumor activity of balsam fir oil: production of reactive oxygen species induced by alpha-humulene as possible mechanism of action. *Planta Med*. 2003; 69: 402–407.
[31] Sylvestre M, Legault J, Dufour D, Pichette A. Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. *Phytomedicine*. 2005; 12: 299-304.
[32] Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J. Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *J Ethnopharmacol*. 2006; 103: 99–102.
[33] Hou J, Sun T, Hu J, Chen S, Cai X, Zou G. Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of *Photinia serrulata.* 2006; *Food Chem.* 103: 355-358
[34] Tatman D, Mo H. Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60

leukemia cells. *Cancer Letters*. 2002; 175: 129-139.

[35] Ren Z, Gould MN. Modulation of small G protein isoprenylation by anticancer monoterpenesin *in situ* mammary gland epithelial cell. *Carcinogen*. 1998; 19: 827-832.

[36] Keller MS. *Mysterious Herbs and Roots*. New York: Peace Press, 1978.

[37] Amini A. *Illustrated Dictionary of Therapeutic Plants and their Traditional Usage in Kurdistan*. Khoramabad: Taqbostan Publication, 1997.

[38] Kamatou GPP, Van Zyl RL, Davids H, Van Heerden FR, Lourens ACU, Viljoen AM. Antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S. radula*. *S Afr J Bot*. 2008; 74: 238–243.