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Chemical composition, antimicrobial and antioxidant properties of the essential oils of *Tulbaghia violacea* Harv L.F.

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***Tulbaghia violacea* Harv L.F. is one of the many medicinal plants used by Zulu traditional healers to treat respiratory tract diseases. Essential oil extracted from the rhizomes of *T. violacea* was evaluated for its chemical composition, antioxidant and antibacterial activities. The gas chromatography/mass spectrometry (GC/MS) analysis of the oils revealed the main constituents of the essential oils of *T. violacea* to be 2,4-dithiapentane (51.04%), ρ-xylene (20.59%), chloromethylmethyl sulfide (8.69%), σ-xylene (7.38%), thiodiglycol (6.43%) and ρ-xylol (5.88%). While the oil showed weak antioxidant activity, the antimicrobial activity of the essential oil carried out on both Gram positive and negative bacteria (using the agar disk diffusion method) showed that the oil of *T. violacea* was effective against 8 of the 16 microorganisms tested, with MIC values ranging from 2.5 to 5.0 mg/ml. The oil had low (1218 and 1641 µg/ml) cytotoxicity levels against HEK293 and HepG2 cell lines, respectively. It is apparent that the bioactivity of the essential oil of *T. violacea* contributes to the use of this plant in folk medicine.**

Key words: Essential oil, antioxidant, antibacterial, *Tulbaghia violacea*.

INTRODUCTION

There is an increasing worldwide attempt to screen plants for the biological activities of their oils, from chemical and pharmacological investigations to therapeutic aspects (Sonbolia et al., 2005; Skaltsa et al., 2003; Tzakou and Skaltsa, 2003). Essential oils of various plants have been seen over the years to posses useful biological and pharmacological properties like antimicrobial (Kezemi et al., 2011; Vale-Silva et al., 2010; Gulluce et al., 2006; Altanlar et al.,1999; Janssen et al.,1987; Kurita et al., 1981), antinociceptive (Quintão et al., 2010; Sulaiman et al., 2009), anti–inflammatory (Mehmet et al., 2007; Chao et al., 2005), vaso-relaxant properties (Chiara et al., 2010) and antioxidant properties (Kadri et al., 2011; Kezemi et al., 2011; Gulluce et al., 2006).

Tulbaghia violacea (wild garlic), a member of the Alliaceae family, is a bulbous plant with hairless leaves arising from a white, fleshy stalk (Van Wyk et al., 1997). It is one of the many plants used by Zulu traditional healers in the treatment of respiratory tract infection, bronchitis, cough and asthma (Van Wyk and Wink, 2004; Hutchings et al., 1996). *T. violacea* has been widely used in African traditional medicine for the treatment of

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ulcers and other stomach ailments, as an aphrodisiac, and as a snake and witchcraft repellent (Scott, 1988). The plant has been recommended as a pulmonary tuberculosis remedy and as an anthelmintic (Scott, 1988). The Zulus use the leaves and flowers as spinach and as a hot peppery seasoning for meat and potatoes. Perhaps, retarding of its emergence as a major role player in the world of naturopathic remedies is the strong smell of garlic when crushed, which has been ascribed largely to alliin, a compound found in true garlic (Van Wyk and Wink, 2004) and perhaps in this plant as well. It has attractive mauve or purple flowers, which can be easily distinguished in the gardens of KwaZulu-Natal and the Eastern Cape provinces of South Africa.

In this study, we investigated the chemical composition, antioxidant and antimicrobial activities of the essential oil of *Tulbaghia* violacea*.* Such knowledge is essential for the complete exploitation (medicinal) of this plant.

MATERIALS AND METHODS

Isolation of the oils

T. violacea Harv L.F. was collected from the Sekhutlong forest, Leribe district, Lesotho. Freshly collected rhizomes were washed and cut into small pieces (2 cm).The rhizomes were then subjected to more than three hours of hydrodistillation using a Clevenger-type apparatus. The essential oils so obtained were dried over anhydrous sodium sulfate, and then stored at 5°C until required.

Gas chromatography/mass spectrometry (GC/MS)

GC-MS of the essential oil was carried out using an Agilent Gas Chromatography (7890 A) equipped with a capillary column (Agilent 190915 30 m × 250 µm × 0.25 µm calibrated) attached to an Agilent mass spectrometer system (5975C VL MSD with Triple Axis Detector). The oven temperature was programmed from 45 to 310°C. Helium was used as the carrier gas at a flow rate of 5 ml/min with a split ratio of 1:200. The essential oil (1 µl) was diluted in hexane and 0.5 µl of the solution was manually injected into the GC/MS. The chemical compositions of the essential oil of the rhizomes of *T. violacea* was determined according to their retention time, and spectrometric electronic libraries (WILEY NIST).

Microorganisms

Bacteria strains used in this study consisted of reference strains identified and obtained from the Microbiology Department, University of Zululand: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 19582), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Pseudomonas aeruginosa* (ATCC 7700), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 4352) and *Serratia marcescens* (ATCC 6830). Also included in this study were environmental strains of *Acinetobacter calcoaceticus anitratus* (CSIR), *Bacillus subtilis* (KZN)*, Shigella flexineri* (KZN)*, Salmonella* spp. (KZN), *Staphylococcus epidermidis* (KZN) and *Enterococcus faecalis* (KZN).

Antibiotic resistant strains of *S. aureus* (B10808), *S. aureus* (P12763), *S. aureus* (P12702), *S. aureus* (P12724), *P. aeruginosa* (T3374), *Streptococcus viridians* (S17141), *K. pneumoniae* (S17298) and *K. pneumoniae* (S17302) were clinical isolates and obtained from the Lancet Pathology Laboratory (Durban South Africa). The stock cultures were maintained at 4°C on Mueller-Hinton agar (Merck catalog number 1.05435.0500.)

Antimicrobial assay

The antibacterial properties of the essential oils were evaluated using the agar disk diffusion method (Van Vuuren and Vijoen, 2006). Bacteria were grown on 20 ml nutrient broth (Merck catalog number 1.05443.0500) at 37°C overnight. The cultures were then diluted to the McFarland No.5 standard (1.0×10^8 CFU/ml). Standard Petri dishes containing nutrient agar were then inoculated with the bacteria suspension (1.0×10^8 CFU/ml). Sterile paper disks (6 mm) were placed on the inoculated plates and 10 µl of 10 mg/ml of the essential oils in 10% DMSO were added to the paper disk. The plates were then incubated at 37°C for 24 h and the zone of inhibition was measured. Tests were performed in triplicate and the mean values reported; Ampicillin and Neomycin were used as positive controls.

The minimum inhibitory concentration (MIC) of the essential oils was determined by the method of Eloff (1998). Nutrients broth (50 µl) was added to all wells of the microtitre plate; 50 µl of the essential oils (10 mg/ml) in 10% DMSO was added to the well in row A and then serially diluted down the rows from row A. The remaining 50 µl was discarded. Bacteria culture (50 µl) of McFarland standard was then added to all the wells and then incubated at 37°C for 24 h. *p*-Iodonitrotetrazolium violet (INT) solution (20 µl of 0.2 mg/ml) was then added to each well and incubated at 37°C for 30 min. The MIC is the lowest concentration at which no visible microbial growth is observed. The minimum bactericidal concentration (MBC) is the lowest concentration of the sample at which inoculated bacterial strains are completely killed. This was confirmed by reinoculating 10 µl of each culture medium from the microtiter plates, which were used for MIC, on nutrient agar plates and incubated at 37°C for 24 h. Bacteria treated with ampicillin and neomycin, were used as positive controls.

Antioxidant activity

The essential oils were screened for antioxidant activity: the (DPPH:1.1-diphenyl-2-picrylhydrazyl; ABTS: 2,2-azino-bis (3-ethylebenzthiazoline-6-sulfonic acid); HO: hydroxyl radicals; SO: super oxide radicals; NO: nitric oxide radicals) scavenging activity, Fe^{2+} chelating activity, total antioxidant capacity, and the SH (sulphurhydryl) content of the essential oils were determined by the methods previously outlined (Opoku et al., 2002, 2007; Simelane et al., 2010)

Unless otherwise stated, ascorbic acid, Trolox and BHT were used as standards. All assays were done in triplicate. The inhibitory effect of the extract on each parameter was calculated as:

$$\text{Inhibition (\%)} = \left(1 - {At}/{Ao}\right) \times 100$$

Where, A_0 is the absorbance value of the fully oxidized control and A_t is the absorbance of the extract. The inhibitory concentration

Table 1. Physicochemical properties of the essential oils of *T. violacea*.

Physicochemical properties	
Percentage yield	0.15%
Physical appearance	Pale yellow
Smell	Strong pungent smell
Refractive index	1.5078

Table 2. Volatile constituents of the essential oil of *T. violacea*

Peak no.	Compounds	R.T[a]. (mins)	Relative abundance (%)
1	Acetamide, 2-cyano	4.16	2.08
2	Chlorodifluoro acetamide	4.20	2.70
3	σ- xylene	4.24	7.08
4	(E)-2-heptenoic acid	5.12	1.10
5	ρ-xylol	5.87	5.88
6	ρ-xylene	6.17	6.43
7	Thiodiglycol	19.24	6.17
8	2,4-Dithiapentane	23.11	51.04
9	Chloromethylmethyl sulfide	29.78	8.62
10	Acetamide	29.67	1.64
11	phthalic acid 2-ethylhexyl isobutyl ester	29.71	0.92
12	Phthalic acid	29.77	1.08
13	phthalic acid, heptyl2-methylallyl ester	29.87	0.32
14	Nonadecane	36.98	2.21
15	Heptacosane	38.70	3.02
16	Tetracosane	40.33	1.96

[a]Retention time.

providing 50% inhibition (IC_{50}) was determined using statistical package Origin 6.1.

Cytotoxicity

MTT cell proliferation assay

The cells used for this assay were the human embryonic kidney cells (HEK293) and human hepatocellular carcinoma cells (HepG2). Cells were cultured in 25 cm^3 flask to confluency, thereafter trypsinised and plated into 48 well plates at seeding density of 2.5 x 10^4 per well. Cells were then incubated overnight at 37°C. Fresh medium (MEM +Glutmax + antibiotic) were then added. The essential oils (50 to 350 µg) were then added in triplicate and incubated for 4 h. The medium was then replaced by complete medium (MEM + Glutmax + antibiotics + 10% fetal bovine serum). After 48 h, cells were subjected to the MTT assay (Mosman, 1983).

RESULTS AND DISCUSSION

The percentage yield (Table 1) of the essential oil (which was pale yellow, with refractive index of 1.508) was 0.80% (v/w).

The chemical composition and constituent percentage of the essential oil of the rhizomes is presented in Table 2. A total of 16 constituents (representing 95.95%) were identified in the oil of *T. violacea*. The major components were 2,4-dithiapentane (51.04%), *p*-xylene (6.43%), chloromethylmethyl sulfide (8.62%), σ-xylene (7.38%), thiodiglycol (6.17%), and ρ-xylol (5.88%). In a recent study (Olorunnisola et al., 2012), 7 compounds (representing 22.48% of the total oil) were identified in the *T. violacea* collected from the Eastern Cape of South Africa. 2,4-Dithiapentane (representing 11.35% of their compounds) was also reported to be the major component of the oil. It is apparent that the major components of the essential oils of the rhizomes of *T. violacea* do not include the usual monoterpenoids and sesqiterpenoids associated with essential oils reported in literature (Tariku et al., 2011; Ahmad et al., *2006;* Dos Santos et al., 2001; Ahmad et al., 1988). The oil of *T. violacea* is rather rich in sulfur-containing compounds that are similar to those found in *Allium sativa* (garlic) (Martinez-Velazquez et al., 2011; El-meleigy et al., 2010;

Table 3. Antibacterial activities of the essential oils of *T. violacea* (zones of inhibition)[a].

Bacteria strain	Neomycin	Ampicillin	5 mg/ml of the essential oils of *T. violacea*	10 mg/ml of the essential oils of *T. violacea*	10% DMSO
Esherichia coli (ATCC 8739)	11.0 ± 2	10.0 ± 0	NA	NA	NA
Psuedomonas aeruginosa (ATCC 19582)	15.3± 1.5	9.0 ± 0.1	11.0± 0.1	12.0 ± 0.1	NA
Staphylococcus aureus (ATCC 6538)	16.0± 8.2	13.0 ± 0	7.0 ± 0.1	6.0 ± 0	NA
Streptococcus faecalis (ATCC 29212)	13.3± 0.6	11.0±0.2	9.0 ± 0	10 ± 0.1	NA
Bacillus cereus (ATCC 10702)	14.7± 2.5	14 ±0.2	6.5 ± 0.1	6.2 ± 0.1	NA
Bacillus pumilus (ATCC 14884)	14.3± 1.2	13.0 ± 0	NA	NA	NA
Psuedomonas aeruginosa (ATCC 7700)	12.0 ± 1.2	10.0 ± 0	7 .0 ± 0.1	9.0 ± 0.1	NA
Enterobacter cloacae (ATCC 13047)	11.3± 1.5	11.0± 1.5	NA	NA	NA
Klebsiella pneumonia (ATCC 10031)	12.3± 0.6	11.0± 0.1	NA	NA	NA
Serratia marcescens (ATCC 6830)	15.7± 0.8	8.0 ± 0.2	NA	NA	NA
Acinetobacter calcoaceticus anitratus (CSIR)	14.3± 0.3	11.0± 0.1	9.0 ± 0.1	10.0 ± 0.1	NA
Bacillus subtilis (KZN)	11.3± 1.5	11.0 ± 0	11.0± 0.1	11.0 ± 0	NA
Shigella flexineri (KZN)	11.2± 1.1	10.0 ± 1	12 ± 0.1	9.0 ± 0.1	NA
Salmonella spp. (KZN)	17.0 ± 1	10.0 ± 0	8.0 ± 0.1	9.0 ± 0.1	NA
Staphylococcus epididirmis (KZN)	10.7± 1.5	9.0 ± 0.1	NA	NA	NA
Enterococcus faecalis (KZN)	15.3± 3.1	13.0 ± 0	11.0± 0.1	11.0 ± 0	NA

[a]Inhibition zone diameters (mm) including diameter of the sterile disc (6 mm): values are given as mean ± SD (3 replicates). ND = not determined; NA = not active. ATCC= American Type Culture Collection, USA. CSIR = Council of Scientific and Industrial Research, S.A. KZN = KwaZulu Natal, S.A. DMSO = dimethyl sulfoxide.

Dieumou et al., 2009; Kimbaris et al., 2008). These compounds apparently contribute to the high SH content of the oil (Table 1).

Table 3 shows the antibacterial activities of the essential oil of *T. violacea*. The essential oil exhibited appreciable antibacterial activity against *P. aeruginosa, S. faecalis, A. calcoaceticus anitratus, B. subtilis* and *E. faecalis*. The other organisms showed a degree of resistance to the oil at the concentration tested. The activity of the oil against antibiotic resistant organisms (Table 4) indicates that the oil inhibited the growth of antibiotic resistant strains of *S. aureus* and *S. viridans*. It is apparent that the oil contains components that could be exploited to combat drug resistant microorganisms. The MIC and MBC values (Tables 4 and 5) however reveal the effect of the oil to be more bacterial static than bactericidal.

The antioxidant activity of the essential oil is presented in Table 6. It is apparent that even though the oil exhibits a concentration dependent activity, it is a poor scavenger of DPPH and ABTS radicals. The oil is however a strong scavenger of NO radicals (IC_{50} value of 2.65 mg/ml). The role of nitric oxide (NO) has been controversial as with both protective and harmful effects. For example a dual role of NO has been implicated in many neurological disorders of the body. Its role in the pathogenesis of major depression and modulatory activity of various antidepressants has been indicated in literature (Lee et al., 2004; Galigniana et al., 1999 Stamler et al., 1992). NO is an important signaling molecule, involved in providing innate immunity against pathogens, signal transduction and protection against oxidative stress. The

Table 4. Zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration of the essential oil of *T. violacea* in antibiotic resistant microorganisms.

Antibiotic resistant Bacteria strains	Antibiotic resistant*	10 mg/ml of essential oil of *T. violacea*	MIC of essential oil of *T. violacea* (10 mg/ml)	MBC of essential oil of *T. violacea* (10 mg/ml)
Staphylococcus aureus (P12702)	CIPRO: Levo Clindamycin	9 ± 0.1	10	ND
Staphylococcus aureus (P12763)	CIPRO: Levo Clindamycin	8 ± 0	>10	ND
Staphylococcus aureus (P12724)	CIPRO: Levo Clindamycin	9 ± 0.1	10	ND
Staphylococcus aureus (B 10808)	Oxa: Clox, Oxa: meth, Gentamicin, Penicillin	NA	>10	NA
S. Viridans (S 17141)	Oxa: meth, Oxa, Clox	7 ± 0.1	>10	ND
Psuedomonas aeruginosa (T 3374)	Cotrimoxazole	NA	NA	NA
Klebsiella pneumoniae (S 17302)	Ampicillin	NA	>10	NA
Klebsiella pneumonia (S 17298)	Ampicillin	NA	NA	NA

*Oxa- Oxacillin; Clox-C loxacillin; Meth- Methicillin; Levo- Levofloxacin; CIPRO- Ciprofloxacin. ND= not determined NA = not active.

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils of *T. violacea.*

Bacteria Strains	MIC of essential oil *T. violacea* (10 mg/ml)	MIC of Ampicillin (10 mg/ml)	MBC of essential oil of *T. violacea* (10 mg/ml)
Escherichia coli (ATCC 8739)	NA	1.25	NA
Psuedomonas aeruginosa (ATCC 19582)	2.5	5	>10
Staphylococcus aureus (ATCC 6538)	10	2.5	>10
Streptococcus faecalis (ATCC 29212)	5	5	>10
Bacillus cereus (ATCC 10702)	10	5	>10
Bacillus pumilus (ATCC 14884)	NA	2.5	NA
Psuedomonas aeruginosa (ATCC 7700)	5	5	>10
Enterobacter cloacae (ATCC 13047)	NA	1.25	NA
Klebsiella pneumonia (ATCC 10031)	NA	2.5	NA
Serratia marcescens (ATCC 6830)	NA	NA	NA

Table 5. Continued.

Bacteria Strains	MIC of essential oil *T. violacea* (10 mg/ml)	MIC of Ampicillin (10 mg/ml)	MBC of essential oil of *T. violacea* (10 mg/ml)
Acinetobacter calcoaceticus anitratus (CSIR)	5	NA	>10
Bacillus subtilis (KZN)	2.5	0.625	>10
Shigella flexineri (KZN)	10	5	>10
Salmonella spp. (KZN)	10	5	>10
Staphylococcus epididirmis (KZN)	NA	10	NA
Enterococcus faecalis (KZN)	5	5	>10

MIC values given as mg/ml for essential oils, ND = not determined, NA= not active, DMSO = dimethyl sulfoxide.

Table 6. Percentage scavenging of DPPH, ABTS, nitric oxide and metal chelation of FE^{2+} by the volatile oils of *T. violacea* (mg/ml) and (SH) sulfhydryl content.

Parameter	DPPH	ABTS	($NO^{\cdot}$)	(Fe^{2+})
Control *T. violacea*	100.0 ± 0.02	100.0 ± 0.01	100.0 ± 0.02	100.0 ± 0.02
5 mg/ml	38.76 ± 0.00	11.74 ± 0.06	32.49 ± 0.00	30.16 ± 0.02
10 mg/ml	40.89 ± 0.00	17.88 ± 0.11	41.02 ± 0.02	42.26 ± 0.00
20 mg/ml	42.15 ± 0.01	19.51 ± 0.08	48.00 ± 0.06	51.15 ± 0.01
50 mg/ml	46.54 ± 0.02	21.77 ± 0.04	51.27 ± 0.01	57.54 ± 0.02
100 mg/ml	49.25 ± 0.01	27.91 ± 0.13	62.77 ± 0.04	67.25 ± 0.01
IC_{50} [b]	>5	>5	3.65	2.90
AA (IC_{50})	1.09	1.71	2.32	-
BHT (IC_{50})	-	-	3.22	-
Trolox (IC_{50})	>5	>5	-	-
Citric A (IC_{50})	-	-	-	2.63
EDTA	-	-	-	2.97
(SH) sulfhydryl content of the volatile oils of *T. violacea*			37.51 µg/g (w/w)	

[a] (n = 3, × ± SEM), [b] IC_{50}– inhibitory concentration, ($NO^{\cdot}$)- nitric oxide radical scavenging; (Fe^{2+}) metal chelating.

high nitric oxide radical scavenging activity of the oil is possibly due to the high SH content. NO reacts with SH groups forming *s*-nitroso derivatives (Galigniana et al., 1999).

The essential oil of *T. violacea* also shows a high Fe^{2+} chelating activity (Table 6). H_2O_2 can react with reduced Fe^{2+} and Cu^{+} to produce highly toxic $OH^{\cdot}$; the uncharged $OH^{\cdot}$ is able to penetrate membranes (Thirupathi et al., 2011; Moller 2001).

Reactive oxygen species are known to cause various diseases in living cells (Lee et al., 2004). The antioxidants results indicate that *T. violacea's* oil may not necessary scavenge pre-existing free radical but, it does show the potential to prevent the generation of free radicals through Fe^{2+} chelating.

Biochemical instability of the alliicin, thiosulfonates and related compounds, as well as its strong odour have probably limited the use of *T. violacea* as a potential food additive or food preservative (Benkebli, 2004). However, the therapeutic properties of garlic (*A. sativum*), which is a close relative of *T. violacea,* have been ascribed to its sulfur compounds (Imani et al., 2002). It is apparent that the sulfur compounds, which are also abundant in *T. violacea,* do contribute to its antioxidant and antimicrobial activities and thus its therapeutic properties.

Since penicillin- and mutation-resistant strains of microbial pathogens are on the increase, there is a need to search for new compounds (that are not penicillin based) that inhibit microbial growth. The antibacterial activity of the essential oil of *T. violacea*, coupled with its antioxidant properties and the low cytotoxicity (IC_{50} values of 1218 and 1641 µg/ml against HEK293 and HepG2 cells, respectively) presents the oil as a good candidate for the search of therapeutic agents. The results obtained suggest the rationale for the use of *T. violacea* in folk medicine.

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