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The influence of extraction methods on the composition and antimicrobial activity of the volatile constituents of *Tulbaghia violacea* Harv. Cultivated in Egypt

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Abstract
Tulbaghia violacea Harv. (Alliaceae), is a small bulbous herb known as “sweet or society garlic" broadly consumed in traditional medicine. The composition and antimicrobial activity of leaf (L) and flower (F) volatiles, obtained by hydro-distillation and solvent extraction, were investigated. The hydro-distilled (decanted oil, DO; and recovered water-soluble oil, RO) and hexane extracted (HE) samples were analysed *via* GC/FID and GC/MS. A total of 64 components, representing 90.63-99.15% of the overall sample compositions, were identified. Sulfur compounds were predominant in all hydro-distilled samples with major 2, 3, 5-trithiahexane and 2, 4, 5, 7-tetrathiaoctane. Meanwhile, oxygenated compounds prevailed in HE being dominated by fatty acids and their esters, mainly hexadecanoic and 9,12-octadecadienoic acids, in addition to an aliphatic ketone (16-hentriacontanone, F). Limonene, 1, 8-cineole, α-terpineol, eugenol, carveol, β-ionone, α-bisabolol and caryophyllene oxide were detected in all samples. All DO and HE samples together with RO of F demonstrated remarkable antimicrobial efficiency. Minimum inhibitory concentrations were determined. Obviously, both composition and bioactivity of the investigated volatiles were clearly influenced by the extraction process applied. Finally, *T. violacea* could be proposed as flavoring agent and a safe preservative in food industries to prevent the growth of foodborne bacteria and fungi and extend the shelf-life of food products.

Keywords: *Tulbaghia violacea Harv, flowers and leaves, volatile Constituents, different extraction methods, antimicrobial activity*

1. Introduction
The genus *Tulbaghia* (Alliaceae) includes twenty one species which are mostly rhizomatous plants and entirely indigenous to Southern Africa. The genus was named after Rijk van Tulbagh (1699-1771), the one-time Dutch Governor of The Cape Province. *Tulbaghia violacea* Harv. (Synonyms: *Tulbaghia cepacea* L.f. and *Omentaria cepacea* Salisb.) is the most popular and widely consumed species of the genus [1]. The plant is a small bulbous herb known as “sweet or society garlic” because its intake is not associated with bad breath as in case of *Allium sativum* [2]. Traditionally, *T. violacea* has been used for the treatment of fever and colds, sinus headache, asthma, pulmonary tuberculosis, and gastrointestinal ailments. South African Zulus use the leaves and flowers as vegetables and the rhizome as an emetic; they also grow this plant around their homes to deter moles and repel snakes [1, 3].
The chemical composition and bioactivities of *T. violacea* have been recently reviewed; published data dealt mainly with its rhizomes, bulbs and leaves [4, 5]. Chemical structures of isolated and identified volatile sulfur-containing compounds, non-volatile flavour precursors, steroidal saponins, flavonoids and carbohydrates were reported. Results of biological evaluation were concerned with the antimicrobial, antioxidant, anticancer, antihypertensive, androgenic, anti-atherosclerotic, antithrombin, antidiabetic, anticoagulant, antihypercholesterolemic and anti-inflammatory effects [4, 5].
Recently, Reinten et al. [6] sorted *T. violacea* among several South African ornamental plants with potential inpact in the international market, both as potted and fresh flowers.
The scarcity of reports concerning the composition of the volatiles of the aerial parts [7-10] of *T. violacea* growing abroad and the lack of those concerning the locally naturalized plant stimulated the performance of this study. In addition, nothing could be traced concerning either the composition or bioactivity of the floral volatiles or the influence of the extraction process on the final product.
The present work aimed to provide a detailed comparative investigation of the composition of the leaf and flower, hydro-distilled and hexane-extracted, volatiles and to evaluate their

Antimicrobial potential. In addition, the composition and bioactivity of the hexane-recovered volatiles, derived from the aqueous layer of the distillate which is usually discarded, were also examined. This was performed in an attempt to select the most efficient herbal product and/or extraction procedure for further industrialization.

2. Materials and Methods

2.1. Plant Material

Leaves and flowers of *T. violacea* Harv. were obtained from plants cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt, during the flowering stage from March to October (2012-2013). The plant was kindly authenticated by Mrs. Therese Labib, Herbarium Section, Orman Garden, and Giza, Egypt. Identity was confirmed by Dr. Mohamed El Gebali (Ph.D., Botanist specialist). Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Cairo University (# 16.6.2014.1).

2.2. *Preparation of Samples*

2.2.1. Hydro-distilled samples

Fresh samples of leaves and flowers of *T. violacea* (one Kg, each) were separately subjected to hydro-distillation in a Clevenger-type apparatus. The supernatant oily layer was separated by decantation to yield the 'decanted' essential oil (**DO**)**.** The milky distilled aqueous layer was collected throughout the process, and then thoroughly mixed with hexane to trap any dissolved volatiles. The pooled hexane extracts were dried (anhydrous sodium sulphate) and concentrated under vacuum at a temperature not exceeding 40 ºC, to yield the 'recovered' essential oil (**RO)** [11, 12].

2.2.2. Solvent-extracted samples

The hexane-extracted (**HE**) volatiles were prepared, from fresh leaf and flower samples (one kg, each), by cold percolation till exhaustion. The solvent was then removed by evaporation under reduced pressure at a temperature not exceeding 40ºC to yield a semisolid product.

Percentage yields of the prepared samples were calculated on fresh weight basis (v/w for DO and RO and w/w for HE), followed by storing in sealed glass vials, at -4ºC, until gas chromatographic analysis and antimicrobial testing.

2.3. GC/FID and GC/MS analyses

GC/FID analysis: Samples were analyzed on an Agilent 6890 Series GC System (CA, USA) equipped with flame ionization detector (FID) and DBI column (30 m, 0.53 mm ID, 1.5 µm film (J&W Scientific). Helium was the carrier gas (1.0 ml/min). The volume injected was 1.0 µl. The Column temperature program was: 40 ºC (1 min), 7.5 ºC/min to 150 ºC, 1.2 ºC/ min to 250 ºC. Injector and detector temperatures were 250 ºC and 280 ºC.

GC/MS analysis: The GC/MS analysis of the essential oils was performed on a Shimadzu GC/MS-QP 5050 A and the operating conditions being the same as those described for GC/FID. The GC/MS instrument was used in the ionization mode EI, under an ionization source 70 eV; the data of mass spectra were acquired in the scan mode in m/z range 40-500.

Identification of the components was achieved by library search database (Wiley 7 Nist 05 Lib. and W8N08 Lib.) and by comparing their retention indices with reference to a homologous series of C_8-C_{20} *n*-alkanes, and their mass spectra with published data [9, 13-16]. Relative percentages of different constituents were calculated based on the GC/FID peak areas without the use of response factor correction and are represented in Table (1).

2.4. Evaluation of Antimicrobial Activity

2.4.1 Microbial strains and culture media

Selected strains of bacteria and fungi *viz.*, Gram-positive bacteria: *Bacillus subtilis* (ATCC 6633)*, Bacillus cereus (ATCC 2*5923)*, Staphylococcus aureus* (ATCC 25923) ; and Gram- negative bacteria: *Escherichia coli, Pseudomonas aeruginosa (ATCC 9027)* and *Salmonella typhimurium* (ATCC 14028) and fungi*: Candida albicans* (ATCC 14028) and *Aspergillus flavus* (nrrl 1957), were supplied by the Microbiology Department, Cairo University Research Park, Faculty of Agriculture, Cairo University, Cairo, Egypt. Bacterial strains were grown on Nutrient agar medium (Oxoid, England); *Candida albicans* (ATCC 14028) on Glucose agar and *Aspergillus flavus* (nrrl 1957) on Potato Dextrose agar.

2.4.2 Standardized single disk method:

The antimicrobial screening was performed by applying a standardized single disk method [17]. Tested samples (**DO**, **RO** and **HE** of **L** and **F**) were dissolved in DMSO and sterile paper discs (5 mm in diameter) were individually charged with 10 µl sample solutions (equivalent to 10 µg of the oil and 50 µg of the extract/disc) and transferred aseptically onto the surface of inoculated agar plates. These were incubated during: 24 hrs at 30-37 ºC for bacteria, 48 hrs at 30 ºC for *Candida albicans* and 36 hours at 25 ºC for *Aspergillus flavus*. A disc impregnated with 10 µl *o*f DMSO was used as negative control. Discs of Ampicillin and Penicillin G (10 µg/disc, each), Polymyxin (130 units/disc) and Nystatin (100 units/disc) were used as positive controls. After incubation, diameters of zones of inhibition were measured (mm) in triplicates.

2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

MICs were determined using several dilutions (w/v) of **DO, RO** and **HE** in DMSO. Aliquots (10 µl) of tested dilutions were, separately, transferred onto sterile paper discs (5 mm in diameter). These were placed onto plates inoculated with suspension of 10^5/ml colonies forming units (CFU) of microbial cultures and incubated at 30 °C for 24-48 hrs. The lowest concentration producing complete inhibition of each test organism was recorded as minimum inhibitory concentration (MIC). Each test was performed in triplicates.

3. Results and Discussion

A number of techniques are applied for isolation of volatiles from raw plant materials including: hydro-, steam distillation, organic solvent, supercritical-CO_2 and ultrasonic extraction processes. The most commonly used methods are hydro- or steam distillation. Noticeably, distillation process results in incomplete recovery of the essential oil, since a part of the oil becomes dissolved in the distillation water which is usually discarded and reduces the overall yield of the essential oil. The collected oily layer is referred to as the decanted oil, while, the dissolved fraction has been recovered by solvent extraction yielding the recovered oil. The yield, physicochemical characteristics and bioactivities of the resulting products are hence expected to be influenced by the method applied. Therefore, this prompted the comparative investigation of

three different samples *viz*., the decanted and recovered oils (**DO** and **RO**) obtained by hydro-distillation and direct hexane extraction (**HE**) of each of the leaf (**L**) and flower (**F**) of *t*he plant.

3.1. Yield and sensory characters:
The distilled samples (**DO** and **RO**) were obtained as yellow to yellowish orange oily liquids and the **HE** as green to yellowish green semi-solids, all with strong garlic-like odour. In fact both organs produced a strong persistent garlic-like odour when bruised thus suggesting that the trivial name "sweet or society garlic" appeared not applicable to *T. violacea* [9].The percentage yields of distilled volatiles derived from leaves and flowers were 0.32 and 0.35% (v/w, fresh weight basis) for the (**DO**) and 0.36 and 0.53% (v/w) for the (**RO**) indicating that a relatively high proportion of the distilled samples is water soluble; meanwhile, the hexane extractives (**HE**) amounted to 3.49 and 2.24 % (w/w), respectively.

3.2 Chemical profiles:
Results of GC/FID and GC/MS analyses, as displayed in Table (1), indicated a qualitative and quantitative variability among the chemical profiles of the **DO**, **RO** and **HE** obtained from fresh leaves and flowers of *T. violacea*. The operating conditions adopted allowed the identification of a total of 64 constituents in the examined leaf and flower samples (43 and 48 in **DO**; 35 and 41 in **RO**; and 44 and 48 in **HIM**) amongst which 21 were common. Components identified represented 92.82-99.15% of the overall composition of the analysed volatiles. These included a variety of sulfur-containing, oxygenated (alcohols, carbonyls, aromatics, fatty acids and esters), and non-oxygenated (hydrocarbons) compounds of either terpenoid or aliphatic nature.
The recorded data (Table 1) demonstrated the prevalence of sulfur-containing components in the hydro-distilled **DO** (79.74 and 57.49 %) and **RO** (88.99 and 76.05%) samples of **L** and **F**, respectively. Among these constituents, The major was 2,3,5-trithiahexane (compound **I**) in **DO** and **RO** of **L** (35.53 and 38.17%) followed by 2,4,5,7- tetrathiaoctane (compound **II**) (28.35 and 29.76%). Meanwhile, the amount of the latter in **F** exceeded that of the former (19.04 vs 29.61%; and 17.01 vs 24.52%, respectively), in addition to, dimethyl disulfide, and dimethyl trisulfide. In conclusion, the strong garlic-like odour of the investigated volatiles (either **DO**, **RO** or **HE**) may be mainly attributed to compounds I & II together with dimethyl disulfide, dimethyl trisulfide [14, 18]. Moreover, a number of sulfur volatiles *viz.,* 2,4-dithiapentane; 2,4,6-trithiaheptane; 2,3,4,6 tetrathiaheptane; 2,3,5,7-tetrathiaoctane; 2,3,4,6,8-pentathianonane; 2,4,5,6,8-pentathianonane; 2,4,5,7,9-pentathiadecane; 2,3,5,6,8,10-hexathiaundecane were detected as minors in the same samples. The sulfur compounds identified in the hydro-distilled samples could thus be considered as thermal degradation products of marasmicin which is produced through the enzymatic cleavage of marasmin in an analogous manner to allicin from alliin [2, 9] i.e. as 'artifacts' generated by heat during distillation process. However, a fraction of the distinctly lower amounts of sulphurous components detected in the corresponding cold solvent-extracted **HE** samples (24.32 and 8.64% in **L** and **F**, respectively) could still be referred to the thermal instability of the parent compound (s) during GC/MS analysis (e.g. in the injector) [9]. It can be observed that most of the sulphur-containing compounds were acyclic oligosulfides containing the CH_3SCH_2-moiety [m/z 61 (100)] and are formed by association of -CH_2S- units as indicated through identification of their precursors [14, 19]. Yet, cyclic polysulfides such as 1, 2, 4-trithiolane were found in lower amounts in all samples meanwhile, 1, 3, 5-trithiane was present in trace amounts in the **DO** and **HE**. In addition, among components tentatively identified were: dipropyl trisulfide, *Bis*-(3-chloropropyl) sulfide and thiodiglycol.
As regards the non-sulfurous components, these consisted of variable mixtures of oxygenated and non-oxygenated constituents (Table 1). The higher amount of oxygenated compounds was recorded in the **HE** samples (60.28 and 63.47% in **L** and **F**, respectively) followed by the corresponding **DO** (17.95 and 27.99%), among them, monoterpenoids *viz.,* α-terpineol and 1, 8-cineole and sesquiterpenoids such as *β*-ionone, caryophyllene oxide, α-bisabolol and hexahydrofarnesyl acetone were detected in all samples. All aromatic compounds were oxygenated and detected in high percentages in the **DO** of **F** (17.02%) and **HE** of both **L** and **F** (18.22 vs 16.85%); eugenol and carvacrol were common components in all samples. Moreover, a number of fatty acids and alcohols, and fatty acid esters were characteristic components of **HE** of both plant organs, in addition to, an aliphatic ketone (16-hentriacontanone) in the **F** are probably responsible of their evident higher content of oxygenated components over other samples (**DO** and **RO** Fatty acids reached up to 13.21% in **L** (**HE**) and 11.11% in that of **F** with hexadecanoic and 9,12-octadecadienoic acids as predominant besides the combined amounts of the methyl and ethyl esters (11.31-11.55%), in addition to an aliphatic ketone (16-hentriacontanone, 13.61%) in **F** (**HE**).
The total amount detected of hydrocarbons was highest in **HE** of **F** reaching up to 20.71%. Monoterpenoids of the group were only represented by limonene in all samples, whereas, sesquiterpene hydrocarbons represented by *β*-caryophyllene and *α*-humulene were detected in **DO** and **HE**. Similarly, **HE** of **F** was found the richest in aliphatic hydrocarbons, reaching up to 14.92%, with major nonacosane (6.42%).
The fact that 2,4,5,7-tetrathiaoctane and 2,3,5- trithiahexane prevailed in the distilled samples is quite in accordance with previously reported data on those derived from the aerial parts (48.2 and 10.5%; Pino et al., 2008) and rhizomes (38.5 and 20.1%; [9] of plants growing abroad. Moreover, a wide variety of components, herein identified, were as well reported in trace amounts by **Pino *et al***. [7] (Table 1). However, essential oils distilled from two different samples of South African *T. violacea* rhizomes [8, 10] were found different in composition from each other and from those investigated in the present study: dimethyl disulfide, dimethyl trisulfide, (methyl methylthio) methyl, 2,4-dithiapentane (11.35%) and (methylthio) acetic acid, 2-(methylthio) ethanol, 3-(methylthio)- and propanenitrile (7.20%) were the detected constituents in oil sample [8]; and 2,4-dithiapentane (51.04%), chloromethylmethyl sulfide (8.62%), thiodiglycol (6.17%) in the other [10].

Table 1: GC/MS analysis of the decanted hydro-distilled (DO), recovered water-soluble (RO) essential oils and hexane extracts (HE) of the leaves (L) and flowers (F) of *Tulbaghia violacea* Harv.

R. T.	RI[e]	Compounds	MF (MW)	Relative %						ID
				DO		RO		HE		
				L	F	L	F	L	F	
3.39	743	Dimethyl disulfide	$C_2H_6S_2$ (94)	1.82	0.50	1.40	0.95	4.52	0.5	MS, RI
4.84	890	2, 4 Dithiapentane	$C_3H_8S_2$ (108)	0.12	2.12	-	-	0.11	0.31	MS, RI
5.91	962	Benzaldehyde	C_7H_6O (106)	0.30	0.26	-	2.34	2.5	0.11	MS, RI
6.11	970	Dimethyl trisulfide	$C_2H_6S_3$ (126)	0.24	0.75	7.10	0.32	1.11	0.22	MS, RI
7.21	1029	dl-Limonene	$C_{10}H_{16}$ (136)	0.04	0.23	0.13	0.29	2.87	2.26	MS, RI
7.22	1033	1,8-Cineole	$C_{10}H_{18}O$ (154)	0.26	0.47	0.11	0.38	3.06	0.71	MS, RI
8.52	1065	1,2,4-Trithiolane	$C_2H_4S_3$ (124)	4.21	0.33	5.58	5.79	2.33	0.44	MS, RI
9.13	1099	Linalool	$C_{10}H_{18}O$ (154)	1.12	0.91	-	-	1.73	0.88	MS, RI
9.39	1108	Phenethyl alcohol	$C_8H_{10}O$ (122)	0.57	0.29	-	-	0.08	0.11	MS, RI
9.68	1131	Thiodiglycol	$C_4H_{10}O_2S$ (122)	0.51	1.01	0.08	0.62	0.01	0.47	MS, RI
9.81	1144	2,3,5-Trithiahexane	$C_3H_8S_3$ (140)	**35.53**	**17.01**	**38.17**	**24.52**	3.92	1.50	MS, RI
10.44	1191	Terpineol <α->	$C_{10}H_{18}O$ (154)	1.69	0.5	2.10	0.58	4.43	3.96	MS, RI
10.83	1215	Dimethyl tetrasulfide	$C_2H_6S_4$ (158)	-	-	-	-	0.06	-	MS, RI
10.93	1228	Carveol <cis>	$C_{10}H_{16}O$(152)	0.14	0.29	0.13	0.23	-	-	MS, RI
10.96	1232	Nerol	$C_{10}H_{18}O$ (154)	0.55	-	0.65	-	0.16	-	MS, RI
11.57	1242	2,4,6-Trithiaheptane	$C_4H_{10}S_3$ (154)	0.86	0.99	0.59	1.06	0.16	0.34	MS, RI
11.81	1243	Carvone	$C_{10}H_{14}O$ (150)	-	-	2.69	2.96	-	-	MS, RI
12.10	1251	Anethole <trans->	$C_{10}H_{12}O$ (148)	-	4.50	-	0.43	-	6.44	MS, RI
12.12	1255	geraniol < trans->	$C_{10}H_{18}O$(154)	1.13	-	0.24	-	0.14	-	MS, RI
12.33	1259	1,3,5,Trithiane	$C_3H_6S_3$ (138)	0.08	0.63	-	-	0.20	0.29	MS, RI
12.67	1298	Carvacrol	$C_{10}H_{14}O$ (150)	0.71	0.98	0.12	0.27	4.36	1.86	MS, RI
12.69	1300	Tridecane	$C_{13}H_{28}$ (184)	-	0.39	-	-	-	0.26	MS, RI
13.01	1312	Dipropyl trisulfide	$C_6H_{14}S_3$ (182)	-	-	-	0.53	-	0.14	MS, RI
13.19	1315	Bis (3-chloropropyl)sulfide	$C_6H_{12}Cl_2S$ (187)	0.51	1.12	0.12	4.43	5.03	3.18	MS, RI
13.31	1355	2,3,4,6 Tetrathiaheptane	$C_3H_8S_4$ (172)	1.11	0.4	1.36	1.09	-	-	MS, RI
13.88	1361	Eugenol	$C_{10}H_{12}O_2$ (164)	1.34	5.32	0.51	0.82	4.14	4.01	MS, RI
14.02	1420	caryophyllene < β->	$C_{15}H_{24}$ (204)	0.17	5.63	-	-	1.93	1.43	MS, RI
14.73	1454	Geranylacetone < *E* ->	$C_{13}H_{22}O$ (194)	0.52	-	0.17	-	2.08	-	MS, RI
14.98	1457	Humulene <α->	$C_{15}H_{24}$ (204)	0.20	1.56	-	-	0.79	2.10	MS, RI
15.11	1466	2,3,5,7-tetrathiaoctane	$C_4H_{10}S_4$ (186)	-	1.09	-	5.07	-	-	MS, RI
15.31	1480	2,4,5,7-tetrathiaoctane	$C_4H_{10}S_4$ (186)	**28.35**	**19.04**	**29.76**	**29.61**	**6.87**	1.25	MS, RI
15.64	1490	β-Ionone<trans->	$C_{13}H_{20}O$ (192)	1.2	0.39	0.66	0.56	0.55	1.12	MS, RI

R.T.	RI[e]	Compounds	MF (MW)	Relative %						ID
				DO		RO		HE		
				L	F	L	F	L	F	
16.20	1581	caryophyllene oxide	$C_{15}H_{24}O$(220)	**DO**	**RO**	**HE**	0.11	0.21	0.13	MS, RI
17.93	1642	Muurolol <epi-α>	$C_{15}H_{26}O$ (222)	L	F	L	F	L	F	MS, RI
18.25	1673	2,3,4,6,8-Pentathianonane	$C_4H_{10}S_5$ (218)	0.45	0.01	0.17	0.35	-	-	MS, RI
18.41	1684	2,4,5,6,8-Pentathianonane	$C_4H_{10}S_5$ (218)	0.82	2.16	0.9	0.34	-	-	MS, RI
18.50	1687	Bisabolol < α ->	$C_{15}H_{26}O$(222)	0.12	1.38	0.01	0.36	0.03	0.34	MS, RI
18.75	1700	Heptadecane	$C_{17}H_{36}$ (240)	-	-	-	-	-	0.25	MS, RI
20.26	1774	2,4,5,7,9-Pentathiadecane	$C_5H_{12}S_5$ (232)	4.32	9.08	2.97	1.02	-	-	MS, RI
21.37	1845	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$ (268)	0.44	0.20	0.03	0.32	0.28	0.18	MS, RI
21.05	1870	2,3,5,6,8,10-Hexathiaundecane	$C_5H_{12}S_6$ (264)	0.81	1.25	0.79	0.35	-	-	MS, RI
21.25	1925	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270)	-	-	-	-	7.59	5.66	MS, RI
21.64	1940	1,2 Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$ (278)	-	2.09	-	1.07	2.1	1.5	MS, RI
21.73	1965	Hexadecanoic acid	$C_{16}H_{32}O_2$ (256)	-	-	-	-	5.18	**9.49**	MS, RI
22.10	1993	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$ (284)	0.79	0.16	0.09	1.57	0.04	0.85	MS, RI
23.25	2095	Phytol	$C_{20}H_{40}O$ (296)	0.12	-	0.33	-	0.19	-	MS, RI
23.32	2099	9,12,15-Octadecatrienoic acid , methylester	$C_{19}H_{32}O_2$ (292)	-	-	-	-	2.14	4.09	MS, RI
23.34	2100	Heneicosane	$C_{21}H_{44}$ (296)	-	0.18	0.03	0.82	-	-	MS, RI
23.84	2113	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$ (280)	1.65	0.11	0.02	0.29	**8.03**	1.62	MS, RI
24.22	2177	9,12, 15-Octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$ (306)	1.95	2.01	0.79	1.69	1.78	0.71	MS, RI
24.73	2188	1, 21-Docosadiene	$C_{22}H_{42}$ (306)	-	-	-	-	-	0.91	MS, RI
25.47	2300	Tricosane	$C_{23}H_{48}$ (324)	-	0.92	-	0.80	-	0.77	MS, RI
26.23	2349	9-Octadecenamide	$C_{18}H_{35}NO$ (281)	-	-	-	-	0.44	-	MS, RI
26.56	2400	Tetracosane	$C_{24}H_{50}$ (338)	0.02	-	0.12	0.23	0.06	-	MS, RI
26.79	2456	Docosanol <1->	$C_{22}H_{46}O$ (326)	-	-	-	-	0.04	1.49	MS, RI
27.20	2471	9-Pentacosene	$C_{25}H_{50}$ (350)	0.09	0.87	-	0.09	0.10	1.33	MS, RI
27.41	2499	1,2 Benzendicarboxylic acid, bis (2-ethylhexyl)	$C_{24}H_{38}O_4$ (390)	0.24	3.58	0.20	1.66	5.04	2.82	MS, RI
27.44	2500	Pentacosane	$C_{25}H_{52}$ (352)	0.91	0.98	-	0.57	0.28	0.09	MS, RI
27.71	2577	Tricosanol <1->	$C_{23}H_{48}O$ (340)	-	-	-	-	1.69	0.09	MS, RI
28.45	2600	Hexacosane	$C_{26}H_{54}$ (366)	-	0.25	-	-	-	2.5	MS, RI
29.31	2700	Heptacosane	$C_{27}H_{56}$ (380)	0.03	0.57	0.20	0.57	-	1.43	MS, RI
30.66	2800	Octacosane	$C_{28}H_{58}$ (394)	-	0.16	-	0.01	-	0.96	MS, RI
31.09	2900	Nonacosane	$C_{29}H_{60}$ (408)	-	0.21	-	0.04	-	6.42	MS, RI
32.94	3304	16-Hentriacontanone	$C_{31}H_{62}O$ (450)	-	1.72	-	-	-	**13.61**	MS, RI
		Sulfur compounds		79.74	57.49	88.99	76.05	24.32	8.64	
		Monoterpene hydrocarbons		0.04	0.23	0.13	0.29	2.87	2.26	

R.T.	RI[e]	Compounds	MF (MW)	Relative %						ID
				DO		RO		HE		
				L	F	L	F	L	F	
		Oxyg. monoterpenes		5.41	2.17	6.09	4.15	11.6	5.55	
		Sesquiterpene hydrocarbons		0.37	7.19	-	-	2.72	3.53	
		Oxyg. sesquiterpenes		4.87	4.8	0.77	1.35	3.34	3.46	
		Oxg. aromatic compounds		3.16	17.02	0.83	6.59	18.22	16.85	
		Other oxyg. components		4.51	4	1.23	3.55	27.12	37.61	
		Aliphatic hydrocarbons		1.05	4.53	0.35	3.13	0.44	14.92	
		Total % Identified compounds		**99.15**	**97.43**	**98.39**	**95.11**	**90.63**	**92.82**	
		No. Identified compounds		**43**	**48**	**35**	**41**	**44**	**48**	
		% yield*		**0.32**	**0.35**	**0.36**	**0.53**	**3.49**	**2.24**	

RI[e]: experimentally determined retention indices on HP-5MS column by injection of a homologous series of *n*-alkanes C_8-C_{20}; ID: identification method; MS: mass spectra; RI: retention indices matching to built-in Wiley Mass Spectral Database (Wiley 7 Nist 05 Lib. and W8N08 Lib.) and published retention indices; Major compounds and other predominant components were marked in bold; Yield*: DO & RO (v/w) and HE (w/w) calculated on fresh weight basis.

3.3 Antimicrobial activity

The antimicrobial activity of DO, RO and HE of flowers and leaves of *T. violacea* was evaluated against selected bacterial and fungal strains. Results depicted in Table (2 and 3) revealed that the Gram-positive bacteria are more susceptible to almost all tested samples than the Gram-negative ones and that *Pseudomonas aeruginosa* was the most resistant.

Indeed apart from leaf **RO**, all samples exerted a remarkable growth-inhibitory action against Gram-positive bacteria with lowest MICs against *Bacillus. Subtilis, Bacillus cereus* and a moderate activity against *Staphylococcus aureus*, as compared to the standard antibacterial drugs (Table 2 and 3). As mentioned by Burt [20], the antimicrobial potential of these samples is mostly related to the presence of oxygenated components either monoterpenoids like linalool, 1, 8 cineol and α-terpineol or phenylpropenoids such as carvacrol, eugenol and anethole. The hydrophobicity of essential oils and their components enables them to partition in the lipids or act on cell proteins embedded in the bacterial cell membrane and mitochondria, rendering them more permeable for ions and other cell contents, and finally leading to impairment of essential cell processes and cell death [21]. The inefficiency of **RO** samples against Gram-positive bacteria may be referred to the prevalence of sulfur compounds (I &II) [22, 23]. In fact, 2, 4, 5, 7-tetrathiaoctane exerted a moderate antifungal effect against *Candida albicans* (MIC 12.5 µg/ml) [18]. Furthermore, the high potency of **HE** samples against *Bacillus subtilis, Bacillus cereus* and their moderate action on *Staphylococcus aureus* may be attributed to the free fatty acids [24], and their methyl esters [25]. The present findings are, however, different from those reported by Soyingbe et al. [10] on the hydro-distilled essential oil of the rhizomes which was found significantly active on *Pseudomonas aeruginosa*, although being consistent with its action against *Staphylococcus aureus*. Moreover, earlier studies revealed a wide variability among antibacterial activity of different leaf and bulb extracts *viz*., aqueous, dichloromethane, ethyl acetate, petroleum ether against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus, K. pneumonia* and *Escherichia coli* [26-29].

Regarding the antifungal effect, all tested samples displayed a pronounced activity against *Aspergillus flavus* which can be related to the presence of limonene, caryophyllene oxide, carvacrol, linalool, eugenol and β-caryophyllene [30] and were ineffective, except for leaf **RO**, on *Candida albicans*. The herein recorded effects are in agreement with those reported for the aqueous extract of the bulbs against *Aspergillus flavus* [31, 32]; although being inconsistent with those previously published concerning *Candida albicans* [28, 33, 34].

Table 2: Antimicrobial Activity of the decanted hydro-distilled (DO), recovered water-soluble (RO) essential oils and hexane extracts of the leaves (L) and flowers (F) of *Tulbaghia violacea* Harv.

Microorganism	Diameter of Inhibition zone (mm)						PC*
	DO		RO		HE		
	L	F	L	F	L	F	
DMSO	NI	NI	NI	NI	NI	NI	
Gram-positive bacteria							
Bacillus subtilis (ATCC 6633)	15±0.33	22.5±3.54	NI	18±2.83	25.5±0.71	28±0	10±0
Bacillus cereus (ATCC 25923)	10.5±0.71	13.7±0.11	NI	9.5±0.71	14.5±0.71	13.5±0.7	8±0.01
Staphylococcus aureus (ATCC 25923)	14.5±1.12	13±0.22	NI	15±1.01	19±1.04	14.5±0.7	35±0.31
Gram-negative bacteria							
Pseudomonas aeruginosa (ATCC 9027)	NI	NI	NI	NI	NI	NI	10±0,02
Escherichia coli (ATCC 8739)	8±1.41	NI	8.5±0.71	NI	NI	NI	18±0.05
Salmonilla typhimurium (ATCC 14028)	NI	NI	8±1.41	NI	NI	NI	17±0.11
Fungi							
Candida albicans (ATCC 14028)	NI	NI	8±0	NI	NI	NI	15±0.04
Aspergillus flavus(nrrl 1957)	15±0.11	20±0.25	10.5±0.4	13.5±0.3	18±0.35	20±0.05	10±0.2

Values are means ± standard deviation (n=3), NI: No inhibition, DMSO: dimethyl sulfoxide: negative control; PC*: positive controls *viz.*; penicillin (10 units/disc) for Gram-positive bacteria, Ampicillin (10 µg/disc) for Gram-negative bacteria, polymyxin (130 units/disc) for *Pseudomonas aeruginosa,* NY statin (100 units/disc) for fungi.

Table 3: Minimum Inhibitory Concentration (MIC) of the decanted hydro-distilled (DO), recovered water-soluble (RO) essential oils and hexane extracts (HE) of the leaves (L) and flowers (F) of *Tulbaghia violacea* Harv.

Microorganism	Minimum Inhibitory Concentration (MIC)					
	DO		RO		HE	
	L	F	L	F	L	F
Bacillus subtilis (ATCC 6633)	0.39±0	1.88±0	NT	1.56±0	3.13±0	0.39±0
Bacillus cereus (ATCC 25923)	0.39±0	1.88±0	NT	0.78±0	12.5±0	1.56±0
Staphylococcus aureus (ATCC 25923)	12.5±0.01	15±0.01	NT	4.69±3.13	12.5±0.11	25±0.25
Pseudomonas aeruginosa (ATCC 9027)	NT	NT	NT	NT	NT	NT
Escherichia coli (ATCC 8739)	12.5±0.12	NT	50±0.01	NT	NT	NT
Salmonilla typhimurium (ATCC 14028)	NT	NT	50±0.02	NT	NT	NT
Candida albicans (ATCC 14028)	NT	NT	50±0.01	NT	NT	NT
Aspergillus flavus(nrrl 1957)	3.13±0.2	7.5±0.11	25±0.31	3.13±0.22	6.25±0,	4.69±2.21

Values are means ± standard deviation (n=3); MIC: minimum inhibitory concentration (µg/ml); NT: not tested

4. Conclusion

The variation in yield, qualitative and quantitative composition of the investigated oil samples compared to those previously reported for the same plant collected in other geographic areas in the world may be attributed to some factors such as time of collection, climate, genetic variability, mode of extraction, etc. Moreover, the great correlation of the components obtained using different techniques for extraction and their biological potency has been confirmed. In conclusion, to obtain a chemical profile representing the total composition of the distilled oils, it was thus advisable to recover the water-soluble volatiles *via* solvent extraction. Moreover, in fact the direct solvent-extraction is favored over distillation for isolation of thermolabile volatiles. This study confirmed the possibility of using *T. violacea* Harv. Essential oils and hexane extracts of the leaves and flowers in food industry as seasoning agent and as a preservative to prevent the growth of foodborne bacteria and fungi. To the best of our knowledge, this is the first report on the chemical composition and antimicrobial activity of the hydrodistilled and hexane extracted floral volatiles of *Tulbaghia violacea* Harv. [35].

Conflict of Interest: The author has no conflict of interest to disclose

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