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Original article

GC-HRTOF-MS metabolite profiling and antioxidant activity of methanolic extracts of *Tulbaghia violacea* Harv

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ABSTRACT

Tulbaghia violacea is a bulbous herb that is used extensively in traditional medicine to alleviate various illnesses. The current study aimed to evaluate the total phenolic and flavonoid contents of methanolic extracts of the bulb, leaves, rhizome, and stem of the plant, *T. violacea,* and to compare the phytochemical profile of these extracts utilizing gas chromatography/high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS). The antioxidant potential of the plant extracts was also determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). All methanolic *T. violacea* extracts were rich in terpenoids, flavonoids, and saponins; however, only leaf extract contained tannins. Cardiac glycosides and anthraquinones were not observed in any of the tested extracts. All plant extracts showed weak antioxidant capabilities in both DPPH and ABTS assays with IC_{50} values from 146.4 ± 1.11 to 303.15 ± 1.98 μg/mL and 136.25 ± 0.03 to 246.09 ± 0.01 μg/mL, respectively. Methanolic bulb extract of *T. violacea* contained significant phenolic content (22.85 ± 3.15 mg GAE/g), followed by rhizomes (17.46 ± 1.75 mg GAE/g), whereas the leaf (9.47 ± 0.64 mg GAE/g) and stem (5.83 ± 0.77 mg GAE/g) extracts had the least phenolic contents. Similarly, the bulb extracts possessed significant flavonoid content (37.59 ± 1.27 QE/g), followed by rhizome (26.40 ± 0.21 QE/g) and leaves (22.67 ± 1.26 QE/g), and then stems with flavonoid content of 8.65 ± 2.11QE/g. These results were significantly different at $P < 0.05$. The GC-HRTOF-MS revealed that stem extract is rich in sulphur-containing compounds (51.2 %), followed by fatty acid amides (23.64 %), esters (10.50 %) and flavonoids (10.51 %). The rhizome extract showed the presence of sulphur-containing compounds (40.11 %), fatty acid amides (37.24 %), fatty acid esters (14.33 %), phenol (3.70 %), butyl alcohol (3.04 %) and sterols (0.77 %), while the bulb extract possessed a high quantity of sulphur compounds (93.34 %) with a lesser amount of fatty acid amide (0.29 %), fatty acid esters (2.91 %), flavonoids (0.81 %) and miscellaneous compounds (1.35 %). Additionally, the leaf extract also possessed sulphur compounds (48.37 %), fatty acid esters (15.77 %) and fatty acid amides (21.97 %), vitamins (9 %), terpenoids (1.37 %), sterols (1.63 %) and phenols (0.19 %). The findings from this study indicate that bulb extract of *T. violacea* holds potential pharmacological properties, which can induce detoxifying enzymes and can protect against reactive oxygen species due to its high number of sulphur-containing compounds.

1. Introduction

Plants present excellent alternatives for the discovery of drugs, nutraceuticals, and feed additives due to the broad spectrum of protective and preventative properties they possess. They contain several other secondary metabolites, which are known for their disease-fighting capabilities when consumed regularly. These phytochemicals include phenolic compounds, alkaloids, terpenes, and essential oils (Awuchi and Twinomuhwezi, 2021). Herbs provide not only essential nutrients to the body, but also phytochemicals

Abbreviations: GC-HRTOF-MS, gas chromatography/high-resolution time-of-flight mass spectrometry.

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that help in the prevention and reduction of chronic diseases, thus promoting general wellbeing (Thakur et al., 2020). Such uses are currently regaining interest around the world, in that many individuals are turning to these products for the treatment of several health ailments (Restani, 2018). Furthermore, medicinal plants have low toxicity and they are good sources of pharmaceutical compounds that act against various diseases (Nyakudya et al., 2020). Generally, the food and allied industries are limiting the use of chemicals, synthetic food and feed additives due to their negative impact on human and animal health, and are opting for natural plant-based additives and preservative agents that can withstand other climatic conditions such as drought (Dikhoba et al., 2019; Lasram et al., 2019).

Tulbaghia violacea Harv. is a bulbous drought-resistant plant in the family Alliaceae and is widely known as wild garlic, society garlic, or sweet garlic (Madike et al., 2019). The leaves and bulbs of *T. violacea* have been employed in herbal medicine to alleviate various diseases, which include tuberculosis, fever, cough, asthma, hypertension, sinus, headache, oesophageal cancer, rheumatism and gastrointestinal disorders (Moodley et al., 2013; Saibu et al., 2015; Madike et al., 2017). Research has demonstrated that *T. violacea* possess antifungal, antibacterial, antioxidant and anti-inflammatory properties (Aremu and Van Staden, 2013; Takaidza et al., 2015; Krstin et al., 2018; Ncise et al., 2021). Mcgaw et al. (2000) revealed that *T. violacea* water and ethanol extracts possess anthelminthic properties. Furthermore, the *T. violacea* leaf extract also showed anticancer effect against several cancer cell lines tested by inducing apoptosis (Motadi et al., 2020). Several sulphur-containing compounds were isolated and identified from *T. violacea* and they include thiosulfinate marasmicin (2,4,5,7-tetra thiaoctan-4-oxide), (R(S)R(C))– S-(methylthiomethyl) cysteine-4-oxide and Methyl alpha-D-glucopyranoside. Thiosulfinate marasmicin has been reported to possess antimicrobial activities, while methyl alpha-D-glucopyranoside has shown anticarcinogenic activity (Ncise et al., 2021). The current research aimed to compare the secondary metabolites of the methanolic bulb, rhizome, stem, and leaf extracts of *T. violacea,* using GC-HRTOF-MS profiling, and explore their antioxidant properties.

2. Materials and methods

2.1. Chemicals and reagents

Ascorbic acid, acetic acid, chloroform, methanol, 1,1-diphenyl-2-picrylhydrazyl salt (DPPH), 2,2′-azinobis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS), sodium bicarbonate (Na_2CO_3), potassium persulphate ($K_2S_2O_8$), sodium nitrite ($NaNO_2$), quercetin, and Trolox were procured from Sigma Aldrich, Germany. Chloroform, sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$), ammonium hydroxide (NH_4OH), and sodium hydroxide (NaOH) were bought from Merck, Germany. Folin-Ciocalteu, aluminium chloride ($AlCl_3$) and gallic acid were purchased from Protea Lab, Johannesburg, South Africa.

2.2. Plant collection and processing

Different plant part materials of *Tulbaghia violacea* were gathered from the Walter Sisulu Botanical Garden in Roodepoort (26°05′33.5″S 27°50′33.6″E), South Africa, in October 2021. The identity of the plant was authenticated by the Nursery Manager, Mr Solomon Nenungwi, of the South African National Biodiversity Institute, Roodepoort in Johannesburg. The voucher specimen (RM01) was filed in the University of Johannesburg herbarium (JRAU). The plant materials were gently cleansed with tap water to eliminate excess dust and soil and then rinsed with distilled water. The leaves, stems, rhizomes, and root bulbs were separated and then dried at room temperature for about 21 days. Thereafter, each plant material was milled to a powder and stored in glass containers in the dark at room temperature until use.

2.3. Sample extraction and preparation

An amount of 10 g of each powder plant material (leaves, stems, rhizomes, and root bulbs) of *T. violacea* was extracted to exhaustion using 80 % methanol by maceration at room temperature for 48 h. The crude extracts were then filtered through Whatman No. 1 filter paper. Methanol was concentrated using a rotary evaporator (Buchi, Germany) and the extract was frozen at −80 °C before freeze-drying in a Telstar Lyoquest freeze drier (Labotec, RSA) for 48 to 96 h, depending on the amount of the extract obtained. Freeze-dried extracts were weighed and stored in a tight glass container away from light at 4 °C until required. The percentage extract yield of the extracts was expressed by dividing the total mass extracted after freeze-drying by the mass of the dried plant sample used for extraction. Stock solutions of 10 mg/mL extract were prepared and dissolved in 80 % methanol for each extract.

2.4. Qualitative phytochemical analysis

All plant extracts were screened for detection of the bioactive constituents, including terpenoids, flavonoids, tannins, saponins, and quinones, executed as described by María et al. (2018), while glycosides, phenols and cardiac glycoside were carried out as per Roghini and Vijayalakshmi (2018), following standard methods.

2.5. Antioxidant activity

2.5.1. DPPH free radical-scavenging assay

DPPH assay was conducted to determine the antioxidant activities of *T. violacea* extracts by the method described by (Phuyal et al., 2020). Ascorbic acid and Trolox were used as the reference standard for antioxidant. The DPPH scavenging activity of the extracts was calculated by using the equation below, and the inhibitory concentration at a 50 % decrease of free radicals (IC_{50}) value was determined. The results were expressed as mean IC_{50} values.

Percentage DPPH scavenged (%) = ((Absorbance $_{control}$– Absorbance $_{sample}$)/ Absorbance $_{control}$) × 100.

2.5.2. The ABTS radical scavenging assay

The assay was conducted as outlined by Dikhoba et al. (2019). Ascorbic acid and Trolox were used as reference antioxidant standards. The ABTS scavenging activity of the extracts were calculated using the equation below and the IC_{50} value was determined. The results were expressed as mean IC_{50} values.

Percentage ABTS scavenged (%) = ((Absorbance $_{control}$– Absorbance $_{sample}$)/ Absorbance $_{control}$) × 100.

2.6. Determination of the total phenolic content

Total phenolic contents of *T. violacea* extracts were determined using the Folin–Ciocalteu (FC) test as described by Sankhalkar and Vernekar (2016). The total phenolic content (mg/mL) was calculated using gallic acid as standard and was expressed as mg equivalents of GA per g of dry matter (mg GAE/g).

2.7. Determination of total flavonoid content

The total flavonoid content of stems, rhizomes, bulbs, and leaves extract of *T. violacea* was determined using the aluminium chloride method according to Sankhalkar and Vernekar (2016). Total flavonoid content was determined from the calibration curve

of quercetin using a linear equation and expressed as mg quercetin equivalent per gram of dry weight (mg QE/g).

2.8. Phytochemical profiling of methanolic extracts by GC-HRTOF-MS

The analysis of secondary metabolites of extracts of *T. violacea* was performed as outlined by Adebiyi et al. (2019) using the Pegasus GC-HRTOF-MS. The compounds were identified using LECO ChromaTOF® software.

2.9. Statistical analysis

Data obtained from this study were examined using the IBM SPSS statistics 27. The experiment was repeated thrice, and the results were presented as mean ± standard deviation (SD). Analyses of variance were performed using one-way ANOVA to determine the significant differences between the mean values ($P < 0.05$).

3. Results

3.1. Phytochemical analysis

The phytochemical screening of methanolic plant extract of stem, rhizome, bulb and leaves of *T. violacea* showed the existence of different secondary metabolites (Table 1). Flavonoids, terpenoids and saponins were the most plentiful secondary metabolites in all the extracts. Phenolics and tannin were only observed in the leaf extract, while absent in all other extracts as evident after adding ferric chloride, which did not change the colour to black or green. Cardiac glycosides and anthraquinones were not found in any of the various extracts. Furthermore, these different plant parts of *T. violacea* varied in their percentage yield extracts, which ranged from 14.88 % to 60.09 % (w/w).

3.2. Antioxidant activity

The results for DPPH and ABTS free radical scavenging effects of the methanolic extracts of *T. violacea* are presented in Table 2. The plant extracts and reference standards (ascorbic acid and Trolox) revealed different antioxidant activities. Ascorbic acid and Trolox showed significant higher antioxidant potency based on both the DPPH and ABTS scavenging assay with the IC_{50} value of 15.83 ± 0.08 and 41.732 ± 0.49 μg/mL at $P < 0.05$, respectively. All tested plant extracts of *T. violacea* showed significant poor DPPH and ABTS radical scavenging activities in comparison with ascorbic acid and Trolox ($P < 0.05$). Their IC_{50} value ranged from 146.4 ± 1.11 to 303.15 ± 1.98 μg/mL in the DPPH assay and 136.25 ± 0.03 to 246.09 ± 0.01 μg/mL in the ABTS assay.

3.3. Total phenolic content

The total phenolic contents of stem, rhizome, bulb and leaves of *T. violacea* plant extract were determined from the calibration curve of gallic acid with the linear regression equation ($y = 10.742x + 0.0337$; $R^2 = 0.9565$). The results in Table 2 indicated that methanolic bulbous extract of *T. violacea* possessed significant higher phenolic content ($P < 0.05$) followed by the rhizomes (17.46 ± 1.75 mg GAE/g), while the leaf (9.47 ± 0.64 mg GAE/g) and stem (5.83 ± 0.77 mg GAE/g) extracts had less phenolic contents.

3.4. Total flavonoid content

The results for the total flavonoid content of methanolic *T. violacea* extracts are listed in Table 2. The flavonoid content of *T. violacea* extracts was established using linear equation ($y = 0.4469 + 0.0048$) $R^2 = 0.9989$, obtained from calibration curve of quercetin. The bulb extracts possessed significant high flavonoid

Table 1
Phytochemical test of different methanolic extracts of *T. violacea.*

Phytochemical tests	Stem	Rhizome	Bulbs	Leaves
Flavonoids				
Basic	+	+	+	+
Acid	+	+	+	+
Terpenoids				
Salkowski test	+	+	+	+
Lieberman Bouchard test	+	+	+	+
Tannins	–	–	–	+
Phenols	–	–	–	–
Saponins	+	+	+	+
Cardiac glycoside	–	–	–	–
Quinones	+	+	+	–
Anthraquinones	–	–	–	–
Extraction yields (% w/w)	60.09	14.88	21.95	53.35

+ = positive test; - = negative test.

Table 2
Inhibitory concentration (IC_{50}) and total phenolic content and total flavonoid content values of the methanolic extracts and reference standard.

Sample	DPPH IC_{50} (μg/mL)	ABTS IC50 (μg/mL)	Total phenolic content (mg gallic acid equivalent/g)	Total flavonoid content (mg quercetin acid equivalent/g)
Stem	290.57 ± 2.91*	221.96 ± 0.90*	5.83 ± 0.77*	8.65 ± 2.11*
Rhizome	157.16 ± 1.76*	136.25 ± 0.03*	17.46 ± 1.75*	26.40 ± 0.21*
Bulbs	146.4 ± 1.11*	246.09 ± 0.01*	22.85 ± 3.15*	37.59 ± 1.27*
Leaves	303.15 ± 1.98*	153.86 ± 0.2*	9.47 ± 0.64*	22.67 ± 1.26*
Trolox	**41.732 ± 0.49***	**38.67 ± 0.06***	–	–
Ascorbic acid	**15.83 ± 0.08***	**52.83 ± 0.08***	–	–

Values are expressed in means ± standard deviation (n = 3). The results show statistical significance (*) at $P < 0.05$.

Table 3
Phytochemical profiling of methanolic leaf, stem, bulb, and root extension extracts of *T. violacea* using gas chromatography/high-resolution time-of-flight mass spectrometry.

	Retention time (min)	Compound Name	Nature of compound	Observed Ion *m/z*	Molecular formula	Peak Area (%)			
						Stem	Rhizome	Bulb	Leaves
1	3,81	Dimethyl trisulfide	Sulphur compound	126	$C_2H_6S_3$	3.70	nd	nd	1.26
2	4,44	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	Butyl alcohol	155	$C_{10}H_{21}N$	nd	1.25	1.00	nd
3	4,56	1-Butanamine, 3-methyl-N-(3-methylbutylidene)-	Butyl alcohol	154	$C_{10}H_{21}N$	nd	1.79	nd	nd
4	5,64	3,5,5-Trimethylhexyl S-2-(dimethylamino)ethyl propylphosphonothiolate	Miscellaneous compounds	264	$C_{16}H_{36}NO_2PS$	nd	nd	1.18	nd
5	5,73	**Disulfide, methyl (methylthio)methyl**	Sulphur compound	140	$C_3H_8S_3$	**7.00**	3.97	**8.69**	**5.63**
6	6,02	**4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-**	Flavonoid	144	$C_6H_8O_4$	**10.51**	nd	0.81	nd
7	7,32	Benzene, 1,3-bis(1,1-dimethylethyl)-	Benzene, phenylpropanes	190	$C_{14}H_{22}$	0.14	nd	nd	0.17
8	8,39	2-Methoxy-4-vinylphenol	Phenol	150	$C_9H_{10}O_2$	nd	3.57	nd	nd
9	8,88	**Methylthio(methylthio-methyl) sulfone,**	Sulphur compound	172	$C_3H_8O_2S_3$	nd	nd	**14.02**	nd
10	9,65	Phosphine, tris(trifluoromethyl)-	Miscellaneous compounds	219	C_3F_9P	nd	0.03	0.01	0.04
11	10,88	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy)tetrasiloxane	Miscellaneous compounds	503	$C_{18}H_{52}O_7Si_7$	nd	nd	0.16	nd
12	11,29	Phenol, 2,5-bis(1,1-dimethylethyl)-	Phenol	206	$C_{14}H_{22}O$	0.17	nd	nd	0.19
13	11,31	Phenol, 2,6-bis(1,1-dimethylethyl)-	Phenol	206	$C_{14}H_{22}O$	nd	0.13	0.04	nd
14	11,46	**2,4,5,7-Tetrathiaoctane**	Sulphur compound	186	$C_4H_{10}S_4$	nd	nd	**34.19**	nd
15	11,47	**Methane, (methylsulfinyl)(methylthio)-**	Sulphur compound	125	$C_3H_8OS_2$	**13.92**	**10.67**	0.97	**41.04**
16	14,22	Tetradonium Bromide	Miscellaneous compounds	269	$C_{17}H_{38}BrN$	nd	nd	nd	0.93
17	14,23	Glycine, N, *N*-dimethyl-, ethyl ester	Amine	131	$C_6H_{13}NO_2$	nd	0.51	nd	nd
18	15,20	**Isomer2: 2,3,5,7-Tetrathiaoctane 3,3-dioxide**	Sulphur compound	218	$C_4H_{10}O_2S_4$	**14.54**	nd	nd	nd
19	16,87	**Hexadecanoic acid, methyl ester**	Fatty acid methyl esters	270	$C_{17}H_{34}O_2$	**6.55**	nd	1.86	**9.35**
20	16,89	2,4,5,6,8-Pentathianonane	Sulphur compound	217	$C_4H_{10}S_5$	2.11	nd	nd	nd
21	17,35	Dibutyl phthalate	Plasticizer	225	$C_{16}H_{22}O_4$	nd	nd	0.10	nd
22	17,52	Nonanamide	Amide	156	$C_9H_{19}NO$	0.98	nd	nd	nd
23	17,56	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid methyl esters	269	$C_{17}H_{34}O_2$	nd	4.85	nd	nd
24	17,59	Dodecanoic acid, ethyl ester	Fatty acid ethyl esters	227	$C_{14}H_{28}O_2$	0.27	nd	nd	0.15
25	17,93	Undecanoic acid, methyl ester	Fatty acid methyl esters	199	$C_{12}H_{24}O_2$	nd	nd	0.06	nd
26	17,99	Neophytadiene	Diterpene	278	$C_{20}H_{38}$	nd	nd	nd	1.37
27	18,59	Tridecanoic acid, methyl ester	Fatty acid methyl esters	227	$C_{14}H_{28}O_2$	nd	0.62	nd	nd
28	18,62	9,12-Octadecadienoic acid, methyl ester	Fatty acid methyl esters	294	$C_{19}H_{34}O_2$	nd	nd	0.99	nd
29	18,62	**9,12-Octadecadienoic acid, methyl ester, (E, E)-**	Fatty acid methyl esters	294	$C_{19}H_{34}O_2$	nd	**8.86**	nd	1.51
30	18,62	Isomer1: 9,12-Octadecadienoic acid, methyl ester, (E, E)-	Fatty acid methyl esters	294	$C_{19}H_{34}O_2$	2.65	nd	nd	nd
31	18,68	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl esters	275	$C_{19}H_{36}O_2$	0.58	nd	nd	nd
32	18,69	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid methyl esters	292	$C_{19}H_{32}O_2$	nd	nd	nd	3.88
33	18,87	1-Decanamine, *N*-decyl-*N*-methyl-	Alicyclic compound	269	$C_{21}H_{45}N$	nd	0.26	nd	nd
34	18,87	Ethylamine, *N*-decyl-*N*-methyl-2-(2-thiophenyl)-	Amine	257	$C_{17}H_{31}NS$	nd	nd	nd	0.56
35	18,91	Tridecanoic acid, 12-methyl-, methyl ester	Fatty acid methyl esters	241	$C_{15}H_{30}O_2$	nd	nd	nd	0.88
36	19,25	Isomer2: 9,12-Octadecadienoic acid, methyl ester, (E, E)-	Fatty acid methyl esters	271	$C_{19}H_{34}O_2$	0.45	nd	nd	nd
37	19,35	Isomer1: 9-Octadecenamide, (Z)-	Fatty acid amide	269	$C_{18}H_{35}NO$	1.30	nd	nd	nd
38	19,52	Hexadecanamide	Fatty acid amide	255	$C_{16}H_{33}NO$	3.00	nd	nd	nd
39	19,53	Dodecanamide	Fatty acid amide	198	$C_{12}H_{25}NO$	nd	3.83	nd	4.93
40	19,60	Isomer1: 2,3,5,7-Tetrathiaoctane 3,3-dioxide	Sulphur compound	219	$C_4H_{10}O_2S_4$	1.00	nd	nd	nd
41	19,61	**2,3,5,7-Tetrathiaoctane 3,3-dioxide**	Sulphur compound	219	$C_4H_{10}O_2S_4$	**8.93**	**25.47**	**35.47**	0.44
42	21,10	**Isomer2: 9-Octadecenamide, (Z)-**	Fatty acid amide	281	$C_{18}H_{35}NO$	**19.34**	nd	nd	nd
43	21,12	**9-Octadecenamide, (Z)-**	Fatty acid amide	225	$C_{18}H_{35}NO$	nd	**33.41**	0.29	**17.04**
44	21,85	Bis(2-(Dimethylamino)ethyl) ether	Amine	156	$C_8H_{20}N_2O$	nd	nd	nd	nd

Table 3 (*continued*)

	Retention time (min)	Compound Name	Nature of compound	Observed Ion *m/z*	Molecular formula	Peak Area (%)			
						Stem	Rhizome	Bulb	Leaves
45	24,60	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Sesquiterpenoids	223	$C_{15}H_{26}O$	1.16	nd	nd	nd
46	26,78	**dl-a-Tocopherol**	Vitamins	430	$C_{29}H_{50}O_2$	nd	nd	nd	**9.00**
47	27,52	Ergost-5-en-3-ol, acetate, (3β,24R) -	Sterols	402	$C_{30}H_{50}O_2$	nd	nd	nd	0.46
48	28,10	β-Sitosterol	Sterols	414	$C_{29}H_{50}O$	0.52	0.77	0.16	1.17
49	28,23	*cis*-3,14-Clerodadien-13-ol	Sterols	290	$C_{20}H_{34}O$	0.54	nd	nd	nd
50	28,70	a-Amyrin	Terpenoids	427	$C_{30}H_{50}O$	0.63	nd	nd	nd

The bold colour indicates the most abundant compound in the sample. Nd means the compound is not determined.

content (37.59 ± 1.27 QE/g), followed by rhizome (26.40 ± 0.21 QE/g) and leaves (22.67 ± 1.26 QE/g), and lastly, stems with flavonoid content of 8.65 ± 2.11 QE/g. These extracts showed significant differences in their flavonoid content ($P < 0.05$).

3.5. Phytochemical profiling of T. violacea extracts using GC-HRTOF-MS

A total of 23 different bioactive compounds were reported from the stem, 16 from the rhizome, 17 from the bulb and 20 from the leaves of *T. violacea* methanolic extracts using GC-HRTOF-MS. Table 3 provides the respective identified chemical composition, *m/z*, molecular formula, retention time, and percentage composition. All extracts of *T. violacea* mostly contained a complex mixture of sulphur-containing compounds, fatty acid esters, fatty acid amide, terpenoids, sterol, flavonoids, and phenols. The major compounds observed in stem extract include disulfide, methyl (methylthio), methyl (7 %), 4 h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (10,51 %) methane, (methylsulfinyl) (methylthio)- (13,92 %), isomer 2: 2,3,5,7-tetrathiaoctane 3,3-dioxide (14,54 %), 2,3,5,7-tetrathiaoctane 3,3-dioxide (8,93 %), and isomer2: 9-octadecenamide, (z)- (19,34 %). For rhizome extract, the major compounds were methane (methylsulfinyl) (methylthio)-(10,67 %), 9,12-octadecadienoic acid, methyl ester, (e,e)- (8,86 %), 9-octadecenamide, (z)- (33,41 %) and 2,3,5,7-tetrathiaoctane 3,3-dioxide (25,47 %). The bulb extract revealed the presence of methylthio (methylthio-methyl) sulfone (14,02 %), 2,4,5,7-tetrathiaoctane (34,19 %), disulfide, methyl (methylthio) methyl (8,69 %) and hexadecanoic acid, methyl ester (6,55 %). Furthermore, leave extract contained methane, (methylsulfinyl)(methylthio)- (41,04 %), hexadecanoic acid, methyl ester (9,35 %), dl-a-tocopherol (9 %), 2,3,5,7-tetrathiaoctane 3,3-dioxide (35,47 %), 9-octadecenamide, (z)- (17,04 %) and disulfide, methyl (methylthio) methyl (5,63 %). The chromatographic profiles and relative abundances of the Plant extracts are presented in figure below. Methanolic stem extract of *T. violacea* contained 23 compounds (Fig. 1 and Fig. 5), of which 51.2 % of the compounds present belonged to the sulphur-containing compound group followed by fatty acid amides (23.64 %) and esters (10.50 %), flavonoids (10.51 %), terpenoids (1.79 %) and sterols (1.06 %). The rhizome extract showed the presence of 16 compounds on GC–MS metabolic profiling (Fig. 2 and Fig. 6). The sulphur-containing compounds (40.11 %) were the major compounds present followed by fatty acid amides (37.24 %), fatty acid esters (14.33 %), phenol (3.70 %), butyl alcohol (3.04 %) and sterols (0.77 %). The sulphur compounds (93.34 %) were also observed as the major compounds present in the bulb methanolic extracts of *T. violacea* (Fig. 3 and Fig. 7) with 17 compounds identified. Other compounds, including fatty acid amide (0.29 %), fatty acid esters (2.91 %), flavonoids (0.81 %) and miscellaneous compounds 91.35 %), were observed but in relatively low quantities. Furthermore, the methanolic leaf extract of *T. violacea* contained a total of 20 compounds (Fig. 4 and Fig. 8) with sulphur-containing compounds (48.37 %) being the most abundant constituents. Additionally, the leaf extract also

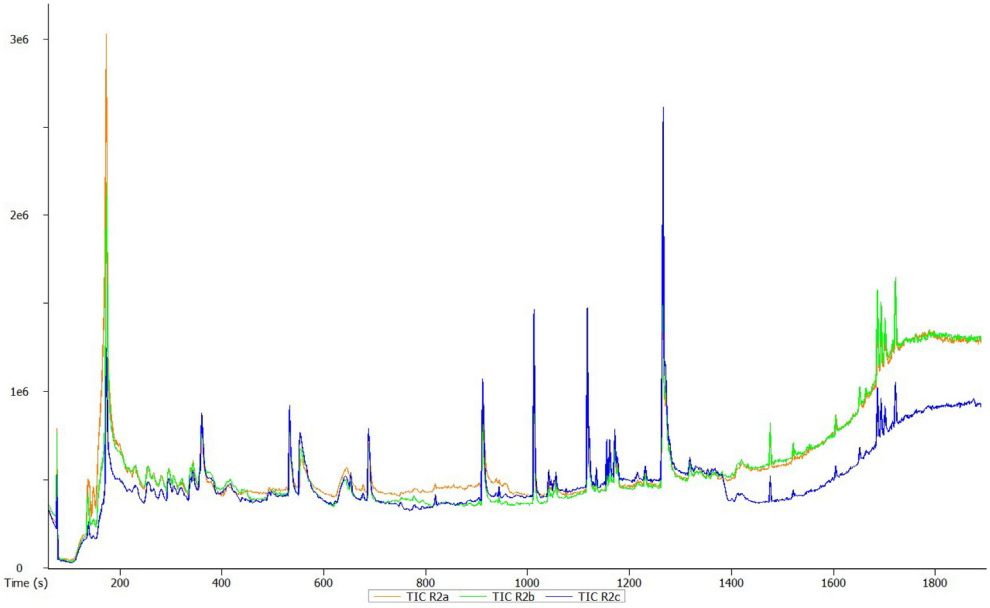

Fig. 1. GC-HRTOF-MS chromatographic profile of methanolic stem extract of *T. violacea.*

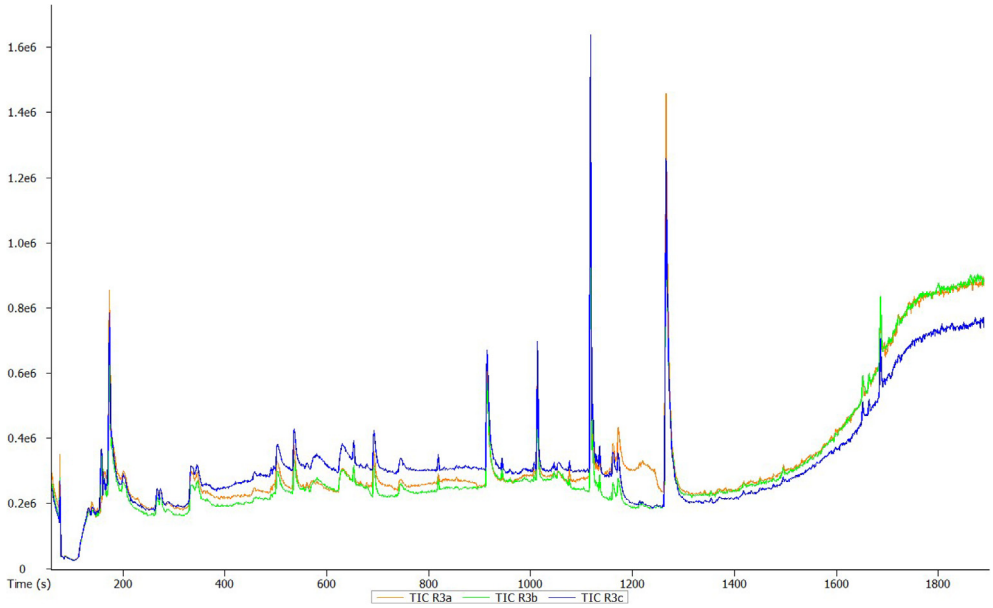

Fig. 2. GC-HRTOF-MS chromatographic profile of methanolic rhizome extract of *T. violacea.*

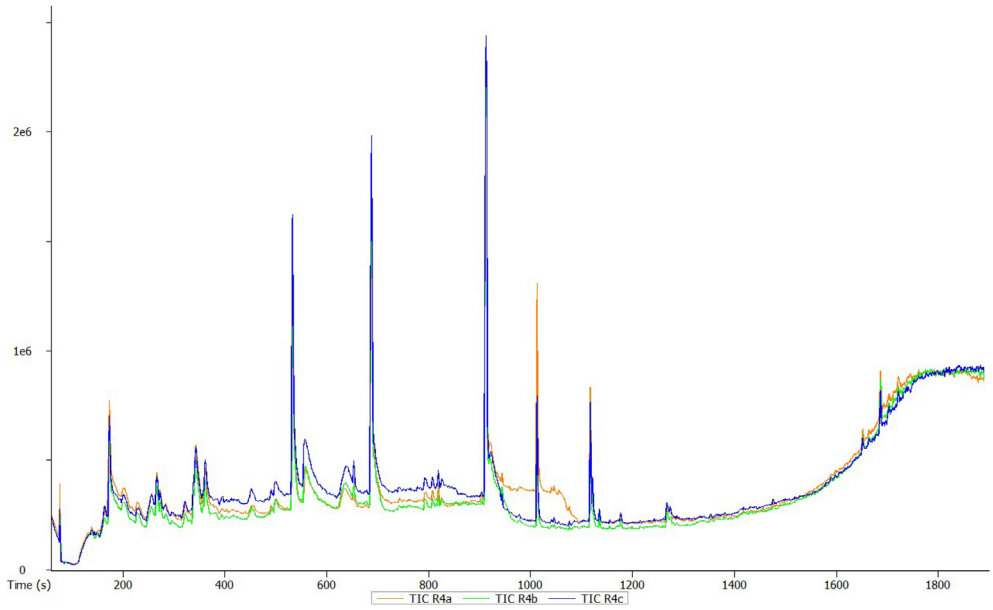

Fig. 3. GC-HRTOF-MS chromatographic profile of methanolic bulb extract of *T. violacea.*

possessed fatty acid esters (15.77 %) and fatty acid amides (21.97 %), vitamins (9 %), terpenoids (1.37 %), sterols (1.63 %) and phenols (0.19 %). Other miscellaneous volatile compounds, such as 3,5,5-trimethylhexyl s-2-(dimethylamino) ethyl propylphosphonothiolate, phosphine, tris (trifluoromethyl)-, 3-isopropoxy-1, 1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and tetradonium bromide were also reported in the present study.

4. Discussion

Various bioactive compounds of plant origin are well known for their biological actions that contribute to human and animal health. Phenolics, flavonoids, terpenoids, alkaloids, steroids, saponins, and essential oils play a significant role in combating many diseases. The current study compared the phytochemical profile, antioxidant, flavonoid and phenolic content of five different *T. violacea* extracts. The study found that methanolic stem, rhizome, bulb and leaf extracts of *T. violacea* are rich in flavonoids, terpenoids and saponins, whereas tannins were only observed in the leaf extract. These discoveries are in line with research by Madike et al. (2017). Tannins are water-soluble phenol with an astringent taste and have displayed important pharmacological and nutraceutical roles. They have antioxidant, antimicrobial, cardioprotective, antidiabetic, antimutagenic as well as antinutrive properties (Sieniawska and Baj, 2017). Cardiac glycosides and anthraquinones were not found in all the extracts and these outcomes are in line with what was stated earlier by (O et al., 2013), but contrary to the findings reported by Ncube et al. (2011) and

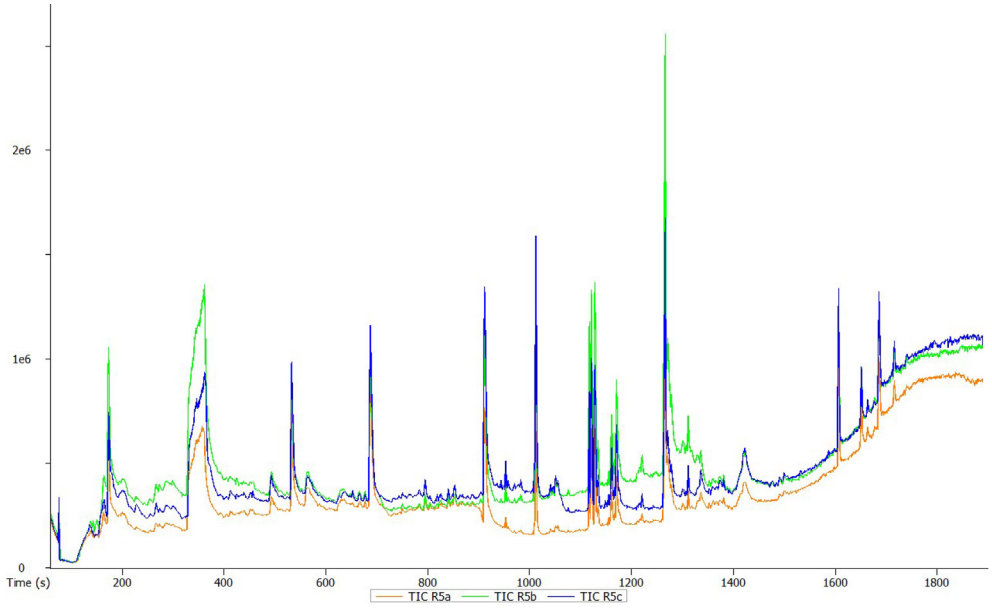

Fig. 4. GC-HRTOF-MS chromatographic profile of methanolic leaves extract of *T. violacea.*

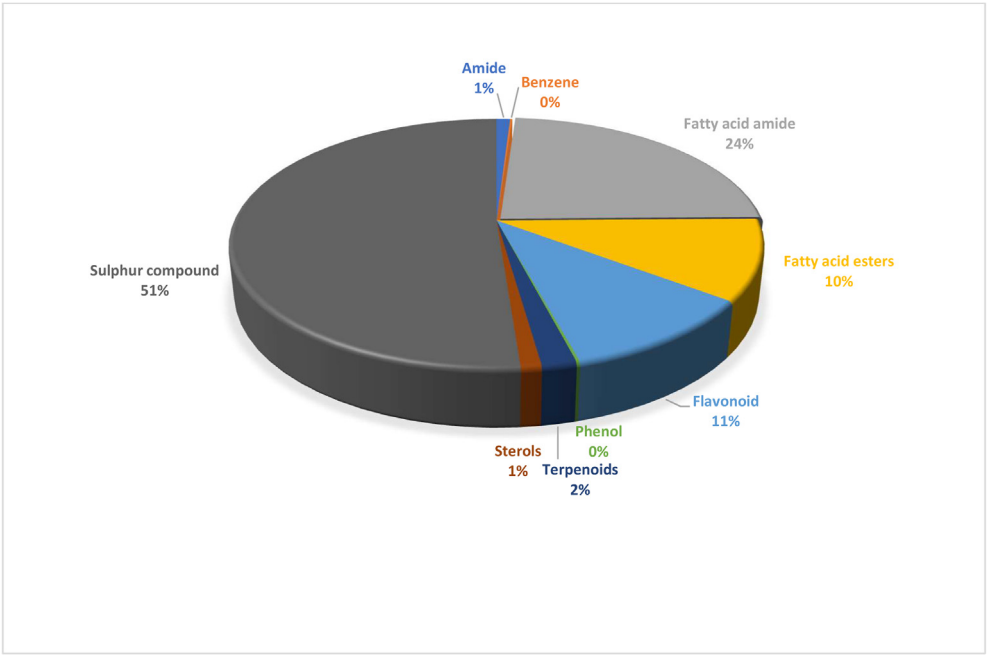

Fig. 5. Distribution of secondary metabolites in stem extract of *T. violacea.*

Madike et al. (2017), who reported the presence of cardiac glycosides in different parts of *T. violacea*. Geographical position and environmental conditions, such as temperature, soil composition, drought, rainfall as well as radiation, have significant effects on the biosynthesis or bioactivity of these compounds in the plant. They affect plant morphology and physiology as well as gene expression. The expression of genes responsible for the biosynthesis of secondary metabolites is regulated by different stress levels (Li et al., 2020). Similar plant species may contain different compound contents and quantities depending on their geographic location (Ghasemzadeh et al., 2018).

Plants that exhibit high antioxidant activities play a significant role in food preservation and human health due to their capacity to scavenge free radicals in a cell. High IC_{50} values mean less antioxidant activities, whereas low IC_{50} values indicate higher antioxidant activities (Dikhoba et al., 2019). Our results show that all the extracts of *T. violacea* had weak antioxidant effects in DPPH and ABTS assays since they had IC_{50} values of more than 100 µg/mL. These findings correspond with the work done by (O et al., 2013), in which the authors observed poor ABTS and DPPH scavenging activity. The antioxidant effect of the plant extracts also corresponds to their phenolic content. In this study, we observed that phenolics were not detected using qualitative phytochemical screening and total phenolic contents were considered low. The absence or low total phenolic contents noted in this study could explain the weak antioxidant effects of *T. violacea* extracts estab-

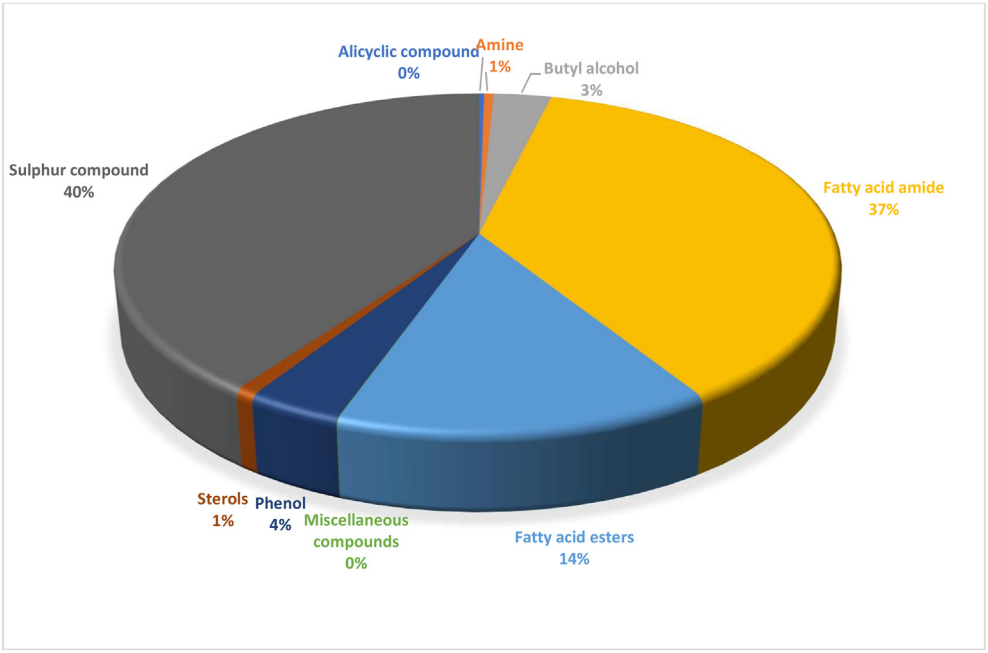

Fig. 6. Distribution of secondary metabolites in rhizome extract of *T. violacea.*

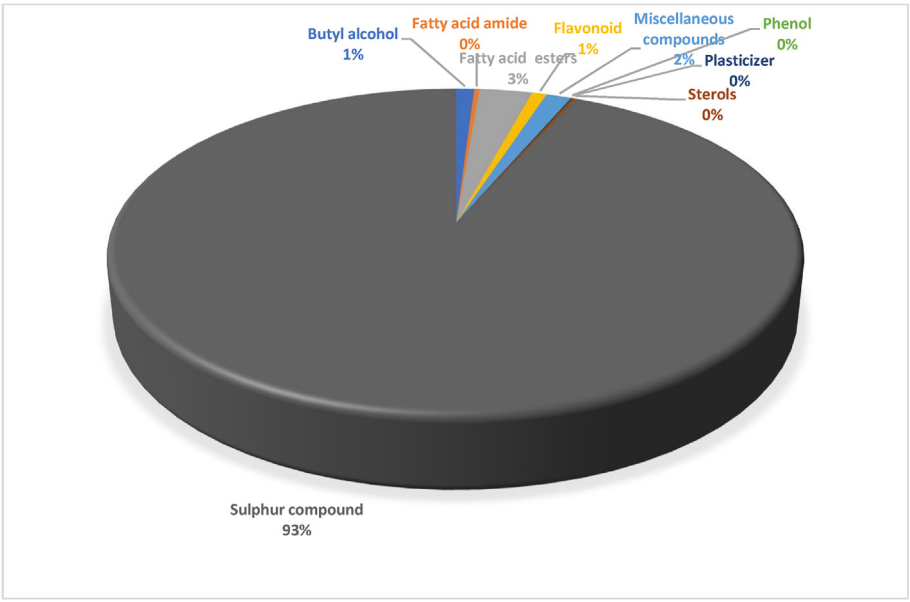

Fig. 7. Distribution of secondary metabolites in bulb extract of *T. violacea.*

lished when conducting both ABTS and DPPH radical scavenging assays. However, the bulb extract showed to have better phenolic and flavonoid contents when compared with those of the leaf and stem extracts. The phenolic contents of methanolic stem and leaf extract of *T. violacea* were low in this study and it is similar to the results reported earlier by Madike et al. (2017). This might likewise be a result of the environmental conditions in which the plant grew.

The GC-HORTF-MS results revealed the occurrence of many sulphur compounds in all extracts of *T. violacea* and only a few of these compounds have been earlier reported in the literature (Pino et al., 2008; Olorunnisola, 2012; Eid, 2015; Staffa et al., 2020). Eid (2015) reported the presence of 2,4,5,6,8-pentathiononane, hexadecenoic acid methyl ester and 9-octadecenamide in *T. violacea* extracts. These sulphur compounds identified in the methanolic extracts of *T. violacea* can be considered as degradation by-products of marasmicin produced via enzymatic cleavage (Eid, 2015). Furthermore, dimethyl trisulfide was also reported in *T. violacea* species by Pino et al. (2008), Olorunnisola (2012), Eid (2015) and Staffa et al. (2020). The medicinal properties of *T. violacea* demonstrated herein are attributed to the abundance of sulphur-containing compounds and fatty acids identified using GC-HORFT-MS. During oxidative stress, which is a condition whereby the amount of free radical generated and destroyed is not balanced, the antioxidant compounds can regulate the process by scavenging the free radical. The sulphur-containing

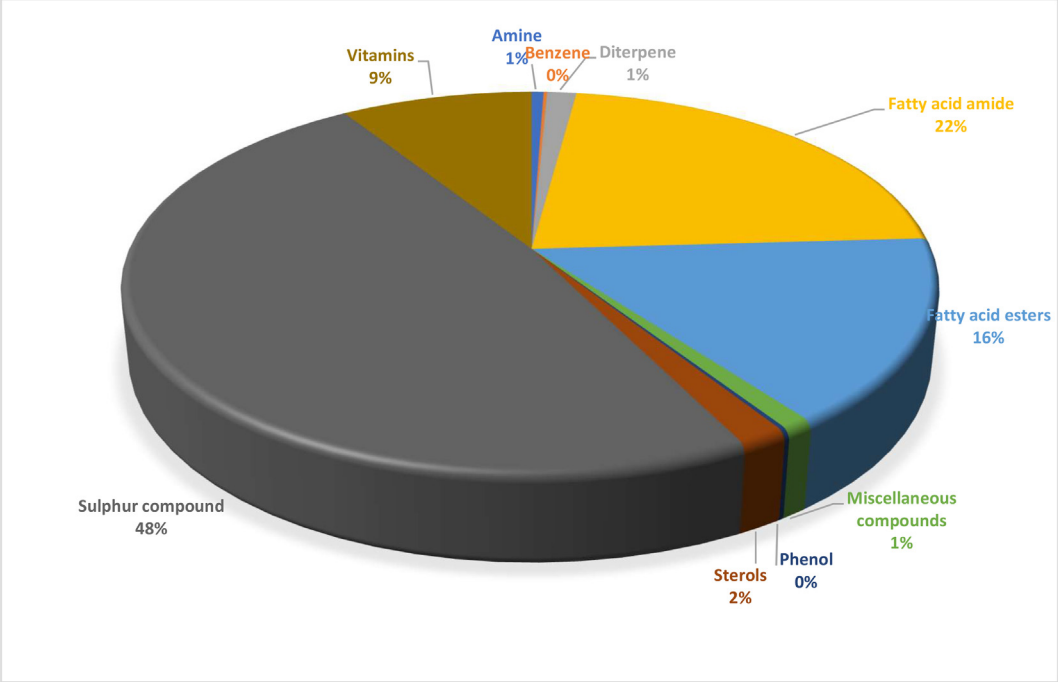

Fig. 8. Distribution of secondary metabolites in leaves extract of *T. violacea.*

compounds are known for their ability to induce phase II detoxification enzymes and protect against free radicals that are linked to carcinogenicity, mutagenicity, cardiovascular, and metabolic diseases (Cerella et al., 2014; Miękus et al., 2020). Fatty acid esters, hexadecanoic acid, and methyl esters were reported to have antioxidant, hypocholesterolemic, nematicide and anticancer activity (Kim et al., 2020; Siswadi & Saragih, 2021). 9,12-octadecadienoic acid, methyl ester is reported to have anti-inflammatory activity, while 9–octadecenamide showed antioxidant activity. Tocopherol, commonly known as vitamin E, has been reported for its antioxidant activity (Siswadi and Saragih, 2021).

5. Conclusion

Different secondary metabolites, including terpenoids, flavonoids, saponin, and tannin, were observed in solvent extracts of the medicinal plant, *T. violacea*, in this study. The results of phytochemical screening showed that methanolic bulb extract of *T. violacea* contains significant total phenolic and flavonoid contents when compared to those of rhizome, leaf and stem extracts. The plant extracts of *T. violacea* were revealed to have weak antioxidant activities in this study. Different compounds were profiled from the bulb (17), rhizome (16), leaf (20) and stem (23) extracts of *T. violacea* plant materials by GC-HRTOF-MS with the bulb extract being a good source of phenolic, flavonoids and sulphur-containing compounds, which may be useful in protection against oxidative damage resulting from free radicals. Nonetheless, future studies should focus on the antioxidant activity of individual bioactive compounds, such as sulphur-containing compounds and the fatty acids that could be isolated, purified and characterised. Other biological studies, such as anticancer, antigenotoxicity and detoxification abilities of extracts from the plant, could also be explored.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102278.

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