African Journal of Pharmacy and Pharmacology Vol. 5(22), pp. 2490-2497, 15 December, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.620
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of *Tulbaghia violacea*

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Accepted 23 November, 2011

The rhizomes of *Tulbaghia violacea* Harv. used in Eastern Cape of South Africa for the treatment of heart diseases and several human ailments was investigated for antioxidant activity. The phytochemical content and toxicity of the fresh and dried rhizomes was also evaluated. Antioxidant activity was determined by spectrophotometric methods such as 2, 2-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide and lipid peroxidation scavenging activities and ferric reducing power assay. Toxicity evaluation was carried out using brine shrimps cytotoxicity test. Results obtained indicated that methanolic extract of the fresh rhizomes contained higher concentration of flavonoid, flavonol, phenolics, tannin and proanthocyanidin than dried sample. Antioxidant studies revealed that the two extracts exhibited potent antioxidant activities in concentration dependent manner. The fresh extract had higher radicals scavenging activity than the dried extract with 50% inhibition of DPPH, hydrogen peroxide and lipid peroxidation at a concentration of 35.0 ± 0.12, 19.3 ± 0.11 and 17.9 ± 0.15 µg/ml, respectively. The dried extract demonstrates lower ferric reducing ability with an absorbance of 0.61 when compared with fresh extract (0.79), butylated hydroxytoluene (BHT) (1.80) and rutin (1.20) standard at 50 µg/ml. The fresh and dried methanolic extracts of the plant exhibited high degree of cytotoxic activity with IC_{50} values of 18.18 and 19.24 µg/ml, respectively. The results obtained in this present study indicated that the rhizome of *T. violacea* may serve as potential source of natural antioxidant, antimicrobial and anticancer agents.

Key words: *Tulbaghia violacea*, antioxidant activity, free radicals, polyphenolic compounds, cytotoxicity.

INTRODUCTION

The maintenance of pro-oxidant and antioxidant homoeostasis status in living cells is the primarily focus of research in recent years. Imbalance in pro-oxidant and antioxidant homoeostasis occasioned by excessive free radicals generation or insufficient antioxidants has been implicated in the development of several human disease conditions, such as atherosclerosis, hypertension ischaemic diseases, Alzhemiers' disease, Parkinsonism and cancer (Narendhirakannam and Rageswari, 2010). Pro-oxidants are free radicals (superoxide, nitric oxide and hydroxyl radicals) produced in normal or pathological cell metabolism (Jadeja et al., 2009; Ponmari et al., 2011) and through exogenous sources, such as human exposure to ionizing radiation, injury, oxidative drugs and pollutants (Erasto and Mbwambo, 2009). Naturally, human body constantly quench and/or scavenge, activate a battery of detoxifying enzymes or inhibit the generation of ROS (Ayoola et al., 2011) through various mechanisms, such as antioxidant enzymes and molecules (Erasto and Mbwambo, 2009). Several synthetic substances, such vitamins, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) (Vinay et al., 2010) are used as antioxidant, but are suspected to be toxic to human and experimental animals (Anagnostopoulou et al., 2006). Therefore, the development and utilization of more effective antioxidants

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of natural origin is very important (Kolar et al., 2011). Moreso, antioxidant evaluation of medicinal plant might give an insight to the mechanism (s) of their pharmacological activities.

Tulbaghia violacea belongs to the family of *Alliacea* which is most commonly associated with garlic. *T. Violacea* is indigenous to the Eastern Cape, South Africa. The leaves and bulbs are widely used as an herbal remedy for various ailments (Bungu et al., 2006). Scientific evidence has demonstrated that *T. violacea* and its various preparations possess pharmacological activities. This includes screening for anthelmintic activity, anticancer and *in vitro* growth inhibition and induction of apoptosis in cancer cells (Bungu et al., 2006; Duncan et al., 1999; McGaw et al., 2000). Despite the fact that the rhizomes of *T. Violacea* are used for the treatment of many diseases, information on antioxidant activity, cytotoxicity or phytochemical contents is relatively scarce. It has been suggested that the pathophysiology of many diseases, such as atherosclerosis involves oxidative stress (Ratheesh et al., 2011). It is then, a good step in the right direction to determine the bioactivities of rhizomes of *T. violacea*.

The present study was designed to investigate and compare phytochemical constituents, free radicals scavenging activities and cytotoxicity of fresh and dried methanolic extracts of *T. violacea* using different *in vitro* experimental model.

MATERIALS AND METHODS

Chemicals

2, 2, Diphenyl-2-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-trizine (TPTZ) purchased from sigma chemicals co Ltd., Ascorbic acid and BHT were obtained from Sisco research laboratories Pvt. Ltd., Mumbai, India. Folin-Ciocalteu reagent, hydrogen peroxide (H_2O_2) was obtained from Merck Limited-India, Mumbai, India. Methanol HPLC grade from Merck, Darmstadt, Germany. Acid and Quercetin of HPLC grade from Sigma chemicals. Brine shrimps for cytotoxicity were obtained from USA. The other chemicals and solvents used in the present study were of analytical grade obtained from local supplier in pure quality.

Collection of plant materials

Whole fresh rhizomes of *T. violacea* Harv. was collected from Alice, Eastern Cape, South Africa. They were collected in April, 2011 and authenticated by Professor D. S. Grierson of Botany Department, University of Fort Hare and was deposited (Sin 2010/2) at the Giffen Herbarium. Plant materials obtained were separated into two groups; one fresh and another oven dry at 40℃ for 3 days.

Preparation and extraction of plant materials

327.4 g of chopped *T. Violacea* rhizome was homogenized in a blender with 1.6 L of 100% methanol at 4℃. The crude extracts were incubated at 37℃ for 15 min, followed by centrifugation at 1500 ×g for 10 min at 4℃ (Mohammad and Woodward, 1986). The supernatant was filtered using Whatman No. 1 filter paper and was concentrated under *vacuo* at 65℃ using rotary evaporator. Since the rhizomes are fresh, the extract did not dry completely. The remaining aqueous solution was freeze dry and stored at 4℃ in the dark. Plant yield was 9.6 g.

The oven dry plant was powdered using a laboratory blender powder. Fifty gram of the powdered plant was extracted with 100% methanol by shaking for 48 h in an orbital shaker. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40℃ using a rotary evaporator (Laborota 4000-efficien t, Heldolph, Germany).

Total phenolics determination

Total phenol contents in the extracts were determined by themodified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10, v/v) and 4 ml (75 g/L) of sodium carbonate. The tubes were vortexed for15 s and allowed to stand for 30 min at 40^0C for colour development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-Vis spectrophotometer. Samples of extract were evaluated at a final concentration of 1.0 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

Total flavonoid determination

Total flavonoids content was measured by aluminium chloride colometric assay (Marinova et al., 2005). 1 ml of the extracts or standard solution of catechin was added to 10 ml volumetric flask containing 4 ml of distilled water, 0.3 ml of 5% $NaNO_2$ was added to the mixture. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At the 6th min, 2 ml of 1 M NaOH was added to the mixture and the total volume was made-up to 10 ml with distil water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm.

Total tannin determination

Tannins were determined using the Folin phenol reagent as reported by Folin and Ciocalteu (Folin and Ciocalteu, 1927). Briefly, 0.1 ml of the sample extract was added with 7.5 ml of distilled water and 0.5 ml of Folin phenol reagent, 1 ml of 35% sodium carbonate solution was diluted with 10 ml distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of tannic acid in mg/g of extract.

Determination of proanthocyanidins content

The total proanthocyanidin were determined using the procedure reported by Sun et al. (1998). A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3.0 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid, the mixture was allowed to stand for 15 min at room temperature, and the absorbance was measured at 500 nm. Total proanthocyanidin contents were expressed as quercetin (mg/g) using the following equation of the curve: Y= 0.5825x, R2 = 0.9277, where x is the absorbance and Y

is the quercetin equivalent.

Determination of total flavonol

Total flavonols were estimated using the method of Kumaran and Karunakaran (2007). To 2.0 ml of the sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h at 20°C. Extract sample were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0255 x, R^2 = 0.9812, where x is the absorbance and y is the quercetin equivalent (mg/g).

Determination of diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method of Liyana-Pathiranan and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical in the extract solution. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of the extract prepared in methanol containing 0.02 to 0.1 mg of the plant extracts and standard drugs BHT and ascorbic acid. The reaction mixture was vortexed thoroughly and was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by the equation: DPPH radical scavenging activity = {(Abs control – Abs sample)/(Abs control)} × 100; where Abs control is the absorbance of DPPH radical + BHT/Ascorbic acid equivalent per 100 g of my plant extract.

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986) with a slight modification. Different concentrations (0.02 to 1.0 mg/ml) of extract (0.5 ml) were mixed with 0.5 ml of 0.2 M phosphate buffer (pH 6.6) and 0.5 ml of 0.1% potassium hexa-cyanoferrate, followed by incubation at 50°C in water bath for 20 min. After incubation, 0.5 ml 10% trichloroacetic acid (TCA) was added to terminate the reaction. The upper portion of the solution (1 ml) was mixed with 1 ml of distilled water and 0.1 ml of 0.01% $FeCl_3$ solution was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against appropriate blank solution. All tests were performed in triplicates. A higher absorbance of the reaction mixture indicated greater reducing power. The absorbance obtained was converted to ascorbic acid equivalents in milligrams per gram fresh and dried material (mg g^{-1}) using ascorbic acid standard.

Determination of H_2O_2 inhibition activity

The H_2O_2 inhibition effect of the extracts was assessed by the method of Ilhami (Ilhami et al., 2005). Briefly, a solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 1.0 ml of the sample was added to a 0.6 ml of hydrogen peroxide solution (40 Mm). The absorbance of the hydrogen peroxide at 230 nm was determined after 10 min at room temperature against a blank solution containing phosphate buffer solution without hydrogen peroxide. BHT and ascorbic acid were used as positive controls. The percentage scavenging of hydrogen peroxide of the samples was calculated using the following formula:

H_2O_2 inhibition capacity (%) = [1 - (H_2O_2 concentration of sample / H_2O_2 concentration of control)] × 100.

Lipid peroxidation inhibition assay

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media, as described by Ruberto et al. (2000). Briefly, 0.5 ml egg yolk homogenate (10% v/v) was added to 0.1 ml of the extract (10 µg/ml). The volume was then made up to 1.0 ml with distilled water. Thereafter, 0.05 ml of $FeSO_4$ (0.07 M) was added and the mixture was incubated at 37°C for 30 min. Then, 1.5 ml 20% acetic acid (pH 3.5) was added, followed by 1.5 ml of 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% of sodium dodecyl sulphate (SDS). The resulting mixture was vortex mixed and heated at 95°C for 1 h. After cooling, 5 ml of butanol was added and the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Inhibition of lipid peroxidation percent by the extract was calculated as [(1-E)/C] × 100, where C is the absorbance value of the fully oxidized control and E is the absorbance in the presence of the extract.

Brine shrimp lethality test

Shrimp eggs were allowed to hatch and mature as nauplii in two days in a hatching tank filled with seawater. The free-swimming nauplii were attracted by a light to a compartment from which they could be collected for the assay proper. Vials containing 4 to 20 µg ml^{-1} samples were prepared by dissolving the extracts in distilled water and transferring the solution to each vial. The solvent was evaporated at room temperature for 72 h and sea water was added to achieve the correct concentration. 15 shrimps were added to each vials via a disposable pipette. The number of deaths out of 15 shrimps per dose was recorded after 24 h and LC_{50} values obtained from the best-fit line slope. The control solution consists of 15 nauplii in the sea water without the extract.

Statistical analysis

Statistical analysis was carried out with Statistical Package for Social Sciences (SPSS). The data was expressed as the mean ± standard deviation and a probability of less than 0.05 ($P < 0.05$) was considered to be statistically significant. Graph was drawn using Microsoft Office excel, 2007 software.

RESULTS AND DISCUSSION

Total phenolics, flavonoid, flavonol, tannin and proanthocyanidin

Phenolics compounds have been reported to be crucial for bioactivities in plants (Nagavani et al., 2010). They serve as antioxidant and exhibit a wide spectrum of medicinal properties, such as anti-cancer, anti-allergic and cardio-protective (Banerjee and Bonde, 2011).

Analysis of phenolics compounds in the fresh and dried methanolic extract of rhizome of *T. Violacea* (RTV) revealed that the extracts possesses high concentration of total flavonoid ranging from (38.9 to 67.9 mg/g quercetin equivalent) followed by total phenolics (18.3 to 38.2 mg/g tannic acid equivalent, flavones (25.3 to 11.5 mg/g quercetin equivalent), tannin (14.6 to 37.4 mg/g tannic acid equivalent) and proanthocyanidin (17.2 to 8.4

Table 1. Polyphenol contents of methanolic extracts of fresh and dried rhizomes of *T. violacea*.

Phenolic	Fresh	Dried
Phenol[a]	38.2 ± 0.01	18.3 ± 0.04*
Tannin[b]	37.4 ± 0.13	14.6 ± 0.09*
Flavonoid[c]	67.9 ± 0.11	38.9 ± 0.15*
Flavonol[d]	25.3 ± 0.00	11.5 ± 0.02*
Proanthocyanidins[e]	17.3 ± 0.21	8.40 ± 0.16*

[a,b]Expressed as mg tannic acid/g of dry plant material. [c,d,e]Expressed as mg quercetin/g of dry plant material. *P < 0.05.

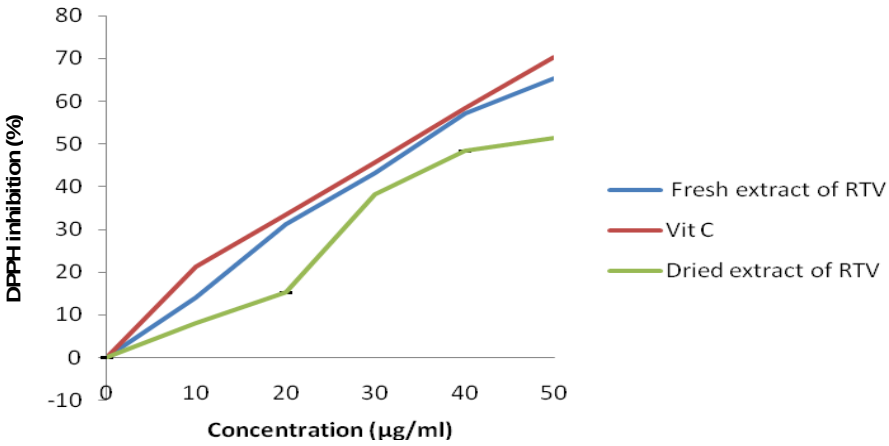

Figure 1. DPPH scavenging activity of methanolic extract of fresh and dried rhizomes of *T. violacea*. Each value is expressed as mean ± SD,(n = 3).

mg/g quercetin equivalent), respectively (Table 1). Our results showed that the concentration of polyphenolic compounds in the dry extract was significantly (P < 0.05) lower than the fresh extract.

The concentration of the aforementioned phytochemicals present in *T. Violacea* followed similar trend reported in three varieties of *Allium sativum* by Narendhirakannan et al. (2010). However, the amounts of phenolic compounds are lesser than what was obtained in the three varieties of *A. sativum.* The differences in the content of metabolites may be due to the nature of the soil, microclimate variations (Millogo-Kone, 2008), processing methods (Choi et al., 2008) and generic, since the same method was used by Narendhirakannan et al. (2010) in the extraction of the three varieties of *A. sativum* was employed in this study. The high levels of phytochemicals in the extracts indicates that rhizomes of *T. violacea* could be a good source of anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators (Okwu and Emenike, 2006). This may therefore, explain the medicinal value of the plant in management and treatment of oxidative stress induces disorder.

DPPH radical scavenging activity

The methanolic extracts of dry and fresh rhizomes of *T. Violacea* (RTV) were tested for antioxidant activity using DPPH radical scavenging assay. The antioxidant ability of plant products to donate hydrogen to DPPH radical, thus converting it into stable molecules has been attributed to phenolic compounds, such as flavonoid, polyphenol, tannins and terpenes (Diouf et al., 2009; Rahman and Moon, 2007). Therefore, phenolics compounds due to their redox properties play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Hasan et al., 2008).The extracts in this study were able to reduce the stable DPPH, thus changing the colour from purple to yellow. Fresh extract of *T. violacea* demonstrated higher percentage (65.3 %) DPPH scavenging activity (Figure 1) when compared with dried extract (51.4%) or other *Allium* species like green onion and yellow onion (Noureddine, 2005), but exhibited lower percentage DPPH inhibition when compared with red onion, purple onion, garlic (Noureddine, 2005) and ascorbic acid standard (70.2%). The strong DPPH activity

Table 2. Comparison of 50% inhibitory concentration for DPPH, hydrogen peroxide radicals scavenging activity and lipid peroxidation.

Sample and standard	DPPH (µg/ml)	H_2O_2 (µg/ml)	Lipid peroxidation (µg/ml)
FRTV	35.0 ± 0.12	19.3 ± 0.11*	17.9 ± 0.15*
Ascorbic acids	33.6 ± 0.22	12.7 ± 0.14	7.99 ± 0.20
DRTV	48.7 ± 0.41*	36.5 ± 0.16*	41.1 ± 0.18*

FRTV represents fresh methanolic extract of rhizomes of *T. violacea*. DRTV represents dried methanolic extract of rhizomes of *T. violacea.* *P < 0.05.

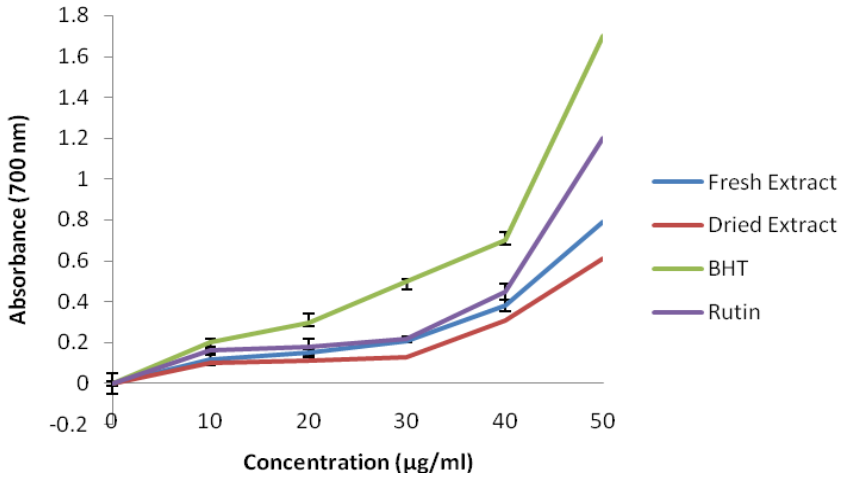

Figure 2. Reducing power of methanolic extract of fresh and dried rhizome of *T. violacea.* Each value is expressed as mean ± SD (n = 3).

(50% inhibition concentration of 35.0 ± 0.12 mg/ml) of the fresh extract rhizome of RTV (Table 2) agreed favourably with the report of Drużyńska and Wojda (2007) in which they submitted that the DPPH radical scavenging activities of fresh extracts of garlic, oregano and rosemary is higher than the dried. The results of the present study indicated that RTV had good antioxidant activity.

Reducing power of extract

Reducing power ability of plant extracts may serve as a significant reflection of the antioxidant activity serve as a significant reflection of the antioxidant activity (Jayanthi and Lalitha, 2011).

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, thereby, acting as a primary and secondary antioxidants (Chada and Dave, 2009). The presence of reductants in T. *violacea* rhizome causes the reduction of the Fe^{3+}/ferricynide complex to the ferrous form. Since, Fe^{3+} has been implicated in the generation of hydroxyl radical ion, it is logical to assume that the plant extracts are capable of inhibiting hydroxyl radical Fe^{3+}/induced generation and might serves as potential antioxidant. The reducing ability of methanolic extracts of dry and fresh rhizome of *T. Violacea* was very potent since increased absorbance indicated increased reducing power (Figure 2). Our results also revealed that the reducing ability of the extracts is concentration dependant. This observation was in agreement with what was reported for other varieties of *A. sativum* bulb (Narendhirakannan and Rajeswari, 2010). Although, the reducing power of the fresh extract was higher than the dry, which was comparably lower than BHT and rutin standard. In view of the reductants activity of the extract, further studies need to be carried out to identify the individual compound(s) that aids in the reducing power.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide (H_2O_2) is one of the major by products of incomplete oxygen metabolism (Ilias and Carlos, 2001). It is not a free radical by definition because it

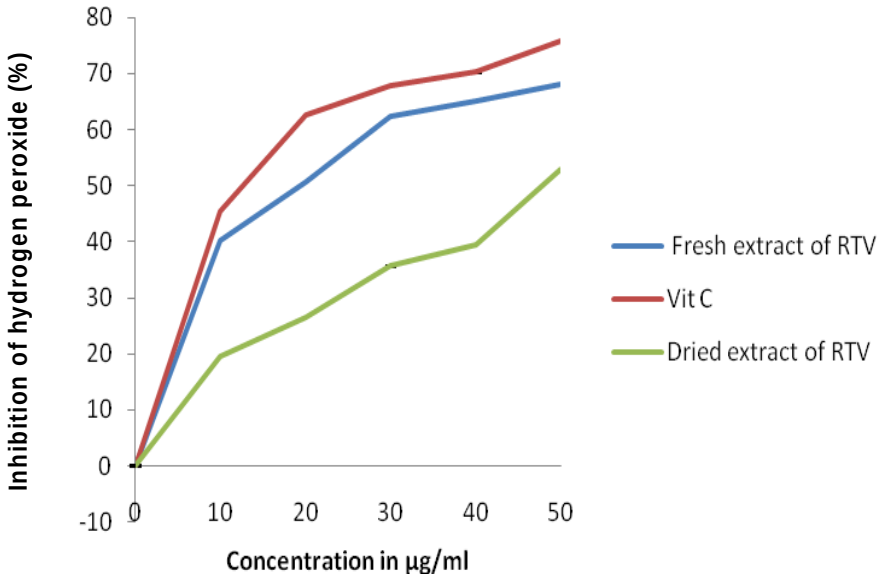

Figure 3. Scavenging ability of methanolic extracts of fresh and dried rhizomes of *T. violacea* (RTV) on hydrogen peroxide. Each value is expressed as mean ± SD (n = 3).

lacks free electrons. Nevertheless, its role in reactive oxygen species (ROS) mediated damage is important because of its chemical versatility and diffusibility (Ilias and Carlos, 2001). H_20_2 is produced from the scavenging activity of superoxide dismutase on superoxide radical (Christophe et al., 2007). In the presence of glutathione peroxidase and reduced glutathione (GSH), hydrogen peroxide is converted to water, thus completely detoxifying ROS (Łukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004; Yilmaz et al., 2004). However, in the presence of reduced transition metals, such iron, H_2O_2 can produce the highly reactive OH•, which can cause extensive damage to DNA, proteins and lipids. Therefore, removing of hydrogen peroxide is very important for antioxidant defence in cell system (Kumar et al., 2011). The methanolic extracts from fresh and dry rhizomes of *T. violacea* demonstrated a significant hydrogen peroxide scavenging ability (Figure 3).

The dry methanolic extract exhibited lower H_20_2 scavenging activity with a 50% inhibition of a concentration of 36.5 ± 0.16 mg/ml when compared with the fresh extract (19.3 ± 0.11 mg/ml) and ascorbic acid standard (12.7 ± 0.14 mg/ml) (Table 2). Similar result was reported for three varieties of *A. sativum* L. Extracts (Narendhirakannan and Rajeswari, 2010). However, the percentage of H_2O_2 activity of *T. violacea* was slightly higher than what was reported for three varieties of *A. sativum* L. extract by Narendhirakannan and Rajeswari (2010). The H_2O_2 scavenging activity of the extract may be attributed to the presence of phenols, which could donate electrons there by neutralizing it into water (Nagavani et al., 2010).

Lipid peroxidation assay

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage (Surapaneni and Vishnu, 2009). The damage has been implicated in the pathophysiology of various human diseases, such as atherosclerosis, diabetes and cancer. The initiation of peroxidation sequence in membrane or polyunsaturated fatty acids is due to the abstraction of a hydrogen atom from the double bond in the fatty acids (Wagner et al., 1994). Malondialdehyde (MDA) is the major product of lipid peroxidation and is used to study the lipid peroxidation process. Incubation of egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation. The ability of the methanolic extract of rhizome of *T. Violacea* to inhibit the process of lipid peroxidation was tested using the method of Ruberto et al. (2000). Table1 shows that the extract demonstrates high percentage inhibition of lipid peroxidation in egg homogenate (66.8%). The low IC_{50} value (17.4 µg/ml) (Table 2) suggested that extract of rhizome of *T. Violacea* possessed high anti-lipid peroxidative agents. This finding is similar to the report that garlic (*A. sativum*) extract significantly reduced lipid peroxidation (Sundaresan and Subramanian, 2005). Our results also showed that the percentage of lipid peroxidation inhibiting activity of the extract and the standard (Ascorbic acid) increases with concentrations. However, the activity of the extract is lower than the standard as reflected by the value of IC_{50}. Phenolic compounds have been reported to be activating lipid free or prevent the decomposition of

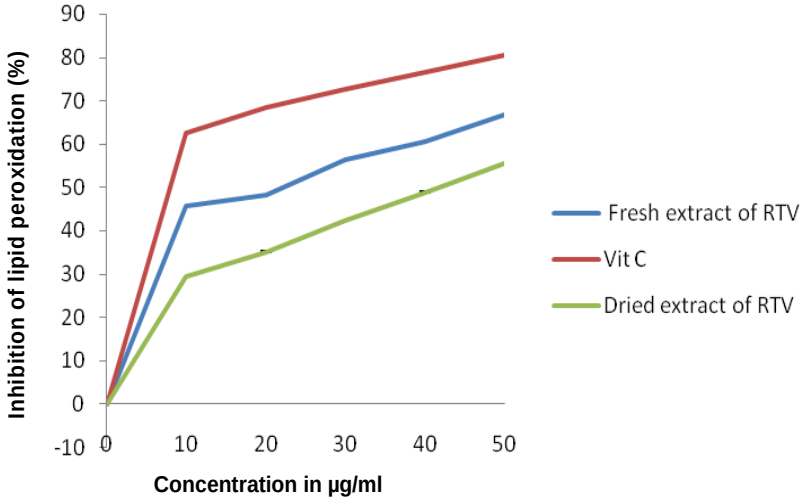

Figure 4. Scavenging ability of methanolic extracts of fresh and dried rhizomes of *T. violacea* (RTV) on lipid peroxidation. Each value is expressed as mean ± SD (n = 3).

Table 3. Brime shrimp lethality test of Methanolic extract of rhizome of *T. Violacea*.

Concentration (µg/ml)	Methanolic extract of *T. violacea* rhizome		
	Average no. of survivors	Average no. of dead	Mortality (%)
4	15.0 ± 0.00	15.0 ± 0.00	0
16	3.0 ± 0.00	12.0 ± 0.00	12
20	0	0	100
control	15	0	0
LC_{50}18.18 µg/ml			

Data were expressed as mean ± SD. LC_{50} values were calculated by extrapolation.

hydrogen peroxides into free radicals ((Maisuthisakul et al., 2007). Hence, the observed anti-lipid peroxidation activity of the extract may be due to the high concentration of phenolic compounds in the extract (Figure 4).

Cytotoxicity assay

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (Mann et al., 2011). The extracts studied in this work showed significant lethality against brine shrimp with IC_{50} (half-inhibition) values 18.18 µg/ml (Table 3). This significant lethality of the crude plant extracts (IC_{50} values less than 100 ppm or µg/ml) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds (Morshed et al., 2011). The level of toxicity observed in this study may have resulted from the presence of multiple compounds in the crude extracts, acting in synergy or independently. Therefore, further isolation of the highly active compound(s) from rhizomes of *T. Violacea* may lead to the discovery of new cytotoxic compounds.

Conclusion

This study suggests that the methanolic extract of rhizome of *T. Violacea* may be a source potent, antioxidant and cytotoxic compounds especially when use fresh. The *in vitro* bioassays provide an ample knowledge of antioxidant, free radical scavenging and cytotoxicity activities of *T. Violacea* and thus can be further investigated for *in vivo* studies.

ACKNOWLEDGEMENT

This study was supported with a grant from the National Research Foundation of South Africa.

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