Comparative Evaluation of *Sutherlandia frutescens* and *Tulbaghia violacea* Antioxidant Potential

ABSTRACT

Aims: To evaluate the antioxidant properties of *Sutherlandia frutescens* and *Tulbaghia violacea* to justify their medicinal uses and values.

Study design: Experimental

Place and Duration of Study: Department of Biochemistry, Faculty of Science, Lagos State University and Department of Biotechnology, University of The Western Cape, Cape Town, between June 2019 to July 2021.

Methodology: The antioxidant and free radical scavenging activity of *Sutherlandia frutescens* and *Tulbaghia violacea* extracts were determined by several standard methods including ferric-ion reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), trolox equivalent absorbance capacity (TEAC) and the thiobarbituric acid reactive substances (TBARs) assays.

Results: All *S. frutescens* extracts exhibited higher FRAP activities (ranging from 687.43 ± 11.90 to 974.31 ± 6.21 µMAAE/g) compared with corresponding extracts of *T. violacea.* Aqueous extract of *S. frutescens* produced the highest trolox equivalent absorbance capacity **(**1603.12 ± 5.50 µMTE/g), copper-initiated prooxidant activity (51.40 ± 1.25 µMTE/g) as well as peroxyl (1049.45 ± 0.54 µMTE/g) and hydroxyl (3911.27±18.67 µMTE/g) scavenging activities. The peroxyl and hydroxyl scavenging activities of aqueous methanolic extracts of *S. frutescens* and *T. violacea* increased in a concentration dependent manner. The inhibition of Fe^{2+}**-**induced microsomal lipid peroxidation showed that aqueous methanolic extracts of *Sutherlandia frutescens* and *Tulbaghia violacea* significantly inhibit this process when compared with ethylacetate, dichloromethane and water only extracts.

Conclusion: The results suggest that *S. frutescens* and *T. violacea* antioxidant capacities depend on the extractive solvent. The antioxidant activity of the plants could be related to inherent phenolic bioactive compounds. However, further study is required to determine the precise mechanism of action and active constituents responsible for the antioxidant properties of these plants.

Keywords: *Antioxidants, Oxidative stress*, *Sutherlandia frutescens, Tulbaghia violacea.*

Abbreviations: AAPH: 2, 2'-Azobis (2-methylpropionamidine) dihydrochloride; ABTS (2,2'-Azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid); DMSO: Dimethyl Sulfoxide; FRAP: Ferric-ion reducing antioxidant power assay; Gli1: Glioma-associated oncogene; HepG2: Hepatoma G2 cell line; H157: non-small cell lung cancer cell line; HT29: Human adenocarcinoma colorectal cell line; LPS: Lipopolysaccharide; MCF7: Michigan Cancer Foundation-7; NO: Nitric oxide; Nrf2: Nuclear factor erythroid 2-like 2 ; ORAC: Oxygen Radical Absorbance Capacity; PTCH1: Transmembrane receptors patched; PUFAs: Polyunsaturated fatty acids ; SAG: Gli/Hh signalling agonist; SF- *Sutherlandia frutescens*; STDEV: Standard deviation; TBARs: Thiobarbituric acid reactive substances; TE: Trolox equivalents; TEAC: Trolox Equivalent Absorbance Capacity; TPTZ: 2,4,6-tri[2-pyridyl]-s-triazine; TV- *Tulbaghia. violacea*; µM AAE/g DW: µM ascorbic acid equivalents per milligram dry weight; µM TE/g DW: µM Trolox equivalents per milligram dry weight (µM TE/g DW)**.**

1. INTRODUCTION

Traditional medicine remains the most patronised practice at primary level of health care worldwide [1]. This has been attributed to accessibility, cultural acceptability, affordability and self-cultivation potential of medicinal plants [2, 3]. Traditional medicine entails the use of medicinal plants as sources of natural products to treat a wide range of infectious diseases such as tuberculosis, malaria and non-communicable diseases such as diabetes, arthritis and stroke [4-7].

Natural products from plants such as fox-gloves, yew and opium poppy have been explored to make drugs such as digoxin, taxol and morphine in the past [8-10]. The emergence of multidrug resistance and suboptimal adherence to synthetic and semi-synthetic antibiotics and anticancer drugs have provided a new dimension to the global challenge of controlling and eliminating diseases such as tuberculosis and cancer by 2035 [11-13]. This has prompted the need for alternative sources of therapy with equity in access and lower drug resistance risk, novel mechanism of action and safety of use [14, 15]. This need is more relevant to low-and middle- income countries of the world grappling with a surge of non-communicable diseases such as cardiovascular disease, Type 2 diabetes mellitus and cancer, in addition to a weak healthcare system [16]. Looking at cancer alone, a projected global increase of 85% in its burden by 2030 means that sub-Saharan Africa is expected to record a million new cases of cancer and over half a million cancer related deaths yearly [17].

South Africa, with a population of 55.6 million in 2016 and an estimated 59 million in 2018 [18], presently has one of the largest population of people living with tuberculosis, obesity and cancer in sub-Saharan Africa [19-21]. The country is also endowed with a plethora of medicinal plants used by the indigenes for the treatment of several diseases [22], including HIV/AIDS, tuberculosis, cancer, arthritis and diabetes [22, 23]. Plant biogeography provides an important platform for documenting medicinal plants used for

therapeutic purposes in a population. Based on the findings from ethnobotanical surveys, more than 1000 species of plants with therapeutic usefulness have been reported in South Africa [22, 24].

Oxidative stress, defined as an imbalance between cellular prooxidant and antioxidant concentrations, in favour of the former, underlie the basis for the initiation and progression of several diseases such as cancer, hypertension and active pulmonary and extra pulmonary tuberculosis [25, 26]. As these diseases are treated by medicinal plants used in South African folklore, scientific evaluation of their antioxidant properties is very important to justify their medicinal uses and values. Antioxidant activity involves the ability to mop up singlet oxygen and scavenge hydroxyl, peroxyl and free radicals [27]. It also involves the ability to bind iron and inhibit reactive oxygen and reactive nitrogen species producing reaction in the cells [28].

Sutherlandia frutescens and *Tulbaghia violacea* are among the medicinal plants prevalent to South Africa [22, 29]. *T. violacea*, also called wild garlic, is used in traditional medicine in South Africa to treat cancer [30]. The leaf extract of the plant has recently been shown to elicit selective cytotoxicity to HepG2, MCF7, H157, and HT29 cancer cell lines by inducing apoptosis [31]. A study by Raji *et al* [32] also revealed the anti-hypertensive activity of the methanolic extract of this plant to be mediated by the stimulation of muscarinic receptor and decrease in aldosterone level in rat.

A study by Ajit *et al;* [33] on extracts from *Sutherlandia frutescens* or “cancer bush” as it is commonly known, has shown how polyphenols in these extracts have effectively inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) and enhanced nuclear factor erythroid 2-like 2 (Nrf2)-mediated antioxidant responses, which are associated with oxidative stress and inflammatory response of transcriptional (signalling) regulation in neurodegenerative disorders. Lin *et al;* [34] have also shown how crude methanol extracts of *S. frutescens* have dose- and time-dependently supressed prostate cancer cell lines, PC3 and LNCaP, *in vitro* by interfering with the Gli/Hh signalling pathway. Specifically, they have shown that Sutherlandioside D compound within the extracts do so by blocking glioma-associated oncogene (Gli1) and the transmembrane receptors patched (PTCH1) gene expression in the presence of a Gli/Hh signalling agonist (SAG). The present study investigated the antioxidant activity of aqueous and organic solvent extracts of *T. violacea,* and *S. frutescens*.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Standards (purity > 99.0%) for antioxidant and inhibition of Fe^{2+}-induced lipid peroxidation assays such as trolox (6-Hydroxyl-2, 5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-Azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt, potassium peroxodisulphate, fluorescein sodium salt, AAPH (2, 2'-Azobis (2-methylpropionamidine) dihydrochloride), perchloric acid, TPTZ (2,4,6-tri[2-pyridyl]-s-triazine, Iron (III) chloride hexahydrate, tris-HCl, sepharose (wet bead diameter, 60-200 µm), copper sulphate and hydrogen peroxide were secured from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Biological activity measurements: All antioxidant assays including FRAP, TEAC and lipid peroxidation were measured by Multiskan spectrum plate reader, while automated ORAC assays were determined by Floroskan spectrum plate reader. Greiner® F Bottom (white and black) 96-well micro-plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were used for all antioxidant assays.

2.2. Plant collection and authentication

T. violacea plant was collected from Van Den Berg village in Stellenbosch, South Africa and deposited at the Department of Biodiversity and Conservation Biology, University of Western Cape for authentication. Also, *S. frutescens* plant was collected from Jonkershoek Nature Reserve, Western Cape and deposited at the Compton Herbarium, South Africa. *T.violacea* and *S. frutescens* plant samples were issued voucher numbers 6975 and NBG145884 respectively.

2.3. Extraction

About 70 grams of powdered *S. frutescens* leaves were extracted successively three times with ethyl acetate (labelled SF-ethylacetate), followed by 50% aqueous methanol (SF-aqueous-methanol (50%)), 75% aqueous methanol (SF-aqueous-methanol (75%)) and water (SF-water) respectively. An amount of 75.5 grams of powdered *T. violacea* leaves was extracted serially three times with dichloromethane (TV-dichloromethane), 50 % aqueous methanol (TV- aqueous-methanol (50%)), 75 % aqueous methanol (TV-aqueous-methanol (75%)) and water (TV-water) respectively. SF-ethylacetate and TV-dichloromethane

were evaporated to dryness at 35 °C using rotary evaporator while other extracts were reduced to dryness using freeze drying method. All stock solutions (10 mg/mL) were prepared by reconstituting the extract in DMSO *(*Dimethyl Sulfoxide).

2.4. Ferric-ion reducing antioxidant power assay (FRAP)

Working FRAP reagent was prepared in accordance to the methods described previously by Benzie and Strain [35]. Absorbance was measured at 593 nm. L-Ascorbic acid was used as a standard and the results were expressed as µM ascorbic acid equivalents per milligram dry weight (µM AAE/g DW) of the test samples.

2.5. Automated Oxygen Radicals Absorbance Capacity (ORAC) Assay

ORAC was measured according to the methods described by Cao and Prior [36]. The method measures the antioxidant scavenging capacity of thermal decomposition generated by (a) peroxyl radical of 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH; $ORAC_{ROO^{\cdot}}$ assay), (b) hydroxyl radical ($ORAC_{OH^{\cdot}}$ assay), generated by H_2O_2-Cu^{2+} (H_2O_2, 0.3 %; Cu^{2+} [as $CuSO_4$], 18 µM, or (c) Cu^{2+} [as $CuSO_4$], 18 µM as a transition metal oxidant at 37 °C. ORAC values were expressed as micromoles of Trolox equivalents (TE) per milligram of test sample, except when Cu^{2+} (without H_2O_2) was used as an oxidant in the assay. In the presence of Cu^{2+} without H_2O_2, test samples acted as prooxidants rather than antioxidants in the ORAC assay. The copper-initiated prooxidant activity was calculated using [(Area $_{Blank}$ – Area $_{Sample}$)/Area $_{Blank}$] × 100 and expressed as prooxidant units; one unit equals the prooxidant activity that reduces the area under the fluorescein decay curve by 1% in the ORAC assay.

2.6. Trolox Equivalent Absorbance Capacity (TEAC) Assay

The total antioxidant activity of test samples was measured using a method as previously described by Pellegrini *et al*;[37]. Absorbance was read at 734 nm at 25 °C in a plate reader and the results were expressed as µM Trolox equivalents per milligram dry weight (µM TE/g DW) of the test samples.

2.7. Inhibition of Fe (II)-Induced Microsomal Lipid Peroxidation Assay

The thiobarbituric acid reactive substances (TBARs) method was used to evaluate inhibition of lipid peroxidation as described by Snijman *et al*., [38] with little adjustment. Rat liver microsomes were isolated from S9 rats using sepharose column with 0.01 M potassium phosphate buffer; pH 7.4, supplemented with 1.15 % KCl at 5 °C. Absorbance was measured at 532 nm and the percentage inhibition of TBARs formation relative to the positive control was recorded.

2.8. Statistical Analysis

Data were expressed as mean ± SEM (Standard Error of Mean). Variations among groups were determined using one way analysis of variance (ANOVA) and Tukey's Post Hoc test.

3.0 RESULTS AND DISCUSSION

3.1. Evaluation of the inhibitory effects of *S. frutesens* and *T.violacea* on Fe^{2+}-induced microsomal lipid peroxidation.

Currently, chemically synthesised antioxidants are prominently used in the food and drug industries, but of late they have been suspected of promoting negative health effects; hence, natural antioxidants are being investigated as healthier substitute. Antioxidants assays are excellent methods for investigating the potentials of plant-derived substances to inhibit the process of oxidative stress that characterises the onset and progression of several diseases.

TBAR, a marker of lipid peroxidation is commonly used to measure the process initiated by free radical attack on polyunsaturated fatty acids (PUFAs), leading to the formation of toxic aldehyde compounds such as malondialdehyde. Evaluation of the inhibitory effects of different extracts of *S. frutesens* and *T.violacea* on Fe^{2+}-induced microsomal lipid peroxidation (Fig. 1) showed that aqueous methanolic extracts (SF-aqueous-methanol (50%), SF-aqueous-methanol (75%), TV-aqueous-methanol (50%) and 75% TV-aqueous-methanol (75%)) inhibit this process effectively when compared to ethylacetate,

dichloromethane and water only extracts. This may be due to the ability of methanol to release considerable amounts of phenolic compounds which have excellent antioxidant activities [39].

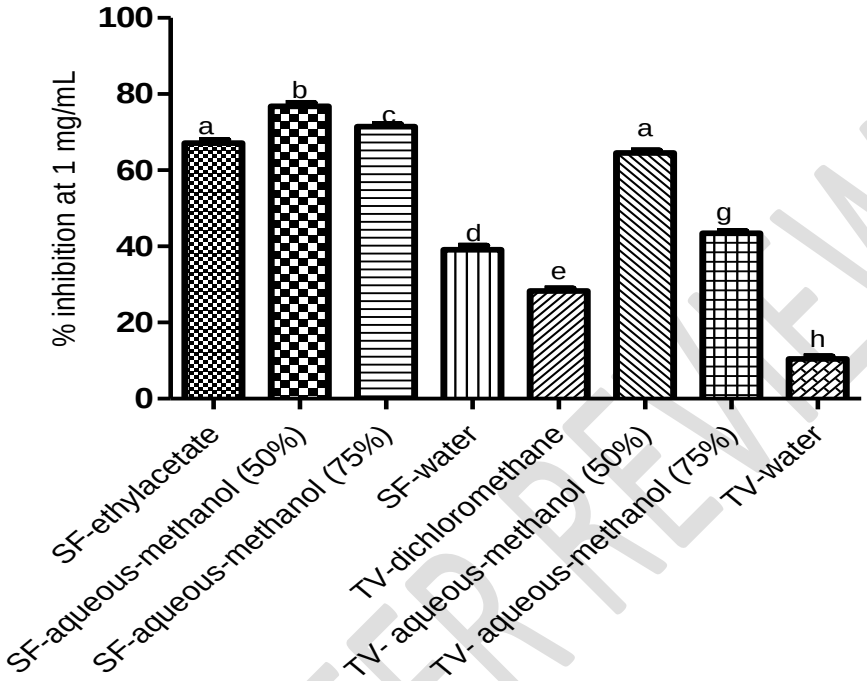

Figure 1: Inhibitory effects of *S. frutescens* and *T. violacea* extracts on Fe^{2+}-induced lipid peroxidation. Data are expressed as mean ± SEM of triplicate samples. Bars with same superscripts are not statistically different at *P*=.05.

Zonyane *et al;* [40] attributed the antioxidant activity of *S. frutescens* to the presence of phenolic compounds such as sutherlandins. The study of Shaik *et al*; [41] revealed that phenolics were the second most abundant phytochemical constituents in the leaves of *S. frutescens*. Madike *et al*; [42] reported that *T. violacea* leaf extracts had higher phenolic content than the stem and root extracts. Studies have shown correlations between antioxidant activity and polyphenolic compounds in methanolic extracts from an array of natural sources, including olive oil [43], rosemary [44], and *Pistacia atlantica* Desf. Fruits [45], grape seeds [46], and the leaves, stem, and root barks of *Moringa oleifera* [47]. Interestingly, Tobwala *et al;* [48] showed that aqueous extracts of *S. frutescens* yielded highest polyphenolic compounds (11.3 ± 0.32; gallic acid equivalent/mg of dried leaves) whilst methanolic extracts were a close second (9.26 ±

0.18; gallic acid equivalent/mg of dried leaves). Some authors state that aqueous extracts can even have a prooxidant (negative) effect, but Tobwala *et al;* [48] showed that both aqueous and methanolic extracts showed potent antioxidant and radical scavenging activities. Contrary to Tobwala *et al;* [48], Koleva *et al;* [49] results appeared more similar to the results found in this study, where methanolic extracts produced a greater antioxidant activity. However, Koleva *et al;* [49] attributed the greater intensity to the species of plant rather than the polarity of the extracting solvent.

3.2. Ferric ion reducing antioxidant power (FRAP) of *S. frutescens* and *T. violacea* extracts

The result of FRAP assay of *S. frutescens* and *T.violacea* extracts is illustrated in Fig.2. FRAP method is based on the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at low pH [50]. In the case of FRAP, aqueous methanolic extracts of *S. frutescens* and *T. violacea* displayed higher ferric ion reducing capacity when compared to their corresponding aqueous extracts. FRAP values of aqueous and aqueous-methanolic extracts of *S. frutescens* were considerably higher compared to values recorded for the corresponding extracts of *T. violacea*.

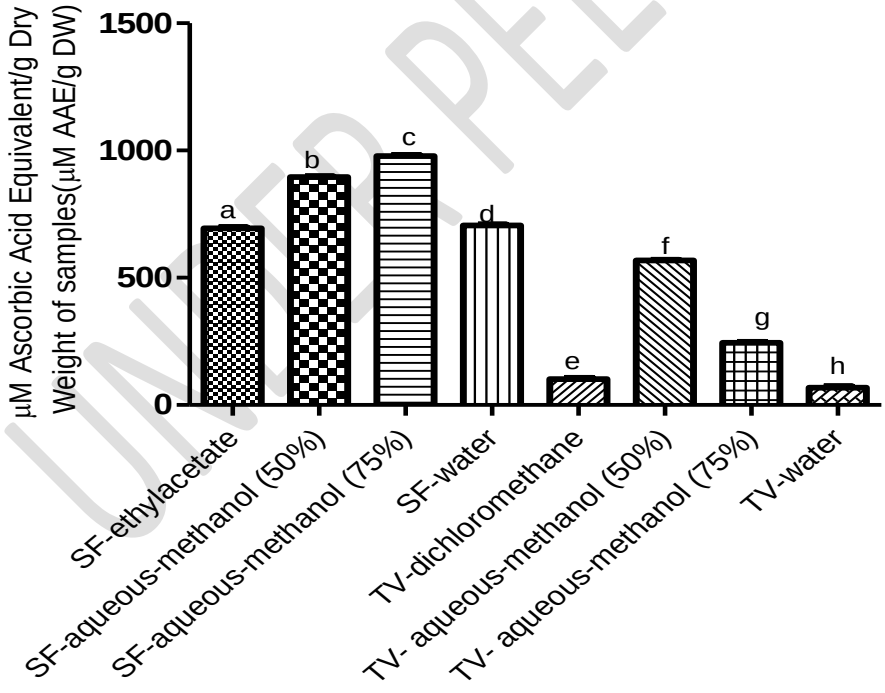

Figure 2: Ferric ion reducing antioxidant power of *S. frutescens* and *T. violacea* extracts. Data are expressed as mean ± SEM of triplicate samples. Bars with the same superscript are not statistically different at *P*=.05.

3.3. Trolox equivalent absorbance capacities of *S. frutescens* and *T. violacea* extracts

The result of TEAC assay of *S. frutescens* and *T.violacea* extracts is presented in Fig.3**.** TEAC assay evaluates the ability of test compounds to reduce the color intensity of a radical cation $ABTS^{\cdot+}$. SF-ethylacetate extract exhibited high TEAC activity, compared to TV-dichloromethane extract, which produced a comparatively low activity. SF-aqueous extract produced the highest TEAC activity, which almost doubled the corresponding activities of TV-aqueous and TV-aqueous-methanolic (75%) extracts. Interestingly, TEAC assay revealed higher antioxidant activities for all *T. violacea* extracts compared to those obtained using the FRAP assay. This is consistent with the findings of Rao *et al*; [51]. Stratil *et al;* [52] reported that TEAC assay yielded higher (about 2.8 folds) antioxidant activity than the FRAP method, as a result of the reactivity of the radical $ABTS^{\cdot+}$ (used in the TEAC method) with phenolic compounds in wines.

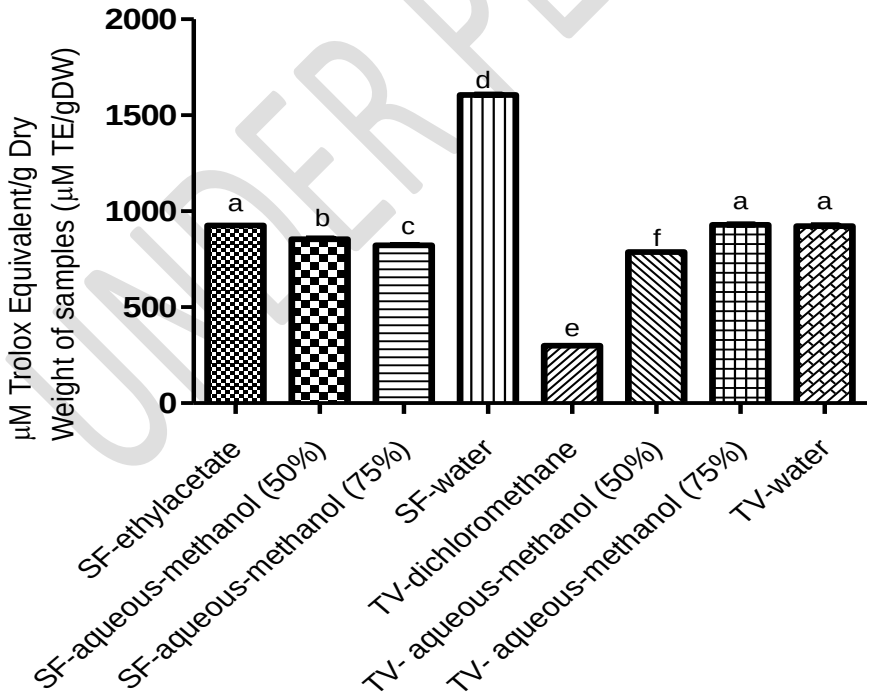

Figure 3: Trolox equivalent absorbance capacities of *S. frutescens* and *T. violacea* extracts. Data are expressed as mean ± SEM of triplicate samples. Bars with the same superscript are not statistically different at *P*=.05.

3.4. Oxygen radical absorbance capacities of *S. frutescens and T. violacea*

Oxygen radical absorbance capacity (ORAC) assay is a well-established method to determine the antioxidant capacity of a substance [53]. In this assay, substances with antioxidant properties are assessed based on the inhibition of oxyradical-induced oxidation of 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) [54]. SF- and TV-aqueous-methanolic extracts produced the highest peroxyl and hydroxyl scavenging activities, which increased in a concentration dependent manner (Table 1).

Table 1: Oxygen radical absorbance capacities of different extracts of *S. frutescens and T. violacea*

Sample	Peroxyl	Hydroxyl	Prooxidant
	µMTE/g ± %STDEV		
SF-ethylacetate	112.17 ± 4.65[a]	80.31 ± 3.19[a]	11.4 ±3.39[a]
SF-aqueous-methanol (50%)	619.38 ± 1.11[b]	1183.29 ± 1.87[b]	25.82 ± 3.02[b]
SF-aqueous-methanol (75%)	1048.09 ± 5.42[c]	3612.7 ± 6.07[c]	45.17 ± 1.25[c]
SF-water	1049.45 ± 0.54[c]	3911.27 ± 18.67[d]	51.40 ± 1.25[c]
TV-dichloromethane	68.50 ± 4.43[d]	44.03 ± 5.11[e]	5.25 ± 3.69[ad]
TV-aqueous-methanol (50%)	471.27 ± 3.57[e]	1948.00 ± 5.62[f]	36.01 ±7.42[bc]
TV-aqueous-methanol (75%)	545.12 ± 3.14[f]	2076.25 ± 10.25[g]	9.28 ± 3.25[ade]
TV-water	517.25 ± 3.91[g]	1970.13 ± 9.13[f]	17.2 ± 3.13[abe]

Data are expressed as mean ± SEM of triplicate samples. Values in the same column with the same superscripts are not statistically different at *P*=.05.

This is similar to the findings of Shin *et al*; [55] on green tea. SF-ethylacetate and TV-dichloromethane extracts had low hydroxyl and peroxyl scavenging activities. SF-aqueous extract, however, exhibited the highest peroxyl and hydroxyl scavenging activities. Fu [56] reported that polar constituents generally demonstrated antioxidant capacities possible due to the presence of phenolics which are capable of

converting free radicals to stable products through hydrogen ion and or electron transfer mechanisms. The high hydroxyl and peroxyl scavenging activities of aqueous extracts were closely matched by their corresponding 75 % aqueous-methanolic extracts. Copper initiated pro-oxidant activity was higher in SF- and TV-aqueous-methanolic extracts, compared to their respective ethylacetate and dichloromethane extracts. However, SF- aqueous extract exhibited the highest pro-oxidant activity.

4.0. CONCLUSIONS

The present study indicates that *S. frutescens* and *T. violacea* extracts displayed antioxidant capacities to extents which varied with the type of extraction solvent used, and may be of health benefit as inhibitors of oxidative stress, capable of mitigating of free radical attack on cells. The antioxidant activity of the extracts under study could possibly be related to phenolic compounds such as sutherlandins. However, for a better understanding of the mechanism of actions for the antioxidant activity demonstrated by these extracts, it would be obligatory to isolate the bioactive compounds responsible for the antioxidant and free radical scavenging activities.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AUTHORS' CONTRIBUTIONS

Gbemisola Saibu designed the study and prepared the first draft of the manuscript. Gbemisola Saibu, January Grant, Olugbenga Kayode and Badmus Jelili wrote the protocol and validated the results. Kanmodi Rahmon, Bello Ahmed and Adeyemo Gideon managed the literature searches as well as data

analysis and presentation. Adu Oluwatosin, Oluwadamilare Iyapo and Mutiu Kazeem reviewed and edited the manuscript. All authors read and approved the manuscript.

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