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In vitro anti-radical activities of extracts of *Solanum nigrum* (L.) from South Africa

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ABSTRACT

Objective: Solanum nigrum (black nightshade) is a wild vegetable with many ethnomedicinal uses. This study evaluated the quantitative phytochemical components, *in vitro* antioxidant and antibacterial activities of extracts of *S. nigrum* with the aim of providing more information on its therapeutic potential in the management of metabolic and infectious diseases.

Methodology and Results: The phytochemicals components, antioxidant and antibacterial activities of the extracts were evaluated using standard laboratory techniques. The phenols content was significantly higher in the ethanol extract of the berry (228.40±3.09 mg/g) than the water extract (132.2±1.23 mg/g) and the percentage saponins content was generally higher in the water extracts than the ethanol extracts of the plant. At 0.5 mg/ml, the water extracts exhibited higher ferrous reducing action and DPPH radicals scavenging activity than the ethanol extracts. The antioxidant activity of the ethanol extracts against nitric oxide radicals was higher than that of the water extracts. The ethanol extracts exhibited 100% antibacterial activity.

Conclusion and Application of results: *Solanum nigrum* showed significant antioxidant and antibacterial activities. The polyphenol components of the plant could be responsible for the antioxidant and antibacterial activities observed in this study. The isolation and characterization of active compounds from various parts of *S. nigrum* could lead to the discovery of novel compounds that could present the plant as a valuable candidate in the management of diseases. The plant could be important in the management of bacterial related infectious diseases. Powder and tincture prepared from the leaves could be used as antibacterial remedy. However, antifungal screening of the plant will further confirm its anti-infective status. Based on its antioxidant activity, the leaves of *S. nigrum* could be a good source of natural antioxidant with considerable therapeutic effect in the management of metabolic diseases such as diabetes, obesity, hyperlipidemia, atherosclerosis, hypertension and cancer. This study forms basis for future research activities on extracts and active compounds of *S. nigrum* for bioactivity in chronic diseases.

Keywords: *Solanum nigrum,* phytochemicals, antioxidant activity, antibacterial activity, natural antioxidant.

INTRODUCTION

Globally, chronic diseases constitute a major cause of mortality and oxidative stress is believed to be the main contributor to the pathogenesis of a number of chronic diseases such as cancer, atherosclerosis, heart failure, myocardial infarction and Alzheimer's disease (Singh *et al*., 1995; Halliwell, 2007; Valko *et al*., 2007). Antioxidants are compounds that delay autoxidation by inhibiting formation of free radicals or

by interrupting propagation of free radical by one (or more) of several mechanisms: scavenging species that initiate peroxidation; chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides; quenching O^{2-} and preventing formation of peroxides; breaking the autoxidative chain reaction and/or reducing localized O^{2-} concentration (Nawar, 1996). Epidemiological research has revealed a lower incidence of many chronic diseases in populations that are regular consumers of vegetables and fruits (Block *et al*., 1992). Vegetable have antioxidant properties. In addition to vitamins A and C, they contain a polyphenol (quercetin) that have strong H^+ donating activity (Weisburger, 1999). Phenolic acids generally act as antioxidants by trapping free radicals and some plant-derived compounds are better antioxidants than BHA (Butylated Hydroxyl Anisole) (Brewer, 2011). Consequently, natural antioxidants may be useful in the treatment and prophylaxis of chronic diseases. The increasing frequency of antibiotic-resistant bacterial infections is a threat to public health worldwide and has necessitated the search for plant-derived antimicrobial compounds as possible alternatives to chemically synthesized drugs to which many infectious microorganisms have become resistant (Gbadamosi, 2012). Functional foods such as fruits and vegetables are promising sources of antioxidant and antimicrobial compounds and are currently receiving the attention of researchers worldwide. *S. nigrum* (Solanaceae) (Fig. 1) is native to Eurasia and was introduced in the Americas, Australasia and South Africa. It is an annual herb up to 70 cm tall. The medicinal use of *S. nigrum* probably goes back more than 2000 years (Mohy-ud-dint *et al*., 2010). The plant is used traditionally as diuretic, tonic, antidiarrhoea, antimalaria, and in the treatment of eye, heart and skin diseases (Karmakar *et al*, 2010). This study investigated the phytochemical contents, antioxidant and antibacterial activities of extracts of berry, leaf, stem and root of *S. nigrum*, to provide further information on its therapeutic potential in the management of infectious and chronic diseases.

Figure 1: Leaves and berries of *Solanum nigrum*

MATERIALS AND METHODS

Plant material and preparation of extracts : Fresh and healthy whole plants of *S. nigrum* were collected from University of Fort Hare (UFH) Research Farm, Alice Campus, Eastern Cape Province of South Africa. A voucher specimen (BVE11/017) of the plant was prepared and deposited at the Griffin's Herbarium of the Department of Botany, UFH, South Africa. The plants were separated into berries, leaves, stems and roots. The plant parts were washed, cut into small pieces and kept in the oven at 30°C until they completely dried. The dried plant materials were ground into powder and stored in air-tight glass bottles at 4°C prior to experiments. The powdered plant parts (200g each) were shaken in 800ml of solvents [C_2H_5OH (ethanol) and H_2O (water)] for 24 h. The ethanol extracts of samples were filtered using Whatman no 1 filter paper and the filtrates were concentrated to dryness using a rotary evaporator (Loborota 4000- efficient, Heldolph, Germany) at 30°C. The extracts collected were allowed to dry to a constant weight at room temperature. The water extracts of various plant parts were freeze dried to constant weight using Savant Refrigerated Vapor Trap, RVT4104, USA.

Chemicals : DPPH (1,1 diphenyl-2-picrylhydrazyl), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), sodium nitroprusside $Na_2[Fe(CN)_5NO]2H_2O]$, potassium ferricyanide $[K_3Fe(CN)_6]$, phosphate buffer, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), ferric chloride ($FeCl_2$), vanillin, aluminium chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), potassium acetate (CH_3CO_2K), ascorbic acid, rutin, glacial acetic acid (CH_3COOH), Folin-

Ciocalteu's phenol reagent. They were purchased from Merck, Gauteng, South Africa. Other chemicals in experiments were of analytical grade.

Determination of total phenols: The total phenolic contents of plant parts of *S. nigrum* were measured using a modified method of Wolfe *et al* (2003). An aliquot of the extract was mixed with 5ml of Folin-Ciocalteu's reagent (previously diluted with water at a concentration of 1:10 v/v) and 4ml of 75g/L of sodium carbonate in a test tube. The mixture was vortexed for 15 s and left to stand for 30 min at 40°C for colour development. The absorbance was read at 765 nm using AJI-C03 UV- VIS spectrophotometer. The results were expressed as mg/g of tannic acid equivalent using the calibration curve:

$Y=0.121x$, $R^2 =0.936512$,

where x is the absorbance and Y is the tannic acid equivalent. The experiment was replicated three times.

Determination of total flavonols: The flavonol content of samples was determined using the protocol described by Kumaran & Karunakaran (2007). The reaction mixture consisted of 2.0 ml of sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50g/L) sodium acetate solution. The mixture was allowed to stand for 2.5 h at 20°C. The absorbance was measured at 440 nm. The result was calculated as mg/g of quercetin equivalent from the calibration curve using the equation:

$Y = 0.0255x$, $R^2 = 09312$,

where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times.

Determination of total flavonoids content: The flavonoids content of the sample was measured using the method of Ordon Ez *et al*, (2006). The sample (0.5ml) was added to 0.5 ml of 2% $AlCl_3$ ethanol solution. The mixture was allowed to stand for 1 h at room temperature and a yellow colour indicated the presence of flavonoids. The absorbance was read at 420 nm. The samples were evaluated at a final concentration of 0.1 mg/ml. The result was expressed as mg/g using the equation:

$Y = 0.0255x$, $R^2 = 09312$,

where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times.

Determination of total proanthocyanidins content: The method described by Sun *et al*. (1998) was used to evaluate the proanthocyanidin in the samples. The extract (0.5 ml of 1 mg/ml) solution was mixed with 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid. The mixture was left for 15min at room temperature and the absorbance was read at 500nm. The result was expressed as catechin equivalent (mg/g) using the calibration curve equation:

$Y = 0.5825x$, $R^2 = 0.9277$,

where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times.

Determination of tannins: The tannin content of samples was determined using modified method of AOAC (1990). Twenty (20) mls of 50% methanol was added to 0.20 g of the sample. The mixture was shaken thoroughly and placed in a water bath at 80°C for 1 h to ensure uniform mixing. The mixture was filtered into a 100 ml volumetric flask, followed by the addition of 20 mls of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 (Sodium carbonate). The solution was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The bluish-green colour developed of the reaction mixture of different concentrations (0-10ppm). The absorbance of the tannic acid standard solutions as well as sample was read at 760 nm using the AJI-C03UV-VIS spectrophotometer. The results were expressed as mg/g of tannic acid equivalent using the calibration curve:

$Y= 0.0593x – 0.0485$, $R^2 = 0.9826$,

where x is the absorbance and Y is the tannic acid equivalent. The experiment was replicated three times.

Determination of alkaloids: The method of Harborne (2005) was used for the quantification of alkaloids in samples. Two hundred (200) mls of 10 % acetic acid in ethanol was added to 5.0 g of powdered plant sample in 250 ml beaker. The mixture was covered and allowed to stand for 4 h. The mixture was filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until precipitation was completed and the solution was allowed to settle. The collected precipitate was washed with dilute NH_4OH and then filtered. The residue was dried and weighed. The alkaloid content was calculated using the formula:

% alkaloid = Final weight of the sample / Initial weight of the extract x 100.

The experiment was replicated three times.

Determination of saponins: The quantity of saponins in samples was determined using the technique of Harborne (2005). The powdered sample (20 g) was added to 100 ml of 20% aqueous ethanol and kept on a shaker for 30 min. The sample was heated on a water bath for 4h at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml on a water bath at 90°C. The concentrate was transferred into a 250 ml separator funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. Then n-butanol extract

was washed twice with 10 ml of 5% aqueous sodium chloride (NaCl). The remaining solution was heated on a water bath. After evaporation, the sample was dried in the oven at 40°C to a constant weight. The saponin content was calculated as follows:

% saponin = Final weight of the sample / Initial weight of the extract × 100

Reducing power assay: The reducing power of the extracts was evaluated using the method of Oyaizu (1986). The reaction mixture contained 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0ml of the extracts and standards prepared in various concentrations (0.025-0.5 mg/ml) in distilled water. The mixture was incubated for 20 min at 50°C and 2.5 ml of TCA (10% w/v) was added to it. It was then centrifuged at 3000rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was read at 700nm. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

ABTS radical scavenging assay: ABTS scavenging activity of the plant extracts was evaluated using the method described by Re *et al* (1999). The working standard solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM of potassium persulphate in equal quantity, the prepared solution was allowed to react for 12 h at room temperature in the dark. The resulting solution was further diluted by mixing 1 ml of $ABTS^+$ solution with 60ml methanol to obtain an absorbance of 0.706 units at 734nm after 7min using a spectrophotometer. The percentage inhibition was calculated as follows:

% $ABTS^+$ inhibition = Abs (control) – Abs (sample)/Abs (control) x 100.

Where Abs (control) is the absorbance of ABTS radical + methanol, and Abs (sample) is the absorbance of ABTS radical + sample (extract or standard).

Nitric oxide scavenging assay: Nitric oxide (NO) scavenging activity of extracts of *S. nigrum* was determined using modified method of Garrat (1964). 2.0 ml of 10 mM of sodium nitroprusside prepared in 0.5 ml phosphate buffer saline (pH 7.4) was mix with 0.5 ml of the plant extracts and standards separately at various concentrations (0.025-0.5 mg/ml). The mixture was incubated at 25°C for 2.5 h after which 0.5 ml of the mixture was mixed with Griess reagent. The solution was incubated at room temperature for 30 min and the absorbance read at 540 nm. Nitric oxide scavenging activity was calculated as follows:

Abs (control) – Abs (sample)/Abs (control) x 100

Where Abs (control) is the absorbance of NO radical + methanol, and Abs (sample) is the absorbance of NO radical + sample (extract or standard).

DPPH scavenging assay: The method of Liyana-Pathiranan & Shahidi (2005) was used for the determination of scavenging activity of DPPH free radicals. DPPH (1 ml of 0.135 mM) prepared in methanol was mixed with 1.0 ml of aqueous extracts (0.025-0.5 mg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min., the absorbance was measured at 517nm. The scavenging activity of plant extracts was calculated as follows:

% DPPH+ inhibition = Abs (control) – Abs (sample)/Abs (control) x 100.

Where Abs (control) is the absorbance of DPPH radical + methanol, and Abs (sample) is the absorbance of DPPH radical + sample (extract or standard).

***In vitro* antibacterial assay of plant extract**s: The isolates of *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 1982), *Serratia marcescens* (ATCC 9986) and *Streptococcus faecalis* (ATCC 29212) were obtained from the South African Bureau of Standard (SABS). *Enterococcus faecalis*, *Escherichia coli* and *Streptococcus pyogenes* were Laboratory isolates. The organisms were maintained on nutrient agar (Biolab, Johannesburg, South Africa) slants throughout the experimental period. The agar dilution method was used for the antibacterial screening. The isolates were grown in nutrient broth (Biolab, Johannesburg, South Africa) for 18 h at 37°C. Each broth culture was diluted (1:100) with fresh sterile nutrient broth. The extracts were tested against organisms at 5.0, 1.0, 0.5 and 0.1mg/ml. 1m1 of each extract was thoroughly mixed with 9ml of sterile nutrient agar in a test tube and poured into sterile Petri dish (100 mm in diameter). The agar was left to solidify. The organisms from the fresh nutrient broth were streaked in radial patterns on the agar plates (Meyer & Afolayan, 1995). 1ml of various extraction solvents in agar served as controls. In addition, plates containing the test organisms in agar without extract were used as control. All experiments were done aseptically and each experiment was replicated three times. The plates were incubated at 37°C for 24-48 hrs. Complete suppression of growth was required for an extract to be declared active. Observations were after 24h and 48h of incubation.

Data analysis: Where applicable, the results were expressed as mean ± standard deviation and subjected to one-way analysis of variance (ANOVA). The mean

values were separated using Duncan's multiple range test at P<0.5 level of significance.

RESULTS AND DISCUSSION

Phytochemical components of extracts of different parts of *Solanum nigrum:* The phytochemical analysis of the water and ethanol extracts of different parts of *S. nigrum* revealed the presence of polyphenols, alkaloids, saponins and tannins (Tables 1 & 2). The flavonols content was highest in the ethanol extract of the stem (40.25± 0.55 mg/g) and the root (37.16 ±1.73 mg/g) had the least, and there was no significant difference in the flavonols content of the ethanol extracts of the different plant parts (Table 1). The flavonoids component in the ethanol extracts of the plant parts was between 1.91±0.16 mg/g and 2.31±0.47 mg/g. The phenolic content was significantly high in the root (307.22±13.06 mg/g). The ethanol extract of the berry had 419.59±17.97 mg/g proanthocyanidins content and the root (92.61±4.98 mg/g) had the least. There was significant difference in the alkaloids, saponins and tannins constituents of ethanol extracts of the different plant parts. The water extract of the berry (39.59±0.88 mg/g) had the highest flavonols component (Table 2). There was no significant difference in the flavonoids content of the water extracts of the plant parts. Phenolic content was highest in the leaf (218.79±1.40 mg/g). Proanthocyanidins content was highest in the water extract of the stem (147.17± 2.99 mg/g), followed by the leaf (118.61±7.00 mg/g) and the root (102.32±7.87 mg/g had the least. The high proanthocyanidins content recorded in leaf water extract in this study is in contrast to the report of Jimoh *et al*. (2010). The difference data could be attributed to place and time of collection of sample, as well as age of the plant. Alkaloids content was highest in the berry (0.13%) and % saponins differed significantly in the plant parts. The water extract of the leaf of *S. nigrum* had highest tannins content of 1.27%. Generally, the polyphenol contents were higher in the ethanol extracts than the water extracts of berry, leaf, stem and root of the plant, except for the flavonoids in the water extracts of the leaf and stem. In addition, saponins were more soluble in the water extracts. The phytochemical constituents of plants have been reported to be responsible for their bioactivity (White *et al*, 1999; Tiong *et al*, 2013). Plant extracts contain phenolic acids, phenolic diterpense, flavonoids and volatile oils that can chelate metals and donate H^+ to oxygen radicals, thus making them extremely effective antioxidants (Brewer, 2011).

Table 1: Phytochemical components of ethanol extracts of different parts of *Solanum nigrum*

Plant parts	Flavonols (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Proantho-cyanidins (mg/g)	Alkaloids (%)	Saponins (%)	Tannins (%)
Berry	40.19^{a} ±3.02	2.11^{a} ±0.16	228.40^{a} ±3.09	419.59^{a} ±17.97	0.11^{a} ±0.00	0.34^{a} ±0.02	0.29^{a} ±0.00
Leaf	38.40^{a} ±1.93	2.31^{a} ±0.47	233.14^{a} ±2.42	186.16^{b} ±11.30	0.06^{a} ±0.04	0.28^{a} ±0.02	0.79^{b} ±0.00
Stem	40.25^{a}±0.55	1.91^{a} ±0.16	242.18^{a} ±0.56	183.09^{b} ±8.43	0.10^{a} ±0.00	1.16^{b} ±0.02	0.84^{c} ±0.00
Root	37.16^{a} ±1.73	1.94^{a} ±0.05	307.22^{b} ±13.06	92.61^{c} ±4.98	0.52^{b} ±0.00	1.08^{c} ±0.02	1.61^{d} ±0.00

Legend: Values are mean of three replicates ± SD. Values within the same column followed by same superscript are not significantly different at P<0.05. Flavonols are expressed as mg quercetin/g of dry sample. Flavonoids are expressed as mg quercetin/g of dry sample. Phenols are expressed as mg tannic acid/g of dry sample. Proanthocyanidins are expressed as mg catechin/g of dry sample.

Flavonoids are a class of secondary plant phenolic with significant antioxidant and chelating properties. They are concentrated in fruits, vegetables, wine tea and cocoa, their antioxidant and cardioprotective effects are attributed to the ability to inhibit lipid peroxidation, chelate redox active metals and attenuate other processes involving reactive oxygen species (Pietta, 2000; Heim *et al*, 2002; Cao *et al*, 2009). In this study, the highest flavonoids content was obtained from the stem water extract (5.62 mg/g). Previous authors (Maiza-Benabdesselam et al, 2007; Tiong et al, 2013) have reported the effective antioxidant activity of alkaloids. In addition, the alkaloids content of *S. nigrum* was highest in its berry extract. Proanthacyanidins are secondary plant metabolites that have antioxidant activity. They are prevalent in some food and dietary supplements such as berries, red grapes and their wines, and seeds. Some reports support improved vascular health after the consumption of proanthocyanidins or foods and supplements that contain them. These effects include vasodilation, presumably because of increased NO production, decrease platelet aggregation and reduced

sensitivity of low-density lipoproteins (LDL) to oxidation (Beecher, 2004; Goncalves *et al*, 2005). The proanthacyanidins content of *S. nigrum* was highest in the ethanol extract of the berries.

Table 2: Phytochemical components of water extracts of different parts of *Solanum nigrum*

Plant parts	Flavonols (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Proantho-cyanidins (mg/g)	Alkaloids (%)	Saponins (%)	Tannins (%)
Berry	39.59^{a} ±0.88	2.06^{a} ±0.20	132.26^{a} ±1.23	108.15^{ab}±7.00	0.13^{a} ±0.00	1.10^{a} ±0.02	0.19^{a} ±0.00
Leaf	26.63^{b} ±1.02	3.63^{b} ±0.44	218.79^{b} ±1.40	118.61^{ab}±7.00	0.05^{b} ±0.00	1.20^{b} ±0.02	1.27^{b} ±0.00
Stem	20.90^{c} ±0.70	5.62^{c} ±0.36	51.39^{c} ±0.78	147.17^{c}±2.99	0.04^{c} ±0.00	1.66^{c} ±0.02	0.56^{c} ±0.00
Root	36.94^{d} ±0.75	1.89^{d} ±0.06	75.07^{d} ±2.39	102.32^{d}±7.87	0.04^{c} ±0.00	1.52^{d} ±0.02	0.73^{d} ±0.00

Legend: Values are mean of three replicates ± SD. Values within the same column followed by same superscript are not significantly different at P<0.05. Flavonols are expressed as mg quercetin/g of dry sample. Flavonoids are expressed as mg quercetin/g of dry sample. Phenols are expressed as mg tannic acid/g of dry sample. Proanthocyanidins are expressed as mg catechin/g of dry sample.

Ferrous reducing antioxidant power of extracts of *Solanum nigrum*: The ferric reducing power activity of ascorbic acid (standard) was significantly higher than that of extracts of different parts of the plant (Fig. 2). There was no significant difference in the reducing action of water and ethanol extracts of berry, leaf, stem and root at 0.025, 0.05 and 0.1 mg/ml. However, at 0.5 mg/ml extracts concentration, the highest (0.36 nm) reducing activity of sample was observed for water extract of the berry, followed by the leaf (0.32 nm) and the ethanol extract of the root (0.13 nm) showed the least ferric oxide reducing activity. The water extracts exhibited higher ferric reducing action than the ethanol extracts at 0.5 mg/ml. Overall, the reducing power of all extracts increased along concentration gradient. The dose-dependent antioxidant activity is in line with the findings of previous authors (Adebooye *et al*, 2008). The antioxidant activity of plants has been reported by previous authors to be connected with the development of reducing power (Sultana *et al*, 2009; Xia *et al*, 2011), the standard antioxidant agents could react directly with peroxides or prevent peroxide formation by reacting with certain precursors (Pattanayak & Sunita, 2008).

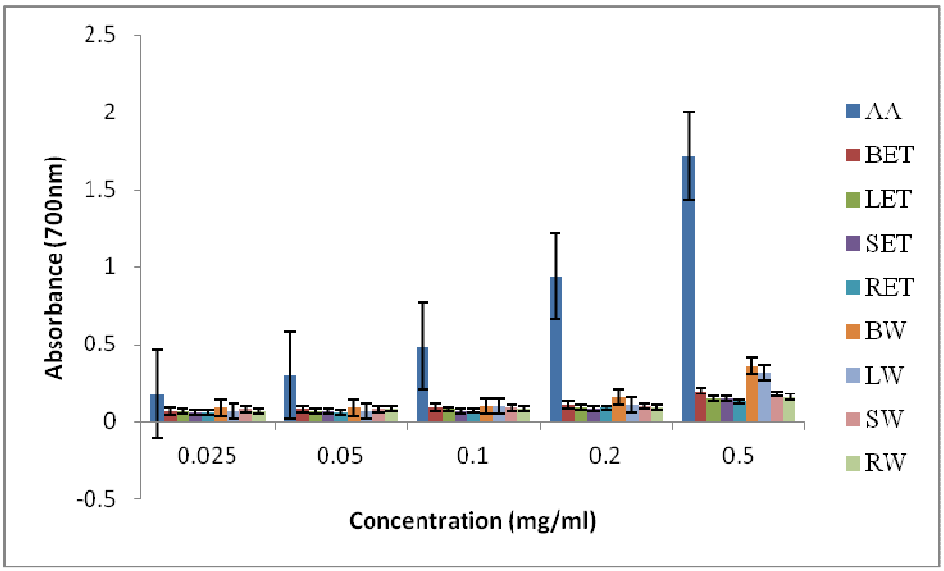

Figure 2: Ferrous reducing power of extracts of different parts of *Solanum nigrum*
Legend: AA=Ascorbic acid; BET=Ethanol extract of berry; LET= Ethanol extract of leaf; SET= Ethanol extract of stem; RET= Ethanol extract of root; BW=water extract of berry; LW= water extract of leaf; SW= water extract of stem; RW= water extract of root.

Antioxidant activity of extracts of *Solanum* nigrum against ABTS radicals: The ABTS scavenging activity of extracts of berry, leaf, stem and root of *S. nigrum* and standards is presented in Fig. 3, rutin exhibited ABTS

scavenging activity at all concentrations used whereas ascorbic acid was inactive at 0.025 mg/ml. The water extract of the stem and root showed no ABTS scavenging activity at 0.05 mg/ml. At 0.2 and 0.5mg/ml, the *in vitro* free radical scavenging activity of the extracts against ABTS radicals was significantly higher than that of standards (ascorbic acid and rutin). The ABTS scavenging activity of ethanol extracts increased along concentration gradient, getting to the peak at 0.5 mg/ml. The ethanol (94.06%) and water (85.62%) extracts of the leaf showed significantly high ABTS scavenging activity at 0.5 mg/ml and the water extract of the root had the least (42.18%) inhibition. The estimated IC_{50} values for standards and highly active extracts were as follows: ascorbic acid (0.20 mg/ml), rutin (0.125 mg/ml), ethanol extract of the leaf (0.188 mg/ml), ethanol extract of the stem (0.063 mg/ml), water extract of the leaf (0.125 mg/ml), and water extract of the stem (0.125 mg/ml). The phenolics and flavonoids contents of the various extracts of *S. nigrum* could have interrupted the ABTS radical chain of oxidation by donating hydrogen and forming stable free radicals, which do not initiate or propagate further oxidation of lipids. The observed ABTS scavenging activity of the extracts conforms to previous report on antioxidant activity of the plant (Karmakar *et al*, 2010).

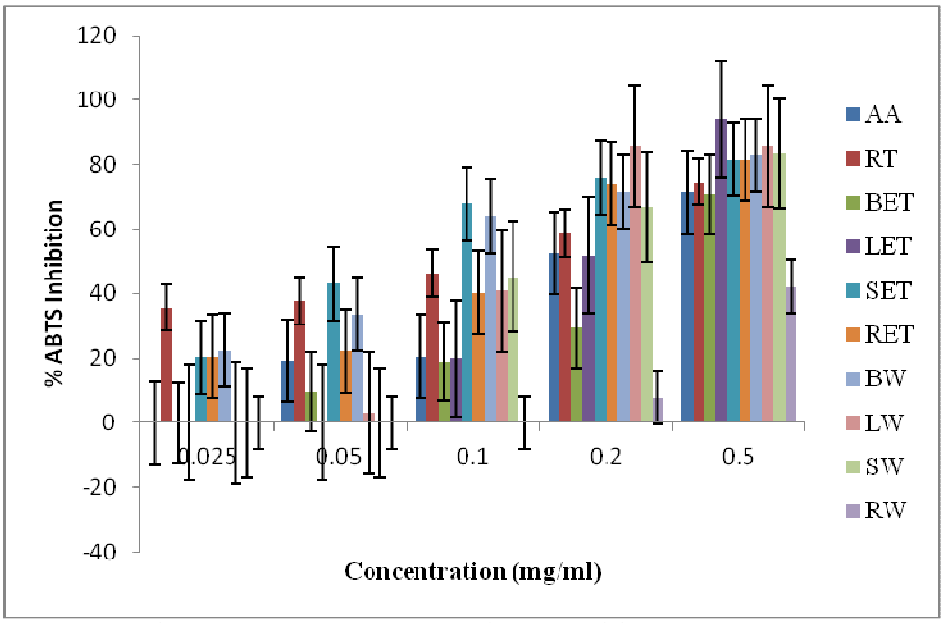

Figure 3: ABTS radical scavenging activities of extracts of *Solanum nigrum*
Legend: AA=Ascorbic acid; RT=Rutin; BET=Ethanol extract of berry; LET= Ethanol extract of leaf; SET= Ethanol extract of stem; RET= Ethanol extract of root; BW=water extract of berry; LW= water extract of leaf; SW= water extract of stem; RW= water extract of root.

Antioxidant activity of extracts of *Solanum* nigrum against nitric oxide radicals: The ethanol extracts of berry, leaf, stem and root of *S. nigrum* exhibited significantly high scavenging activities against nitric oxide radicals compared with the standards, and water extracts (Fig. 4). The water extracts were inactive on nitric oxide radicals at 0.025-0.1 mg/ml, however they showed little activity at 0.5 mg/ml with the stem extract (95.7% inhibition) being most active on nitric oxide radicals. Generally, the ethanol extracts were the most active against nitric acid radicals. The nitric oxide radical scavenging activities of extracts and standards were in the order: ethanol extracts > ascorbic acid > rutin > water extracts. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages and neurons and it is involved in the regulation of various physiological processes. The excess concentration of nitric oxide in living system is associated with diseases such as atherosclerosis and hepatic toxicity (Ross, 1990; Mondal *et al* 2005). The standards and ethanol extracts showed significant antioxidant activity inhibiting the generation of anions from nitric oxide.

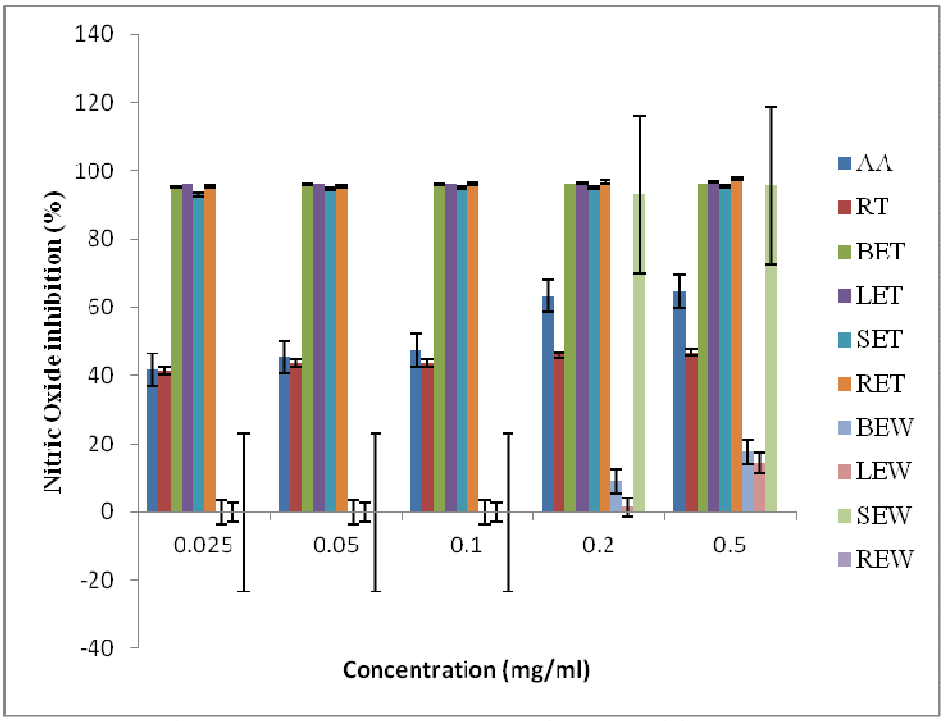

Figure 4: Nitric Oxide radical scavenging activities of extracts of *Solanum nigrum*
Legend: AA=Ascorbic acid; RT=Rutin; BET=Ethanol extract of berry; LET= Ethanol extract of leaf; SET= Ethanol extract of stem; RET= Ethanol extract of root; BW=water extract of berry; LW= water extract of leaf; SW= water extract of stem; RW= water extract of root.

Antioxidant activity of extracts of *Solanum* nigrum against DPPH radicals: The % inhibition of DPPH radical by *S. nigrum* extracts and standards indicated that the standards (ascorbic acid and rutin) were the most active on DPPH radicals (Fig. 5). Of all extracts, the water extract of the leaf showed the highest scavenging activity against DPPH radicals, its activity increased along concentration gradient (0.025-0.5 mg/ml), being highest (87.16%) at 0.5 mg/ml. The DPPH radical scavenging activity of the ethanol extract of the root increased along concentration gradient and gave 54.69% inhibition at 0.5 mg/ml. Overall, the water extracts of berry, leaf, stem and root of the plant exhibited higher DPPH radical scavenging activity than the ethanol extracts. The estimated IC_{50} value for the water extract of the berry was 0.223 mg/ml. The inhibition of DPPH radicals was in the order: ascorbic acid > rutin > water extracts > ethanol extracts. Although there was no correlation between the phytochemical constituents of extracts and the inhibition of DPPH radicals, but the result showed that the extracts of *S. nigrum* were electron donors and could react with free radicals, converting them to more stable products and terminating the radical chain reaction (Oktay *et al*, 2003). The antioxidant activity could be accountable for the pharmacological effects of the plant in the management of diseases caused by oxidative stress such as diabetes and hypertension (Atanu *et al*, 2011).

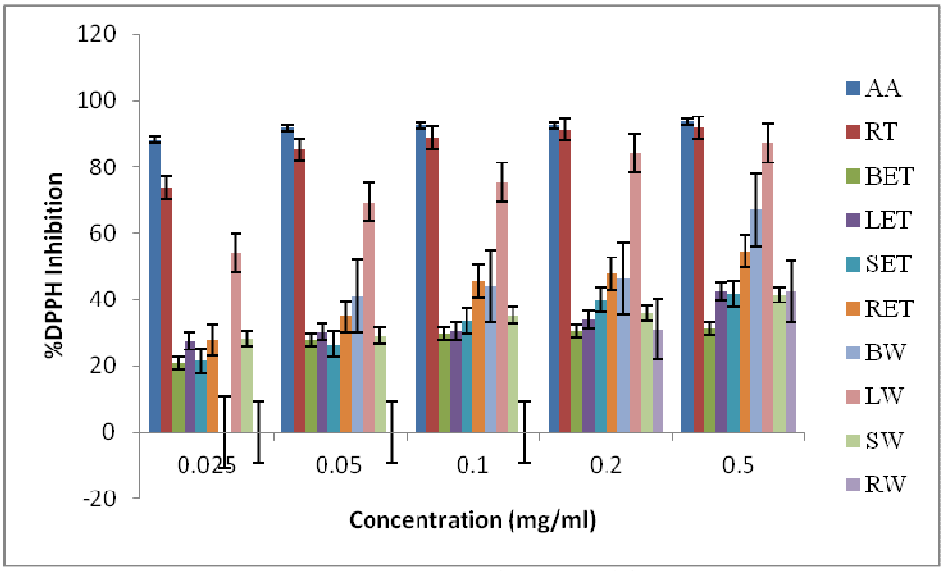

Figure 5: DPPH radical scavenging activities of extracts of *Solanum nigrum*
Legend: AA=Ascorbic acid; RT=Rutin; BET=Ethanol extract of berry; LET= Ethanol extract of leaf; SET= Ethanol extract of stem; RET= Ethanol extract of root; BW=water extract of berry; LW= water extract of leaf; SW= water extract of stem; RW= water extract of root.

Antibacterial activity of extracts of *Solanum nigrum:* The water extracts of different parts of *S. nigrum* showed no activity against all bacterial isolates used in this study. The ethanol extracts showed significant antibacterial activity against all isolates (Table 3). All isolates were susceptible to the ethanol extract of the berries at the lowest concentration used (0.1 mg/ml) except *S. pyogenes* that gave a MIC value of 1.0 mg/ml. The ethanol extract of the leaves inhibited the growth of all bacterial isolates at the lowest concentration of extract (0.1 mg/ml). All bacterial species were susceptible to the ethanol extract of the stem at 0.1 mg/ml concentration of the extract except *S. pyogenes* (1.0 mg/ml). The ethanol root extract gave a MIC of 0.1 mg/ml against all test organisms apart from *S. pyogenes* that recorded a MIC of 5.0 mg/ml. The significant antibacterial activity of ethanol extracts of different part of *S. nigrum* observed in this study agrees with the reports of previous authors (Kaushik *et al*, 2009; Zubair *et al*, 2011; Parameswari *et al*, 2012, Gbadamosi & Afolayan, 2015). The observed inactivity of the water extracts against the test organisms might be due to the solubility of phytochemicals of its different parts in water (Ramya *et al*. 2012).

Table 3: Antibacterial activity of the ethanol extracts from different parts of *Solanum nigrum*

S/N	Bacterial isolate	Gram +/-	MIC (mg/ml)[a]			
			Berry	Leaf	Stem	Root
1.	*Bacillus cereus*	+	0.1[b]	0.1	0.1	0.1
2.	*Bacillus pumilus*	+	0.1	0.1	0.1	0.1
3.	*Enterococcus faecalis*	+	0.1	0.1	0.1	0.1
4.	*Streptococcus pyogenes*	+	1.0	0.1	1.0	5.0
5.	*Staphylococcus aureus*	+	0.1	0.1	0.1	0.1
6.	*Escherichia coli*	-	0.1	0.1	0.1	0.1
7.	*Klebsiella pneumoniae*	-	0.1	0.1	0.1	0.1
8.	*Pseudomonas aeruginosa*	-	0.1	0.1	0.1	0.1
9.	*Serratia marcescens*	-	0.1	0.1	0.1	0.1
10.	*Streptococcus faecalis*	-	0.1	0.1	0.1	0.1

[a]Minimum inhibitory concentration.
[b]Lowest concentration of extracts tested

CONCLUSION

The multiple antioxidant activities of extracts *S. nigrum* observed in this study was evident as the extracts showed significant reducing power, nitric oxide scavenging activity, and inhibition of DPPH and ABTS free radicals. The polyphenol contents could be accountable for the antioxidant and antibacterial activities of this plant. Furthermore, the antioxidant value of the plant could be responsible for its reported pharmacological effects in the management and treatment of metabolic diseases such as hypertension, arthritis, atherosclerosis and hepatic toxicity. As plants of the same species collected from different geographical regions may show varying antimicrobial activities, this study confirms the efficacy of South African species of *S. nigrum* in the management of bacterial infections. However, the potency of the extract depends on the extraction solvent.

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