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Phytonutrients, Minerals and *in vitro* Antioxidant Capacity of Leaf and Stem Bark Powders of *Senna spectabilis*

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Senna spectabilis is a small, rounded deciduous tree commonly found in the lower parts of Eastern province of Kenya. The plant is known to treat dysentery, menstrual cramps, whooping coughs and diabetes mellitus among the natives of Mbeere in Kenya. The present study investigated the amounts of phytonutrients, phytochemicals, mineral elements and *in vitro* antioxidant capacity of the leaf and stem bark powders of *S. spectabilis*. HPLC-UV/VIS, standard phytochemical methods, energy dispersive X-Ray fluorescence system, and 1, 1-diphenyl-2-picrylhydrazyl methods were used. Significant levels of retinol, α -tocopherol, thiamine, nicotinamide, β -carotene, lycopene, and β -cryptoxanthin were found in both leaf and stem bark powders. The antioxidant activity was 63.36, and 28.12%, for the leaf and stem bark powders, respectively. The free radical scavenging activity for the stem bark and leaf powders was 90.95%, and 88.98%. Also important mineral elements were found. The study findings support the claimed efficacy in the management of various diseases among the natives.

Keyword: Antioxidant Activity, Phytonutrients, Mineral Elements, *Senna spectabilis*.

1. Introduction

Senna spectabilis D.C belongs to family Fabaceae-Caesalpinioideae, is a small, rounded deciduous tree approximately 7 to 10 m in height, and has a trunk whose diameter is approximately 30 cm. The plant has characteristic smooth grey bark with horizontal markings, many warts and short fissures, which gets rougher with age. The twigs are stout, brown with light dots, finely hairy and the young parts are soft. The leaves alternate, up to 40cm, are compound with 4 to 15 pairs of leaflets, each up to 7.5 cm^[1-2].

Senna spectabilis is widely distributed in the tropical areas of Africa, Asia, Australia, Latino and South America^[3]. In Africa, it is found in Angola, Burundi, Cameroon, Chad, Kenya, Nigeria, Tanzania, Togo, and South Africa among others^[1, 4]. In Kenya, *Senna spectabilis* is distributed in Siakago, Ngong hills, Loitokitok and semi-arid regions of southern part of Embu County.

Senna spectabilis is widely grown as an ornamental plant in tropical and subtropical areas, and has been commonly used in traditional

medicine since time immemorial. Among the Brazilian, it is used for treatment of flu and cold, as a laxative and purgative^[5]. Among the people of Cameroon, it is used to treat constipation, insomnia, epilepsy, anxiety and dysentery among many other diseases^[2]. In Nigeria, it is used to treat syphilis^[3]. Studies have shown that the extract of *Senna sp.* inhibits lipid peroxidation of bovine brain phospholipids due to its strong antioxidant activity^[6].

Despite extensive use of *S. spectabilis* among the natives of lower eastern province of Kenya in management of diabetes, whooping cough and menstrual cramps, among others, there is no scientific data to support the medicinal claims made by the traditional medicinal practitioners. The present study was set to investigate the phytochemical compositions, mineral elements and *in-vitro* antioxidant activity of *Senna spectabilis* leaves and stem barks and correlate these constituents with its medicinal usage among the local communities.

2. Materials and Methods

2.1 Chemicals and Plant Materials

All the reagents used in this study were of analytical grade and were sought from various companies. The solvents were obtained from E. Merck Germany, and were HPLC grade. The ascorbic acid, β -carotene, β -cryptoxanthin, and lycopene were purchased from Sigma Aldrich, USA. Thiamine HCL Lot No 36020 was obtained from Serrra Heidelberg, Germany. The α -tocopherol standard was obtained from Fluka, Switzerland, and niacinamide Lot 37F-0018, was obtained from Sigma Aldrich, USA.

Green leaves and stem barks of *Senna spectabilis* were collected in March 2011 from their natural habitats in Kambara village, Siakago in Embu County of Kenya. The plant was authenticated by Dr. Omwoyo, a taxonomist at the department of plant and microbial sciences, Kenyatta University, Kenya and a voucher specimen (ref. no. GMK03/2011/2) deposited at the Kenyatta University Herbarium for future reference. The leaves and stem barks were dried under shade for two weeks and crushed in a mechanical mill into fine powder.

2.2 Determination of Phytochemicals

The phytochemicals were screened in powdered medicinal plant materials using procedures earlier described^[7-8], with slight modifications. The following classes of phytochemicals were determined; phenols, alkaloids, flavonoids, steroids, tannins, cardiac glycosides, phylobatannins, saponins, and reducing sugars.

2.3 Antioxidant Activity

The antioxidant activity of the leaf and stem bark powder was determined according to the method described^[9] with slight modifications. Briefly, 10 mg of the powdered plant parts was weighed and mixed with 10 mL methanol, and the mixture was stirred for 30 min. The resulting suspension was filtered through Whatman No. 1 filter paper, and the filtrate was used in the determination of antioxidant activity. β -carotene solution (4 ml) prepared by dissolving 0.1 mg of β -carotene in 1 mL of chloroform, 40 mg of linoleic acid and 400 mg of Tween- 20 were transferred to a round-bottomed flask and mixed by shaking gently.

The mixture was evaporated at 50°C in a rotary evaporator to get rid of chloroform. Then, 100 mL of de-oxygenated distilled water was slowly added to the residue, and vigorously mixed into a stable emulsion. Then, 800 μ L of the extract was added to 3 mL aliquots of β -carotene/linoleic acid emulsion. The blank comprised of the β -carotene/linoleic acid emulsion dissolved in methanol without the extract. Absorbances were read at 470 nm wavelength three times immediately at time zero using a spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotomer Shimadzu Japan). The mixture was then incubated at 50°C for 90 min and absorbances read after every 15 min with methanol in β -carotene/linoleic acid emulsion as a control. Butylated hydroxylated toluene (BHT) was used as a positive control.

2.4 Free Radical Scavenging Activity by DPPH Assay

The free radical scavenging activities of the methanol extracts of the leaves and stem bark powders was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method ^[10]. In this

method, a stock solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 mL of methanol. The solution was kept at 20°C until required. The working solution was prepared by diluting DPPH stock solution with methanol till the absorbance was noted to be

0.980 ± 0.02 at 517 nm. Then, 3 mL of the working solution was mixed with 100 µL of a methanol extract of the medicinal plant (1 mg/mL). After incubating the mixture in the dark for 30 min, absorbance was read at 517 nm.

$$\ln(a/b) \times 1/t = DR_{\text{Sample or Standard}} \quad \text{Equation 1}$$

Where:

Ln is the natural log,

a is the initial absorbance (470 nm) at time zero and

b is the absorbance (470 nm) at 100 min and *t* is time.

Antioxidant activity (AA) expressed as percent of inhibition relative to the control was calculated as follows;

$$AA = (DR_{\text{Control}} - DR_{\text{Sample or Standard}} / DR_{\text{Control}}) \times 100. \quad \text{Equation 2}$$

The scavenging activity was calculated by using the formula;

$$\text{Percent scavenging activity} = \frac{(\text{Absorbance of the blank} - \text{Absorbance of the sample})}{\text{Absorbance of the blank}} \times 100\%$$

Equation 3

2.5 Retinol and α -tocopherol

Two grams of plant powder was suspended in 50 mL of methanol and into this suspension, 0.25 g of ascorbic acid and 5 mL of 50% sodium hydroxide were added. The mixture was blanketed with nitrogen and saponified in a water bath at 60°C for an hour with intermittent shaking after every 20 min. After saponification, the flasks were cooled in a running stream of cold water. Then, 50 mL of distilled water was added to the sample.

Retinol and α -tocopherol were extracted from the sample using 70 mL of n-hexane containing 30 mg/kg of BHT. The phases were allowed to separate and the aqueous phase drained to the round bottomed flask and the n-hexane layer into a conical flask covered with aluminium foil. The procedure was repeated two times with 50 mL of

n-hexane. The organic phase containing the retinol and α -tocopherol was evaporated in a rotary evaporator under reduced pressure and at temperature below 50°C. The resulting extract was dissolved in 10 mL of methanol, filtered and 10 µL was used in the determination of retinol and α -tocopherol by HPLC-UV/VIS (Shimadzu, Inc. Japan) method.

The standard was prepared by dissolving 100 mg of α -tocopherol standard (Fluka Biochemica, purity ≥97.0% HPLC grade) in 100 mL of absolute ethanol. The absolute concentration of the standard stock was determined by use of a UV-VIS spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotometer Shimadzu, Japan) at 291 nm. The molar coefficient of extinction of α -tocopherol in absolute ethanol is 75.6 m²/mol. The concentration of the standard stock solution was determined as follows from three absorbances as follows;

$$\text{Concentration of } \alpha\text{-tocopherol } (\mu\text{g/ml}) = \frac{\text{Mean Absorbance} \times 10^4}{75.8} \quad \text{Equation 4}$$

The HPLC system was set as follows; flow rate of 1.2 mL/min, column oven temperature of 25°C, and injection volume of 10 µL and run time of 11.50 min. the mobile phase consisted of HPLC grade water and methanol mixed in the ratio of 70: 30 (v/v). For the fluorescence detector, the excitation and emission wavelengths were set at 290, and 330 nm, respectively. Between the standards and samples, a blank comprising of filtered mobile phase was injected to prevent carry over. The standard working solution and the samples were injected three times and the mean peak area was used in the determination of the concentrations.

For retinol, 100 mg of vitamin A-palmitate standard 92% pure was obtained from Fluka Biochemica, Switzerland. This was dissolved in 100 mL of absolute ethanol. Three determinations of absorbance were carried out and the mean absorbance at wavelength of 324.3 nm was recorded for determination of the actual concentration of the standard stock solution. The molar coefficient of extinction of vitamin A-palmitate in absolute ethanol is 1830 m²/mol, and was used in the determination of the concentration of the standard stock solution.

The HPLC system was set as follows; flow rate of 1.0 mL/minute, column oven temperature 35°C, and injection volume of 10 µL and run time of 6.0 min. The UV-VIS detector wavelength was set at 325 nm. The standard stock solution and the samples were injected three times and the mean peak area was used in the determination of the concentrations.

The mobile phase consisted of HPLC grade water and methanol mixed in the ratio of 70: 30 (v/v). The mobile phase was sonicated and then filtered into a reservoir ready for use. Waters Spherisorb ODS-1 column of particle size 5 µm, 250 mm long and internal diameter 4.6 mm was used. The column was balanced with mobile phase until a good baseline suitable for analysis was obtained. The concentration was determined from peak areas as previously described in our laboratory^[11].

2.6 Beta-carotene, beta-cryptoxanthin and lycopene

The plant materials were analyzed for β-carotene, β-cryptoxanthin and lycopene according to the described method^[11]. HPLC system fitted with a uv/vis detector was used. The mobile phase consisted of 90: 10, methanol: acetonitrile with 0.05% (v/v) of Triethanolamine (TEA). The flow rate was set at 2.5 mL/min, the oven temperature at 25°C and detector wavelength at 451 nm. β-carotene (2 mg) ≥ 93% pure standard was dissolved in 10 mL absolute ethanol and actual concentration determined spectrophotometrically. The wavelength of maximum absorbance was recorded and the mean absorbance and the molar extinction coefficient of β-carotene in absolute ethanol were used in the determination of the actual concentration of the working standard as shown in equation 3, and the 2560 m²/mol as the molar extinction coefficient of β-carotene in absolute ethanol. Samples and the standards were analyzed in triplicates.

Lycopene (1 mg) standard purchased from Sigma Aldrich, USA (L9879, >90% purity, Lot No 040M5162V) was dissolved in 5 ml of n-hexane and vortexed until it dissolved completely. The solution was scanned in a UV-VIS spectrophotometer at a wavelength range of 450 to 510 nm to determine the wavelength of maximum absorbance. The absorbance at the wavelength of maximum absorbance was determined in triplicates and the mean calculated for determination of the actual concentration of the standard using molar extinction coefficient of lycopene in n-hexane as 3450 m²/mol. 1 mL of the standard stock was diluted 10 times to make a working solution.

β-cryptoxanthin (1 mg) standard purchased from Sigma Aldrich, USA (CAS472-70-8; C40H56O, >98% purity) was dissolved in 5mL of absolute ethanol and vortexed until it dissolved completely. The solution was scanned in a UV-Vis spectrophotometer at a wavelength range of 430-490 nm to determine the wavelength of maximum absorbance. The absorbance at the wavelength of maximum was determined in triplicates and the mean calculated for determination of the actual concentration of the

standard using molar extinction coefficient of beta-cryptoxanthin in absolute ethanol as 2356 m²/mol. 1 mL of the standard stock was diluted 10 times to make a working solution.

Mobile phase for HPLC was prepared by mixing methanol: acetonitrile and tetrahydrofuran in the ratio of 70: 25: 5 (v/v), respectively. The mixture was sonicated to remove air bubbles. The extraction solution was prepared by mixing methanol and tetrahydrofuran in 50: 50 (v/v), and this was also used as a blank.

The Waters Spherisorb (ODS-5 μ , Lot No 122, Part No 8364, length 250 mm x 4.6 mm, Serial No 05021098.1) HPLC column was conditioned at oven temperature of 25°C, flow rate of 1.0 mL/min and wavelengths 451 nm (β -carotene), 471 nm (lycopene), and 452 nm (β -cryptoxanthin).

The standards and the samples were analyzed in triplicates. Peak areas were used in determination of the amounts. Single point calibration was used in quantitation and the amounts were recorded as μ g/100g of dry matter \pm standard deviation.

2.7 Ascorbic Acid

Ascorbic acid was determined in the extracts as total L+ and D+ - ascorbic acids in 2% metaphosphoric acid using HPLC fitted with a UV-VIS detector. Briefly, 1 g of the milled plant material was extracted in 10 mL of 2% metaphosphoric acid for 1 hour. The extraction was done in amber flasks covered with aluminium foil and sonicated at room temperature. The extract was filtered with Whatman filter paper No 540 and further filtered with 0.54 μ m membrane filter ready for analyses with HPLC.

The mobile phase comprised of 50 mM potassium dihydrogen phosphate and pH 2.4. This was filtered and then sonicated to remove air bubbles before use. The wavelength was set at 265 nm, flow rate at 2.0 ml/minute and oven temperature at 15°C. A Phenomenex column (C₁₈) 175 x 3.20 mm x 5 μ m internal diameter was used. The ascorbic acid 99.7% pure was used as a standard.

Serial dilution of the ascorbic acid standard ranging from 0.4 to 5.3 mg/100g was used in the

construction of the calibration curve. Then, 10 μ L of each sample and standard level was injected into the HPLC system and peak areas recorded.

2.8 Thiamine and Niacinamide

One gram of the powdered plant material was dissolved in 25 mL of extraction solution comprising of 50 ml acetonitrile, 10 mL of glacial acetic acid 100 mL HPLC grade water, while shaking on a water bath at 70°C for 40 minutes. The samples were cooled and filtered and the final volume adjusted to 50 mL with the extraction solution.

The standard for thiamine was prepared by dissolving 27 mg of thiamine hydrochloride in 25 mL of HPLC grade water. The linear working range for the standard was 0.896 to 1.792 μ g/mL. For niacinamide, 42 mg of niacinamide was dissolved in 25 mL of HPLC grade water. The linear range working dilutions of the standard was 1.376 to 5.504 μ g/mL.

The mobile phase consisted of methanol and 5 mM heptanesulphonic acid, sodium salt and 0.1% triethylamine in ratio of 25: 75 v/v with the pH of 3.0 adjusted with concentrated orthophosphoric acid. A Cronus HPLC column (Lot No CC011569, NF-00909 Lichrospher 100RP-18EC) of 5 μ m x 25 x 0.46 mm was used, with a flow rate set at 0.8 mL/min and UV-VIS detector wavelength at 254 nm. 10 μ L of each standard dilution and the sample extracts was injected three times and peak areas recorded at the retention times for vitamins B1 and B3, respectively.

2.9 Mineral composition of *Senna spectabilis* powders

10 g of plant powder and 5 g of soluble starch were mixed and pressed into 7 mm thick discs with 4.1 cm in diameter in a mold with a force of 200 kN for 5 seconds. In order to retain the original dry mass of the samples, the discs were stored in a desiccator. The mineral element concentrations were determined by energy dispersive X-ray fluorescence (EDXRF), using Ray EDX-720, EDXRF spectrometer Shimadzu with a Rh anode and Lit 100, PET, Ge and OVO 55 crystals, Si (Li) detector, ¹⁰⁹Cd and ⁵⁵Fe

annular sources and multichannel analyzer with a built-in minicomputer. The following elements were quantified; V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, and Mo, and the results were expressed as $\mu\text{g/g}$ of the dry matter.

3. Results and Discussions

3.1 Phytochemical Compositions

The phytochemicals detected in the leaf and stem bark powders of *S. spectabilis* were tannins, phenols, saponins, phylobatannins, flavonoids, steroids, alkaloids and cardiac glycosides. The cardiac glycosides was detected in trace amounts, however, reducing sugars were not detected in the plant powders (Table 1). Results in Table 2 shows that leaf powders had higher amounts of total phenols, flavonoids, saponins and tannins compared to the stem bark powders. The presence of phenolic compounds in the leaf and stem bark powders of *S. spectabilis* are responsible for the antimicrobial properties against pathogenic bacteria and this explains its use in the traditional medicine in the treatment of bacterial infections. Alkaloids and their synthetic derivatives are used as elementary therapeutic agents because of their analgesic, antispasmodic and bactericidal effects^[12]. Saponins also referred to as the bitter phenolic compounds are known to bind to cholesterol and block its uptake by the intestines and facilitate its excretion^[13].

Flavonoids enhance the effects of vitamin C and both functions as antioxidants. They are abundantly found in the leaves and stem bark powders of *S. spectabilis* and perhaps are responsible for synergistic antioxidant activity important in the management of diabetes mellitus. They protect blood vessels especially the tiny capillaries that carry oxygen and

nutrients to cells and are believed to slow down the development of cataracts in persons who have diabetes^[14]. Ascorbic acid protects low-density lipoproteins *ex vivo* against oxidation and may function in the similar manner in the blood and it also stabilizes folate in food and in plasma^[15]. The steroids and phylobatannins present in this plant make it a good source of steroidal compounds which are potent precursors for the synthesis of sex hormones and these could be responsible for the claimed activity in the management of menstrual cramps. The oil from *S. spectabilis* seeds is applied externally for skin diseases^[4]. Due to the huge amounts of antioxidants this plant is used in treatment of wounds and in restoring inflamed tissues by increasing blood supply to the wounded skin^[16].

3.2 Phytonutrients

Other vitamins such as nicotinamide present in this plant play very important biochemical roles in the body. Nicotinamide an amide of nicotinic acid is the precursor of NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ which play essential metabolic roles including energy metabolism, amino acid metabolism and detoxification of drugs and other substances, thus making this plant useful. Furthermore, nicotinamide acts as an antioxidant by preventing NAD^+ depletion during DNA repair by inhibiting poly (ADP-ribose) polymerase (PARP) which also modulates major histocompatibility complex (MHC) class II expression; inhibits free radical formation and facilitates beta cell regeneration *in vivo* and *in vitro*. Additionally, its protection from macrophage toxins is crucial in prevention of type I diabetes^[17].

Table 1: Phytochemicals from the leaf and stem bark powders of *S. spectabilis*

Plant part	Class of phytochemicals							
	Tannins	Alkaloids	Saponins	Steroids	Phenols	Phylobatannins	Cardiac glycosides	Reducing Sugars
Leaf	++	+	++	+++	++	++	+	ND
Stem bark	++	+	++	++++	++	++	+	ND

Key: + = slightly present; ++ = moderately present; +++ = highly present; ++++ = very highly present; ND = not detected

Table 2: Quantity of the phytochemicals in the leaf and stem bark powders of *S. spectabilis*

Plant part	Class of phytochemicals				
	Total phenols	Alkaloids % m/m	Saponins % m/m	Flavonoids	Tannins
Leaf	4.01 ± 0.03	6.2 ± 2.0	21.4 ± 4.0	14.71 ± 0.03	0.436 ± 0.001
Stem bark	1.78 ± 0.04	6.2 ± 3.0	14.2 ± 6.0	9.78 ± 0.04	0.419 ± 0.001

Results are expressed as Mean ± standard deviation (SD) of three determinations for each powder. Total phenols and tannins were expressed as mg gallic acid equivalents per g dry powder, while other measured phytochemicals were expressed as mg per g of dry powder.

The presence of high quantities of tocopherols such as vitamin E in leaf and stem bark powders confirms the capacity of this plant in providing protection from free radicals and products of oxygenation. Vitamin E works in conjunction with other antioxidant nutrients present in this plant materials to quench free radicals. Vitamin E also inhibits lipoyxygenase, an enzyme responsible for the formation of pro-inflammatory leukotrienes^[18].

The presence of carotenoids such as β-carotene and lycopene and xanthophylls such as β-cryptoxanthin in the leaf and stem bark of *S. spectabilis* may explain its use in traditional medicine in treatment of flu, whooping cough and management of diabetes mellitus. α, β and ε carotene function as vitamin A precursors with β-

carotene is the most active precursor of vitamin A^[19].

As depicted in Table 3, the quantities of retinol, α-tocopherol, thiamine, nicotinamide, β-carotene, lycopene (Ψ-carotene), and β-cryptoxanthin are higher in the leaves compared to the stem barks. Ascorbic acid was the most abundant phytonutrient (235540 ± 400 µg/100g) and beta-cryptoxanthin the least (479.0 ± 4.0 µg/100g) in the leaf powders; in the stem bark, ascorbic acid was the highest (80610.0 ± 40.0 µg/100g) while nicotinamide was not detected. For ascorbic acid, the linearity of the calibration curve was 0.9998 and triplicate injections had a percent coefficient of variation (% CV) of ≤ 2 %. For thiamine and nicotinamide calibration curve the linearity was 0.9960 and 0.9899, respectively.

Table 3: Quantity (µg/100g dry matter) of the phytonutrients in the leaf and stem bark of *S. spectabilis*

Macronutrient	Plant part		Retention time (Rt) (minutes)
	Leaf	Stem bark	
Ascorbic acid (Vitamin C)	235540.0 ± 400.0	80610.0 ± 40.0	1.034
Retinol (Vitamin A)	44070.0 ± 2.0	11.0 ± 0.2	4.752
α-Tocopherol (Vit E)	17950.0 ± 10.0	870.0 ± 10.0	9.270
β-Carotene	10580.0 ± 5.0	95.0 ± 8.3	2.452
Ψ-Carotene (Lycopene)	7050.0 ± 0.1	8.0 ± 0.2	2.039
β-Cryptoxanthin	479.0 ± 4.0	13.0 ± 0.1	3.542
Vitamin B ₁ (Thiamine)	510.0 ± 30.0	350 ± 7	3.025
Vitamin B ₃ (Nicotinamide)	694.0 ± 1.0	ND	3.520

Results are expressed as mean ± standard deviation (SD) of three determinations for each extract. Each vitamin concentration is expressed as µg/100g dry matter. ND stands for not detected.

3.3 Antioxidant Activity

It has been demonstrated that phytochemicals rich in hydroxyl moieties in their chemical structures, particularly phenolic and polyphenolic

compounds acts as antioxidants or free radical scavengers^[20]. These have been found to act by transferring electrons or hydrogen atoms to the oxidants^[19]. The percent *in-vitro* antioxidant

activity of the leaf and stem bark powders of *S. spectabilis* were 63.36% and 28.12%, respectively as compared to 59.10% of butylated hydroxylated toluene used as the control.

The antioxidant activity of *S. spectabilis* was also evaluated using the 1, 1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. It was found that the stem bark and leaf powders exhibited 90.95 and 88.98% radical-scavenging activities, respectively at 1.0 mg/ml. The ascorbic acid used as the standard in DPPH assay exhibited 96.50% activity.

3.4 Mineral Elements

S. spectabilis contains significant amounts of novel mineral elements known to possess medicinal value. As depicted in Table 4, among the mineral elements quantified Mo, V, Ni, Cr, and Cu were higher in the leaf than in the stem bark powders of *S. spectabilis*, while Se, Fe, Zn, Mn, and Co were more abundant in the stem barks than in the leaves. The mineral elements

zinc, vanadium, molybdate, manganese and chromium^[20-21] are involved in glucose homeostasis and are therefore critical in the management of diabetes. Zinc and chromium are cofactors for insulin synthesis and this makes this plant powders crucial in management of diabetes mellitus^[22,21].

Copper plays a vital role in various metabolic processes. The presence of copper in the leaf and stem bark of *S. spectabilis* makes this plant useful against hypochromic anemia. The activity of a copper-zinc dependent enzyme, superoxide dismutase a powerful antioxidant which protects cells against free radical injury just like the manganese is dependent on superoxide dismutase^[21]. Selenium presence in this plant protects against the reduction of the activity of the antioxidant enzyme, glutathione peroxidase in humans. Epidemiologically, low dietary selenium is associated with the development of cancer and cardiovascular disorders^[23].

Table 4: Mineral element composition in the leaf and stem bark of *S. spectabilis*

Mineral	Mineral Content (mg/kg)	
	Leaf	Stem bark
Se	1.41 ± 0.36	1.83 ± 0.20
Fe	1.27 ± 1.01	2.45 ± 0.28
Zn	1.15 ± 0.26	1.25 ± 0.50
Cu	0.75 ± 0.50	0.64 ± 0.78
Cr	30.06 ± 8.75	17.08 ± 2.29
Ni	7.00 ± 0.84	6.86 ± 1.38
Mn	1.58 ± 1.33	2.14 ± 1.64
Co	17.67 ± 0.60	19.04 ± 1.07
V	3.88 ± 2.95	0.57 ± 0.66
Mo	88.36 ± 0.91	87.85 ± 1.44

Results are expressed as mean ± standard deviation mg per kg of dry powder of the three determinations for each plant sample.

In summary, this study has demonstrated that *S. spectabilis* is rich in important phytochemicals and mineral elements that could be associated with its medicinal use in the local settings. Based on the findings it can be assumed that the aqueous stem bark and leaves extracts possess medicinal value as claimed by the local communities. However, further studies are needed to determine the bioactive fractions of the

extracts and the efficacy and toxicity profiles in humans.

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