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Phytonutrient, Mineral Composition and *In vitro* Antioxidant Activity of Leaf and Stem Bark Powders of *Pappea capensis* (L.)

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Abstract: Phytochemicals, phytonutrients and mineral composition of medicinal plants have increased the use of plants as nutraceuticals and therapeuticals. The aim of this study was therefore to determine phytochemical, phytonutrient and mineral composition and *in vitro* antioxidant activity of *Pappea capensis* (L.). Leaf and stem bark of *P. capensis* were collected, air dried under shade and then crushed into powder. The powder was used in the determination of ascorbic acid, alpha-tocopherol, thiamine, nicotinamide, retinol, beta-carotene, beta-cryptoxanthin and lycopene concentrations using HPLC. Phytochemicals were screened and quantified according to standard methods and the mineral composition was determined using Energy Dispersive X-Ray Fluorescence (EDXRF) system. Results show that except for alkaloids which were higher in the stem bark than the leaf extracts, flavonoids and saponins were higher in the leaf than in the stem bark extracts; all the phytonutrients quantified in the leaf and stem bark extracts were higher in the leaves compared to the stem barks except for ascorbic acid which was higher in the stem barks than the leaves; among the minerals quantified Cr, Mn, V and Al were higher in the leafs than in the stem barks; Se, Fe, Cu, Zn, Mo, Co, Ni, As, Hg and Pb were similar in both the leaf and stem bark of *P. capensis*. In conclusion, *P. capensis* contains phytochemicals, phytonutrients and mineral elements that contribute to its effectiveness as a traditional medicine.

Key words: Phytonutrients, mineral composition, antioxidant activity, *Pappea capensis*

INTRODUCTION

Medicinal plants are used by about 80% of the world population, primarily in the developing countries for primary health care. These plants have stood the test of time, because they are thought to be cheap, safe, effective and are culturally acceptable (Kamboj, 2000; Ren *et al*., 2004). Their medicinal value is due to a variety of phytochemical, phytonutrient and mineral elements that are part of the physiological functions of living flora and are therefore believed to have better compatibility with the human body (Kamboj, 2000; Ren *et al*., 2004). Therefore, it is essential to investigate the phytoconstituents, mineral elements and vitamins present in the medicinal plant to assess their potential medicinal values (Koche, 2010).

Jacket plum or wild plum, *Pappea capensis* (L.) tree belongs to the Litchi family Sapindaceae. The tree grows up to 3.9 m tall and can be deciduous or evergreen depending upon the prevailing environmental conditions (Van Wyk and Gericke, 2000; Mng'omba *et al*., 2007; 2008). This plant is fairly adapted to a wide range of ecological areas and it is known to be drought-tolerant thus able to grow in marginal lands. The leaves are simple and oblong, hard-textured and wavy. New leaves are an attractive pinky-bronze when they emerge in spring and this contrasts well with the dark green of the old leaves (Mng'omba *et al*., 2007). *P. capensis* is widespread in southern Africa from the northern Cape through the drier Karoo, eastern Cape, KwaZulu-Natal, to the northern provinces, as well as Mozambique, Zimbabwe and northwards into eastern and southern tropical Africa (Mng'omba *et al*., 2007, 2008; Fivaz and Robbertse, 1993; van Wyk and Gericke, 2000). In Kenya, it is distributed in Lukenya hills, Ngong hills, northern Kapenguria and semi-arid regions of southern part of Embu County such as Siakago.

It produces fleshy leaves which can be processed into vinegar, jelly and jam (Palmer and Pitman, 1972). Seeds are rich in edible, non-drying and fairly viscous oil which constitutes about 74% and is used for making soap and oiling guns (van Wyk and Gericke, 2000). It is a good fodder for livestock and produces edible fruits. Among Kenyan communities the boiled stem barks are used traditionally to treat whooping cough and sparingly the leaves are used in the management of diabetes mellitus.

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While medicinal plants typically contain several different bioactive compounds that may act individually, additively or in synergy to improve health (Gurib-Fakim, 2006), the phytochemical, phytonutrient and mineral composition and *in vitro* antioxidant activity of *P. capensis* growing at Kambara village, Siakago in Embu County of Kenya is unknown. Therefore, the objective of the present study is to evaluate the phytochemical constituents, vitamins and mineral elements present in *P. capensis* from Kambara village, Siakago in Embu County of Kenya.

MATERIALS AND METHODS

Collection of plant materials: Green leaves and stem barks of *P. capensis* were collected in March 2011 from Kambara village, Siakago in Embu County of Kenya. The plant was authenticated by a taxonomist at the Department of Plant and Microbial Sciences, Kenyatta University, Kenya and a voucher specimen deposited at the Kenyatta University Herbarium for future reference.

Preparation of the plant powder: The leaves and stem barks were dried under shade for two weeks and crushed in a mechanical mill into fine powder.

Determination of antioxidant activity: The powder (500 g) was extracted with 2.5 L of water for 24 h. The resulting extract was filtered, concentrated and dried in vacuo at 40-45°C and 0.8MPa in a Buchi evaporator, R-114.

The antioxidant activity of the extracts was determined according to method by Kaur and Kapoor (2002) with slight modifications. Briefly, 10 mg of the sample was mixed with 10 mL methanol and stirred for 30 min. The suspension was filtered through Whatman No. 1 filter paper and the final solution used for the antioxidant activity study. 4 ml of beta-carotene solution (0.1 mg in 1 mL chloroform), 40 mg of linoleic acid and 400 mg of Tween-40 were transferred to a round-bottomed flask. The mixture was evaporated at 50°C by means of a rotary evaporator to remove chloroform. 100 mL of oxygenated distilled water was slowly added to the residue and vigorously agitated to give a stable emulsion. Then, 800 µL of the extract was added to 3 mL aliquots of beta-carotene/linoleic acid emulsion. Absorbance was immediately read at time zero at 470 nm using a spectrophotometer. The mixture was then incubated at 50°C for 90 min and absorbance read after every 15 min using methanol as a control. A blank, devoid of beta-carotene, was prepared for background subtraction. Butylated Hydroxylated Toluene (BHT) was used as a standard and the samples were assayed in duplicates. The Degradation Rate (DR) was calculated according to first order kinetics, using the equation:

$$\ln (a/b) \times 1/t = DR_{sample} \text{ or } DR_{standard} \quad (1)$$

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) is expressed as percent of inhibition relative to the control, using the formula:

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

Determination of alpha-tocopherol and retinol by HPLC: Two grams of plant powder was dissolved in 50 mL of methanol. To the mixture, 0.25 g of ascorbic acid was added and 5 mL of 50% sodium hydroxide. The mixture was blanketed with nitrogen and saponified in a water bath at 60°C for 1 h 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 alpha-tocopherol were extracted from the sample using 70 mL of n-hexane containing 30 ppm BHT. For optimal extraction, the separating funnel was gently shaken while avoiding pressure build up. 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 extract was then evaporated in a rotary evaporator under reduced pressure and temperature below 50°C. The remaining extract was reconstituted in 10 mL of methanol, filtered and 10 µL injected into HPLC for the determination of alpha-tocopherol and retinol. 100 mg of alpha-tocopherol standard (Fluka Biochemica, purity $\geq$97.0% HPLC grade) was dissolved in 100 mL of absolute ethanol. The concentration was determined using UV-VIS spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotomer Shimadzu, Japan) at 291 nm, the wavelength of maximum absorbance for alpha-tocopherol dissolved in absolute ethanol. The molar extinction coefficient of alpha-tocopherol in absolute ethanol is 75.6. The concentration of the stock standard solution was determined using the formula:

$$\text{Concentration of } \alpha\text{-tocopherol } (\mu g/ml) = \frac{\text{Mean absorbance} \times 10^4}{75.8} \quad (3)$$

Three determinations of absorbance were made and the mean absorbance recorded. The concentration of the working standard solution was 1.174 µg/mL.

Analysis for alpha-tocopherol was carried out in triplicates by injecting 10 µL of the filtered sample and the standard. The HPLC system was set as follows: flow rate of 1.2 mL/minutes, column oven temperature 25°C and injection volume of 10 µL and run time of 11.50 min. For the fluorescence detector, the excitation wavelength of 290 nm and emission wavelength of 330 nm

were set. 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 vitamin A, 100 mg of vitamin A-palmitate standard (Fluka, Lot 1319695 of 92% purity) 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. The molar extinction coefficient of 1830 for vitamin A-palmitate in absolute ethanol was used. The concentration of the working stock solution for vitamin A was 2.09 µg/mL.

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

HPLC mobile phase was prepared by mixing methanol and HPLC grade water in the ratio of 77:3. The mobile phase was sonicated and then filtered into a reservoir ready for use. Waters Spherisorb ODS-1 column of particle size 5 µ, 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. Concentration of vitamin A in the plant samples was determined using the formula:

$$\text{Concentration (mg/100 g)} = \frac{A_s \times Cal_T \times V_s \times V_{st} \times 100}{A_T \times M \times V_{is} \times 1000} \quad (4)$$

Where:

- A_s = The peak area of the sample
- Cal_T = The concentration of the standard solution in µg/mL
- V_s = The total volume of sample test solution in mL
- V_{st} = The injection volume of the standard solution in µL
- A_T = The peak area of the standard solution in µg/mL
- M = The mass in grammes of the sample
- V_{is} = The injection volume for the sample test solution in µL
- 1000 = The conversion factor from µg to mg
- 100 = The factor for the calculation of the mass concentration per 100 g.

Determination of beta-carotene, beta-cryptoxanthin and lycopene: The samples were analyzed for beta-carotene with HPLC-UV detector in a mobile phase of 90:10, Methanol: acetonitrile with 0.05% (v/v) of Triethanolamine (TEA). The flow rate was set at 2.5 mL/min, at 25°C and detector wavelength of 451 nm. 2 mg of beta-carotene (Sigma, purity ≥93%) standard was dissolved in 10 mL absolute ethanol and actual concentration determined spectrophometrically. The wavelength of maximum absorbance was recorded and the mean absorbance and the molar extinction coefficient of beta-carotene in absolute ethanol were used in determination of the actual concentration of the working standard as shown in equation 3 and the 2560 as the molar extinction coefficient of beta-carotene in absolute ethanol. Samples and the standards were analyzed in HPLC-UV in triplicates and the mean peak areas, standard deviations and % coefficient of variation were determined.

Lycopene (1 mg) standard purchased from Sigma Aldrich, USA (L9879, >90% purity, Lot No 040M5162V) was dissolved in 5ml 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 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. 1 mL of the standard stock was diluted 10 times to make a working solution. beta-cryptoxanthin (1 mg) standard purchased from Sigma Aldrich, USA (CAS472-70-8; C40H560, >98% purity) was dissolved in 5 mL of absolute ethanol (Merck Chemicals Ltd, South Africa) 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. 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 (THF) in the ratio of 70:25:5(v/v). 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-5µ, 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 (beta-carotene), 471 nm (lycopene) and 452 nm (beta-cryptoxanthin).

The standards and the samples were analyzed in triplicates and mean peak areas, standard deviation and % coefficient of variation recorded. Single point calibration was used in quantitation and the amounts were recorded as mg/100 g of dry matter ± standard deviation.

Determination of ascorbic acid: Ascorbic acid was determined in the extracts as total L+ and D+ -ascorbic acids with HPLC-UV method in 2% metaphosphoric acid and ascorbic acid standard purity 99.7% as reference standard. Briefly, 1 g of the milled plant material was extracted in 10 mL of 2% metaphosphoric acid for 1 h. 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 µm membrane filter ready for injection into the HPLC.

Potassium dihydrogen phosphate (50 mM) was prepared in HPLC grade water and the pH adjusted to 2.4 with concentrated orthophosphoric acid. The mobile phase was filtered and then sonicated to remove air bubbles. The wavelength was set at 265 nm, flow rate at 2.0 ml/minute and oven temperature at 15°C. A Phenomenex column (C_{18}) 175 x 3.20 mm x 5 µ internal diameter was used.

The baseline was attained by balancing the column with mobile phase and extraction solution as a blank. Serial dilution of the ascorbic acid standard was prepared at a concentration range of 0.4-5.3 mg/100 g. The linearity of the curve was determined and the limit of detection and quantitation were determined from the standards. Then 10 µL of the samples were injected into the HPLC system and peak areas recorded.

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

A standard for vitamin B1 (thiamine HCl) was prepared by dissolving 27 mg of thiamine hydrochloride (Lot No 36020, Serra Heidelberg, Germany) in 25 mL of HPLC grade water. The linear range for the working dilutions of the standard was 0.896-1.792 µg/mL. For vitamin B3 (Niacinamide), 42 mg of nicotinamide (Lot 37F-0018, Sigma Aldrich, USA) was dissolved in 25 mL of HPLC water. The linear range for the working dilutions of the standard was 1.376-5.504 µg/mL.

The HPLC mobile phase was prepared by mixing absolute methanol-5 mM heptanesulphonic acid sodium salt and 0.1% triethylamine (25:75 v/v). The pH was adjusted to 3.0 with concentrated orthophosphoric acid. The mixture was filtered and degassed ready for use. A Cronus HPLC column (Lot No CC011569, NF-00909 Lichrospher 100RP-18EC 5 µ x 25 x 0.46 mm) was used in an isocratic mobile phase system, flow rate set at 0.8 mL/min and UV-Vis detector wavelength at 254 nm. 10 µL 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.

Determination of the mineral composition of *Pappea capensis* powders using Energy Dispersive X-Ray Fluorescence Spectroscopy (EDXRF) system: 10 g of plant powder 5 g of starch were mixed and pressed into 7 mm disc in thickness and 4.1 cm in diameter in a mold with a force of 200 kN for 5 sec. In order to retain the original dry mass of the samples, the discs were stored in a desiccator or an oven (40-50°C) till EDXRF analyses.

The mineral element concentrations were determined from the plant material by Energy Dispersive X-Ray Fluorescence (EDXRF) analyses using Ray EDX-720, EDXRF spectrometer Shimadzu with a Rh anode and Lit 100, PET, Ge and OVO 55 crystals, Si (Li) detector, ^{109}Cd and ^{55}Fe annular sources and multichannel analyzer with a built-in minicomputer. The following elements were quantified; Na, Mg, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se and Mo and the results were expressed as µg/g of the dry matter.

RESULTS

The phytochemicals detected in the leaf and stem bark powders of *P. capensis* (L.) were tannins, phenolics, saponins, phylobatannins, terpenoids, flavonoids, steroids, cardiac glycosides (in trace amounts) and alkaloids. Reducing sugars were not detected in this plant powder (Table 1). Results in Table 2 show that other than alkaloids and tannins which were higher in the stem bark than the leaf powders, flavonoids and saponins were higher in the leaf powders.

As depicted in Table 3, the quantities of retinol, alpha-tocopherol, thiamine, nicotinamide, beta-carotene, lycopene (psi-carotene) and beta-cryptoxanthin in the leaf and stem bark of *P. capensis* are higher in the leaves compared to the stem barks except for ascorbic acids. alpha-tocopherol was the highest phytonutrient (47540±40 µg/100 g) and lycopene the least (10±2 µg/100 g) in the leaf; in the stem bark, ascorbic acid was the highest (40300±50 µg/100 g) while nicotinamide and beta-cryptoxanthin were the least (not detected).

For ascorbic acid, the linearity of the calibration curve was 0.9998 and the Limit of Detection (LOD) and Limit of Quantitation (LOQ) were 410 and 1250 µg/100 g, respectively. The triplicate injections had a percent coefficient of variation (% CV) of $\leq$2%. For thiamine and nicotinamide calibration curve the linearity was 0.9960 and 0.9899, respectively.

The percent *in vitro* antioxidant activity of the leaf and stem bark of *P. capensis* were 54.82% and 46.04%, respectively compared to the BHT standard which was 59.10%.

As depicted in Table 4, among the mineral elements quantified Cr, Mn, V and Al were higher in the leaf than in

Table 1: Phytochemicals from the leaf and stem bark powders of *P. capensis*

	Class of phytochemicals							
Plant part	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 *P. capensis*

	Class of phytochemicals				
Plant part	Total phenols	Alkaloids	Saponins	Flavanoids	Tannins
Leaf	7.43±0.85	18.0±4.0	201.0±8.5	127.3±15.3	0.333±0.002
Stembark	5.81±0.41	67.3±7.0	125.0±3.6	106.7±20.8	0.589±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

Table 3: Quantity of the phytonutrients in the leaf and stem bark of *P. capensis*

	Plant part		
Macronutrient	Leaf	Stem bark	Retention time (Rt) (minutes)
Ascorbic acid (Vitamin C)	19310±70	40300±50	1.034
Retinol (Vitamin A)	30±1	5±1	4.752
alpha-Tocopherol (Vitamin E)	47540±40	400±10	9.270
beta-Carotene	650±3	67±1	2.452
psi-Carotene (Lycopene)	10±2	6±0.1	2.039
beta-Cryptoxanthin	20±6	ND	3.542
Vitamin B_1 (Thiamine)	510±30	350±7	3.025
Vitamin B_3 (Nicotinamide)	694±1	ND	3.520

Results are expressed as Mean ± Standard Deviation (SD) of three determinations for each extract. Each vitamin concentration was expressed as µg/100 g dry matter. ND stands for not detected

Table 4: Mineral element composition in the leaf and stem bark of *P. capensis*

	Mineral content (µg/100 g)	
Mineral	Leaf	Stem bark
Se	287.1±13.1	285.5±16.0
Fe	352.4±42.6	298.1±72.6
Zn	236.5±27.8	213.6±69.6
Cu	216.2±50.7	172.9±17.4
Cr	5545.4±491.5	2350.1±632.4*
Ni	1297.9±134.7	1100.6±184.5
Mn	6681.0±129.9	320.7±35.6*
Co	2624.8±236.4	2783.1±188.7
V	1144.7±60.3	434.3±109.0*
Mo	13744.8±68.2	13023.7±286.6
Al	897426.8±12805.7	354700.1±101467.3*
As	3123.3±379.6	2662.1±707.4
Hg	760.9±153.4	575.3±113.4
Pb	159.3±10.9	182.0±48.4

Results are expressed as Mean±SD for three determinations for each plant sample using EDXRF. *p<0.05 by t-test

the stem bark of *P. capensis*; Se, Fe, Cu, Zn, Mo, Co, Ni, As, Hg and Pb were similar in both the leaf and stem bark of *P. capensis*.

DISCUSSION

The phytoconstituents alkaloids, flavonoids, steroids, saponins, tannins, phenols, phylobatannins, cardiac glycosides, alpha-tocopherol, retinol, ascorbic acid, beta-carotene, psi-carotene (lycopene), beta-cryptoxanthin, thiamine and nicotinamide and minerals Se, Fe, Cu, Zn, Mn, Cr, Ni, V, Mo and Co present in leaf and stem bark of *P. capensis* are responsible for the different nutraceutical and therapeutic uses in traditional medicine including antimicrobial activity against various pathogenic microorganism (Ettebong and Nwafor, 2009).

The oil from *P. capensis* seeds is applied externally for skin diseases (Fahey, 2005) and this is due to the presence of terpenoids; terpenoids strengthen the skin, increase the concentration of antioxidants in wounds and restore inflamed tissues by increasing blood supply (Hawkins and Ehrlich, 2006). 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 (Okwu, 2001; Edeoga *et al*., 2005).

The presence of phenolic compounds in the leaf and stem bark of *P. capensis* indicates its antimicrobial properties against pathogenic bacteria (Khoobchandani *et al*., 2010; Gulfraz *et al*., 2011). The use of leaf decoctions of *P. capensis* in the treatment of diabetes mellitus may be explained by the presence of terpenoids. Terpenoids also improve lung function

(Hawkins and Ehrlich, 2006) and therefore make *P. capensis* a potential drug for use in the management of painful respiratory problems such as dyspnoea and oligopnoea. Alkaloids and their synthetic derivatives are used as basic therapeutic agents because of their analgesic, antispasmodic and bactericidal effects (Harisaranraj *et al*., 2009); alkaloids exhibit marked physiological activity when administered to animals.

Tannins present in this plant with their stringent properties are reported to exhibit antiviral, antibacterial and antitumor activity and are also used as diuretics (Aiyelaagbe and Osamudiamen, 2009; Gulfraz *et al*., 2011); they also hasten the healing of wounds and inflamed mucous membranes (Harisaranraj *et al*., 2009). Tannins are used in the treatment of intestinal disorders such as diarrhoea and dysentery and urinary tract infections (Fahey, 2005; Akinpelu and Onakoya, 2006). Flavanoids enhance the effects of vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumours, viruses and other microbes, allergies and inflammation. 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 (Harisaranraj *et al*., 2009; Okwu, 2004; Del-Rio *et al*., 1997; Salah *et al*., 1995). They also protect against platelet aggregation (Okwu and Omodamiro, 2005; Harisaranraj *et al*., 2009; Okwu, 2004).

Saponins which are bitter phenolic compounds are produced by plants as a deterrence mechanism to stop attacks by foreign pathogens making them natural antimicrobials (Okwu and Emenike, 2006). Saponins bind cholesterol and block its uptake by the intestines and facilitate its excretion, foam in aqueous solutions and precipitate and coagulate red blood cells (Okwu and Josiah, 2006; Gulfraz *et al*., 2011); this plant can therefore be used to stop bleeding and to treat wounds and to reduce the risk of heart disease (Harisaranraj *et al*., 2009). Saponins have also the ability to kill or inhibit cancer cells (Harisaranraj *et al*., 2009, Okwu, 2005; Nwinuka *et al*., 2005; Okwu and Emenike, 2006, Okwu and Nnamdi, 2008). Calcium a macro element present in this plant is necessary for blood coagulation and for the integrity of the intracellular cement substances (Harisaranraj *et al*., 2009).

Cardiac glycosides present in *P. capensis* have been shown to aid in treatment of congestive heart failure and cardiac arrhythmia. This could be another reason why this plant is widely used in traditional medicine. Cardiac glycosides inhibit the Na^+/K^+-pump. The increase in the level of sodium ions in the myocytes, leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions used in heart muscle contraction resulting in the improvement of cardiac output and reduction in the distention of the heart. The glycosides also possess strong antibacterial properties. Antibiotics such as streptomycin, neomycin, kanamycin, paromomycin, gentimycin and tobromycin are glycosides (Gafar *et al*., 2010; Dangoggo *et al*., 2001).

Natural ascorbic acid is vital for the body performance (Okwu and Josiah, 2006; Aiyelaagbe and Osamudiamen, 2009; Gulfraz *et al*., 2011). Vitamin C is an antioxidant which acts as an electron donor for 8 human enzymes; three of which participate in collagen hydroxylation and two in carnitine biosynthesis; of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine norepinephrine, one is necessary for amidation of peptide hormones and one is involved in tyrosine metabolism. Vitamin C protects low-density lipoproteins *ex vivo* against oxidation and may function similarly in the blood. A common feature of vitamin C deficiency is anaemia. The antioxidant property of vitamin C stabilizes folate in food and in plasma. Vitamin C promotes absorption of soluble non-haem iron by chelation or by maintaining the iron in the reduced (ferrous, Fe^{2+}) form. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends on the amount of inhibitors, such as phytates and polyphenols, present in the meal.

Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. A striking pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances (Harisaranraj *et al*., 2009). Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia and pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism (Harisaranraj *et al*., 2009). This function of ascorbic acid also accounts for its requirement for normal wound healing. The high levels of copper in the leaves compared to that in the stem bark of *Pappea capensis* may explain the reduced levels of vitamin C in the leaves. Exposure of vitamin C to high levels of copper or iron destroys it (Harisaranraj *et al*., 2009).

Other vitamins; thiamin and nicotinamide present in this plant have very important biochemical roles in the body. The two primary functions of thiamin are alpha-keto acid decarboxylation and transketolation. Decarboxylation reactions are an integral part of carbohydrate metabolism. Thiamin is involved in the alpha-keto acid decarboxylation of pyruvate, alpha-ketoglutarate and the branched-chain alpha-keto acids (leucine, isoleucine and valine metabolites). Transketolation is involved in the pentose phosphate pathways. Thiamin is converted to its active form, thiamin pyrophosphate. The thiamine-dependent enzymes are important for the biosynthesis

of neurotransmitters and for the production of reducing substances used in oxidative stress defenses, as well as for the biosynthesis of pentoses used as nucleic acid precursors. Thiamin plays a central role in cerebral metabolism. Its deficiency results in dry beriberi, a peripheral neuropathy, wet beriberi, a cardiomyopathy with edema and lactic acidosis and Wernicke-Korsakoff syndrome, whose manifestations consist of nystagmus, ophthalmoplegia and ataxia evolving into confusion, retrograde amnesia, cognitive impairment and confabulation (Fattal-Valevski, 2011).

Nicotinamide an amide of nicotinic acid is the precursor of NAD^+/NADH and $NADP^+$/NADPH which play essential metabolic roles including energy metabolism, amino acid metabolism and detoxification of drugs and other substances. Its deficiency causes pellagra, a disease consisting of bilateral symmetrical lesions on both sides of body and hands. Pellagra is characterized by hyperpigmentation and thickening of the skin, inflammation of the tongue and mouth and digestive disturbances including indigestion, anorexia, diarrhea, irritability, amnesia and delirium. 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*; additional protection from macrophage toxins is involved in prevention of type I diabetes. Specifically, nicotinamide via PARP inhibition protects pancreatic islet cell lysis after exposure to oxygen free radicals; nicotinamide also stimulates GABA receptors without binding to the receptor sites; has an anti-inflammatory action towards neutrophil chemotaxis. Due to its inhibition of ADP-ribosylation, nicotinamide suppresses cytokine mediated induction of nitric oxide synthase in cells thus effecting interleukin-1 exposed chondrocytes resulting in decreased inflammation; is involved in production of steroid hormones in the adrenal gland (Monograph on Niacinamide, 2002).

The presence of high quantities of tocopherols such vitamin E in leaf and stem bark of *P. capensis* confirms it's potential in providing protection from free radicals and products of oxygenation. Specifically, tocopherols detoxify lipid peroxy radicals, as well as block the reactivity of singlet oxygen radicals. Vitamin E works in conjunction with other antioxidant nutrients to quench free radicals. Vitamin E also inhibits lipoxygenase, an enzyme responsible for the formation of pro-inflammatory leukotrienes. Gamma-, delta- and alpha-tocopherols inhibit platelet aggregation through inhibition of protein kinase C and increased action of nitric oxide synthase. Gamma- and alpha-tocopherol inhibits production of protein kinase C and collagenase, two enzymes that facilitate cancer cell growth (Monograph on Tocopherols, 2002).

The presence of vitamin A in the leaf and stem bark of *P. capensis* may explain it use in traditional medicine. Vitamin A exists in plants as the precursor carotenoid family. Beta-carotene is cleaved to retinyl esters and retinoic acid in the enterocyte of the small intestine and packaged into chylomicrons along with retinol from preformed vitamin A for transport to the liver for storage as retinol in hepatic stellate cells. When needed, retinol is transported to tissues bound to retinol binding protein (a zinc-dependent protein). Zinc deficiency disturbs normal retinol metabolism and supplementation with zinc treats retinol-resistant night-blindness. In the eye, retinol is oxidized to retinaldehyde, the basis of the visual pigments rhodopsin and iodopsin. It is also oxidized to retinoic acid, the parent compound of natural retinoids. Two specific isomers, all-trans-retinoic acid and 9-cis-retinoic acid, bind to specific receptors in the nucleus of target cells and assist in regulating the cell replication cycle through transcription factors-tumor suppressor proteins p53 and p105, which are both strong inhibitors of uncontrolled cell growth.

Retinoids, through their interaction with nuclear retinoic acid receptors in tissue, control gene expression and promote normal proliferation and differentiation of epithelial tissue, particularly mucous membrane epithelium. Vitamin A functions in the immune system in the modulation of diverse pathways: in the expression of mucins and keratins, lymphopoesis, cytokine production, neutrophil maturation and function, the functional expression of natural killer cells, monocytes and macrophages, T and B lymphocytes and immunoglobin production.

The presence of carotenoids such as beta-carotene and lycopene and xanthophylls such as beta-cryptoxanthin in the leaf and stem bark of *P. capensis* may explain it use in traditional medicine. Alpha, beta and epsilon carotene function as vitamin A precursors. Beta-carotene is the most active precursor of vitamin A. Xanthophylls protects vitamin A, other carotenoids and vitamin E from oxidation. The vitamin A precursors as well as other carotenoids such as gamma-carotene, lycopene and lutein are protective against lung, breast, uterine, colorectal and prostate cancer. Zeaxanthin and lutein may help to prevent loss of sight in persons over fifty years. Xanthophylls protect the skin from sunlight. Beta-cryptoxanthin is thought to have a protective effect on the female reproductive tissues such as the vaginal, uterine and cervical tissues.

The mineral elements contained in *P. capensis* are important in human nutrition. Zinc, vanadium, molybdate, manganese and chromium are mineral elements involved in glucose homeostasis and are used in the management of diabetes. Zinc and chromium are cofactors for insulin (Gloria *et al*., 2010; Kimura, 1996). These elements are required by many enzymes as co-factors (Gloria *et al*., 2010; Ozcan, 2004).

Copper (Cu) plays a vital role in various metabolic processes. The presence of copper in the leaf and stem bark of *P. capensis* makes this plant protect against hypochromic anemia which is associated with defects of iron mobilization due to a combined defect of both ceruloplasmin ferroxidase activity and intracellular iron utilization and neutropenia; the activity of a copper-zinc dependent enzyme, superoxide dismutase a powerful antioxidant which protects cells against free radical injury just like the manganese dependent superoxide dismutase; Menkes disease caused by copper deficiency which in infants is characterized by poor growth, white brittle hair with peculiar twisting, arterial defects, focal cerebral degeneration and mental retardation (Tuormaa, 2000).

Severe copper deficiency in infants results in pathological bone fractures (cross-links collagen), cardiovascular disorders (cross-links soluble elastin and collagen) and emphysema-like lung condition which are associated with reduced activity of a copper dependent enzyme, lysyl oxidase: the peroxidative damage seen in both lung and cardiovascular pathology (arterial and cardiac aneurysm), could be directly associated with excessive free radical formation due to a reduced superoxide dismutase (CuZnSOD) activity. It would also protect against neurological problems such as ataxia, seizures and episodic apnea which could be caused by lack of myelination leading to reduced nerve cell formation during embryonic development. Since copper is toxic, it is required in very small amounts. High copper levels leads to diverse disorders such as suicidal intent, hypotension, heart disease, premenstrual tension, postpartum depression, paranoid and hallucinatory schizophrenias, childhood hyperactivity and autism, nausea, vomiting, diarrhea, jaundice, hematuria, anuria, coma and death (Tuormaa, 2000; Pfeiffer, 1979).

Selenium presence in this plant protects against the reduction of the activity of the antioxidant enzyme, glutathione peroxidase in humans. It prevents the occurrence of Keshan disease and juvenile cardiomyopathy in countries where the soil is low in this essential mineral. Epidemiologically low dietary selenium is associated with the development of cancer and cardiovascular disorders (Tuormaa, 2000).

The presence of zinc in this plant protects infants against poor growth, hypogonadism and reduced immunity. In children, zinc protects against autism, dyslexia, apathy, lethargy, irritability and childhood hyperactivity. In adults, zinc protects against the development of both senility and Alzheimer's disease. Zinc also protects against reproductive failures: infertility, miscarriage, intrauterine growth retardation, small head circumference and an increased number of congenital malformations. In males, zinc guards against low sperm count, slow sperm motility, malformed sperm and infertility (Tuormaa, 2000).

The presence of elements As, Hg, Pb and Co is of great disadvantage to consumers since they are highly toxic even at low concentrations (Asaolu *et al*., 1997; Oloyede, 2005). Aluminum presence in *P. capensis* may be due to environmental contamination including air pollution, modern agriculture and industrial practices and contaminated food or water supplies (Barnes and Bradley, 1994; Bradley and Bennett, 1995). An excessive aluminium accumulation in children causes hyperactivity, a reduced intelligence and anti-social behaviour. In adults, it is associated with heart disease, cancer and infertility and with criminality (Bryce-Smith and Waldron, 1979). In addition, high maternal aluminium leads to miscarriage, a reduced birth weight and a number of fetal malformations (Barnes and Bradley, 1994; Bradley and Bennett, 1995; Tuormaa, 1994).

However, the action of each element can either be potentiated, or reduced, by the presence of another. This is also why the ratio between the concentrations of any given mineral found in body chemistry determines whether or not deficiencies or toxicities occur. The requirement and hence the nutritional adequacy of a particular mineral depends on other minerals already present in the body chemistry. An interaction between minerals is either positive (synergistic) or negative (antagonistic). An example of a synergistic interaction is between copper and iron as both are required for the promotion of hematopoesis. An example of an antagonistic interaction is between iron and zinc because an excess of one reduces/affects the presence of the other. This phenomenon takes place when competing ions possess the same, or very similar, electron configuration.

Antagonistic interactions are also seen between selenium: cadmium, selenium: mercury, manganese: iron, zinc: cadmium and zinc: copper. The antagonism between copper and zinc is of special concern because zinc is centrally involved in over 80 different enzyme system functions, including events relating to cell division and nucleic acid synthesis (Tuormaa, 2000). Thus zinc deficiency is associated with numerous mental, physical and reproductive disorders (Tuormaa, 2000).

In conclusion, *P. capensis* contains phytonutrients and mineral elements associated with prevention and treatment of various diseases and disorders thus providing the biochemical basis of its ethno-pharmacological use in traditional medicine.

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