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ORIGINAL ARTICLE

Evaluation of nutritional and phytochemical properties of *Eucalyptus camaldulensis*, *Hibiscus sabdariffa* and *Morinda lucida* from Ogun State, Nigeria

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This study investigated nutritional and phytochemical properties of three multipurpose savannah plants: *Eucalyptus camaldulensis, Hibiscus sabdariffa* and *Morinda lucida*. Proximate, mineral and anti-nutrient contents of leaves, stems and roots of the plants were determined. Data obtained were analysed using Statistical Analysis System (SAS). One way Analysis of Variance (ANOVA) was conducted to determine significant difference between parameters. Means were separated using Duncan's Multiple Range Test at $p < 0.05$. Results showed that the nutritional and anti-nutrient contents showed significant difference ($p < 0.05$) across leaves, stems and roots of the plants. Crude protein (13.83 mg/100g) and total nitrogen (2.21 mg/100g) were significantly ($p < 0.05$) higher in *E. camaldulensis* leaves, fat (1.48 mg/100g) and moisture (11.42 mg/100g) in *M. lucida* leaves while ash (7.66 mg/100g), crude fibre (15.84 mg/100g) and carbohydrate (75.30 mg/100g) were significantly ($p < 0.05$) higher in *H. sabdariffa* stems and roots. Potassium (277.71 mg/kg) and calcium (321.55 mg/kg) were significantly higher in ML leaves while higher sodium (51.05 mg/kg) was recorded in *H. sabdariffa* leaves. Tannins (0.55 mg/100g), oxalate (0.32 mg/100g), phytate (0.39 mg/100g) and trypsin inhibitor (0.33 mg/100g) were significantly higher ($p < 0.05$) in the roots of the three plants than stems. Flavonoids (0.86 mg/100g), saponins (3.46 mg/100g), steroids (0.58 mg/100g) and anthocyannins (0.15 mg/100g) were significantly higher ($p < 0.05$) in *M. lucida* leaves. In conclusion, although nutritional, phytochemical and anti-nutrient contents varied within leaves, stems and roots of the plants but, *M. lucida* leaves contained highest amounts of mineral and phytochemicals.

Key words: Nutritional properties, phytochemical properties, Eucalyptus camaldulensis, Hibiscus sabdariffa, Morinda lucida

The use of plant products as food or medicine is beneficial to large populations such as Nigerians. This is because most plants contained chemotherapeutic and nutritional constituents suitable for synthesis of useful drugs and health improvement (Oluwatosin and Justine, 2010 and Ojewumi and Kadiri, 2014). Typical examples of such plants are *E. camaldulensis* (River red gum)*, H. sabdariffa* (Roselle) and *M. lucida* (Brimstone) which are savannah plants known for several nutritional and pharmacological uses (Bamayi *et al.,* 2004). According to Kadiri *et al*. (2014), the plants characterize versatile uses in food and textile productions, medicine and dye to mention but a few. Also, Ghulam *et al.* (2013) reported that most medicinal plants consumed contain nutritional compositions as well as pharmacological properties essential for human physiological functions. Although various investigations have been conducted on plants metabolites, yet literatures have indicated less attention on the quantity and variations of plant primary and secondary metabolites in the different parts of plants under study.

Physiological evaluation of quantity and variation of primary and secondary metabolites of these plants is necessary because many plants are consumed without adequate knowledge about their metabolites which when consumed in excess could limit the plants either as food or medicine. Also results of this study could severe as rational approach to validate the multi-purpose claims of the plants. In view of these, the present study was carried out to evaluate nutritional and phytochemical properties of *E. camaldulensis*, *H. sabdariffa* and *M. lucida.*

MATERIALS AND METHODS

Sample collection

Fresh leaves, stem- barks and roots of *E. camaldulensis H. sabdariffa* and *M. lucida* were collected from various farms in Abeokuta, Ogun State Nigeria. The plant materials were identified at Forestry Research Institute, Ibadan, Nigeria, after which they were air-dried, grounded into powder and used for analysis.

Proximate analysis of *E. camaldulensis H. sabdariffa* and *M. lucida* leaves, stems and roots

Crude fibre: One gram each of de-fattened samples of powder of three parts of the three plants was boiled in 20ml of 1.25 % H_2SO_4 (sulphuric acid) for 30 minutes. After this, the content was filtered, washed with hot distilled water and boiled in 200 ml of 1.25% sodium hydroxide for about 30 minutes. Spotless beaker was dried at 100±5^oC overnight, cooled in a desiccators and weighed to a constant weight. Then, the spoutless beakers with its content was put in a muffle furnace at 932^0F - 1112^0F for 2-3 hour, cooled in a desiccators and weighed. Crude fibre was determined using formula **(1)** (AOAC 2000)

Crude protein: Total nitrogen (N) was determined using Micro-Kjeldahl method in (2009)

Protein (%) was determined using the mathematical relationship below.

$$\text{Protein (\%)} = \frac{\text{V} \times 1.4 \text{ x } 6.25 \text{ x } 0.1\text{N Hcl x Vol (used)}}{\text{W} \times \text{A} \times 1000} \text{X100}$$

where;

V -Titter value. 1.4 -Weight of nitrogen expressed in gram in the formula. 6.25 = Protein factor. W -Weight of sample. A -Aliquot digested sample used for distillation

Crude fat: One gram each of crushed dried sample was taken in paper thimble kept in a pre-weighed flask of fat extractor. Eighty (80ml) of petroleum ether was added and refluxed for 8hours. The flask was cooled, weighed and crude fat was determined using formula **(2)**.

Moisture: Moisture was determined using hot air oven method (3).

Ash content: Ten grams (10.g) of the each samples was added to a reweighed crucible, weighed, placed in a muffle furnace at 932^0F for 4hours, cooled in desiccator and reweighed. Ash content was determined using mathematical relationship;

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} X100$$

Carbohydrate: Available carbohydrate was calculated using formula below;

Carbohydrate (%) = 100 - (crude fibre + crude fat + ash + crude protein) % according to AOAC (2000)

Mineral Analysis of *E. camaldulensi, H. sabdariffa* and *M. lucida* leaves, stems and roots

Minerals in each sample of the plants were determined after wet digestion of 3.0 g of each part of each plant with a mixture of $HNO_3/HClO_4/H_2SO_4$ in the ratio 9:2:1 v/v, respectively. Mineral such Mg, Ca, P, Fe, Cu, Zn and Na were determined using atomic absorption spectrophotometer. The K and Na of the samples were determined using atomic emission spectrometer and phosphorus by colorimetric method of AOAC (1990).

Determination of Vitamins in *E. camaldulensi, H. sabdariffa* and *M. lucida* leaves, stems and roots

Vitamin A: Vitamins A was determined according to method of AOAC (2000). Two (2 g) of sample of each plant was weighed into a flat bottom reflux flask, 10ml of distilled water was added and shaken to form a paste after which 25ml of alcoholic KOH solution was added and a reflux condenser attached. The mixture was heated using boiling water bath for one hour, shaken, cooled rapidly and about 30 m1 of water was added after which hydrolysate obtained was transferred into a separatory funnel. The solution was extracted thrice with 250ml quantities of chloroform. In addition, 2g anhydrous Sodium sulphate was added to the extract to remove traces of water. The mixture was then filtered into l00ml volumetric flask and made up to mark with chloroform. Standard solution of B-carotene Vitamin A ranged from 0 – 50 µg/mi and chloroform was used by dissolving 0.003g of standard L-carotene in l00ml of chloroform. The above gradients of different standard solutions prepared were determined with reference to their absorbance from which average gradient was taken to calculate Vitamin A (B-carotene in µg/ l00g) using Spectrophotometer (Metrohm Spectronic 21D Model) at a wavelength of 328nm.

Vitamin B (Niacin): About 5 g of the sample was treated with 50 ml of 1 N H_2SO_4 and shaken for 30 minutes. Thereafter, 3 drops of the ammonia solution were added to the sample and filtered. Afterwards, 10 ml of the filtrate was added into a 50 ml volumetric flask and 5 ml of 0.02 N H_2SO_4 470 nm according to AOAC (2000), Hussian *et al.* (2006) , Iqbal *et al.* (2011)

Vitamin C: One gram of each sample was weighed in a 25 ml conical flask. Then, 10 ml of oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and placed in the sample for 24 hours to provide the required reaction time. After 24 hours, the samples were filtered through using 0.45 µm filter paper. Then, 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added.

Subsequently, metal phosphoric acid was added separately with acetic acid (0.5 ml), H_2SO_4 (5 % v/v) solution (1 ml) and ammonium molybdate solution (2 ml) each volumetric brown flask and the volume made up to 25 ml with distilled water. The absorbance was measured at 760 nm in a UV/visible spectrophotometer.

Determination of anti-nutrients in *E. camaldulensi, H. sabdariffa* and *M. lucida*

Phytic acid: Phytic acid was determined according to method of Sofowora, (1993). Two (2g) of each sample was weighed into 250m1 conical flask. 100mls of 2% Hydrochloric Acid was added to soak each sample in the conical flask for 3 hours and filtered through a double layer of hardened filter paper. Then, 50rnl of each filterate was placed in 0.50ml conical flask and 107mls distilled water was added in each case to give proper acidity. Thereafter, 10mls of 0.3% Ammonium Thiocyanate (NH_4SCM) solution was added into each solution as indicated. This was titrated with standard iron (lll) chloride solution which contained 0.00 195g Iron per ml. The end point was slightly brownish-yellow which persisted for 5 minutes. The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value x } 0.00195 \text{ x } 1.19 \text{ x } 100 \text{ x } 3.55}{\text{Wt. of sample}}$$

Tannin: Approximately, 0.50g of sample was measured into a 50ml beaker and 20ml of 50% methanol was added, covered with paraffin and placed in a water bath at 77-80ºC for 1 hour and shaken to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No. 41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5m; Folin-Denis reagent and 10ml of 1% Sodium carbonate were added and. mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min. The bluish-green colour was developed at the end and treated similarly as 1ml sample above. The absorbance of the tannic acid standard solutions as well as samples was read after colour development on a spectronic 21D

spectrophotometer at a wavelength of 760nm. % Tannin was calculated using the formula **(4)**.

Trypsin inhibitor: One gram of each sample was dispersed in 50 ml of 0.5 M Sodium Chloride solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 min. The supernatant was filtered and the filtrate was used for the assay. Two millilitres of the standard trypsin solution were added to 10 ml of the substrate of each sample. The absorbance of the mixture was taken at 410 nm using 10 ml of the same substrate as blank

Oxalates: Approximately, 2 g powder sample of each bean was boiled in 40m1 of water for 30 minutes in a reflux condenser and 10ml of 20% Sodium carbonate was added and boiled for another 30 minutes. The mixture was filtered and liquid extract washed with hot water until the wash water does not show any alkaline reaction. The combined wash water was filtered to a small volume and cool. With constant stirring, hydrochloric acid (HCL) (1:1) in dropwise was added until final acid concentration of neutralization was about 4% and the extract was filtered into a 250 ml flask to make up to mark and kept overnight. Aliquot of this filtrate was taken in a 400m1 beaker, diluted with water to 200m1 and made just ammoniacal, and re-acidified with Lacotic Acid. In the cold, medium, 10 ml of a 10 % calcium chloride solution was added and stirred well to include calcium oxalate precipitate to appear and allowed to settle overnight. Clean supernatant liquid was decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. Then, precipitate was dissolved in HCL (1:1). Oxalic acid was re-precipitated by adjusting pH with ammonium hydroxide solution. The contents were boiled, allowed to settle overnight and oxalic acid was determined by titrating against 0.05N Potassium permanganate solution.

Calculation was performed using the formula **(5)**

Cardiac glycosides

Buljet's reagent according to Michael and Emmanuel (2012) was used to evaluate the cardiac glycoside content in the examined plant parts. 1g of each powdered sample was soaked in 100 ml of 70 % alcohol for 2 hours before filtration. Using lead acetate and Na_2HPO_4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

Cyanogenic glycosides:

About **f**ive grams (5 g) of each sample were weighed into a 250 cm^3 round botton flask and 200cm^3 of distilled water was added and allowed to stand for 2 hours for autolysis to occur. An anti foaming agent (tannic acid) was added and full distillation carried out in a 250 cm^3 conical flask containing 20cm^3 of 2.5 % NaOH (Sodium hydroxide). To 100 cm^3 of each distillate containing cyanogenic glycoside, 8cm^3 of 6 M NH_4OH (ammonium hydroxide) and 2 cm^3 of 5 % KI (Potassium Iodide) was added, mixed and titrated with 0.02 M $AgNO_3$ (silver nitrate) using a micro-burette against a black background. Permanent turbidity indicated the end point. Cyanogenic glycoside content of the sample was calculated as:

$$Cyanogenic\,glycoside = \frac{Titre\,value(Cm3)\,x10\,Bx\,extract\,volume}{Aliquot\ \ volume(Cm3)\,x\,sample\,weight\,(g)}X100$$

Determination of phytochemicals in *E. camaldulensi, H. sabdariffa* and *M. lucida* leaves, stems and roots

Phytochemical contents of the samples were determined according to the methods of Harborne, (1973) and Awoyinka *et al*., (2016)

Alkaloids: Using distillation and titrimetric method described by of Harborne, (1973), 2 g of finely grounded simple was weighed into a 100 ml beaker and 20 ml of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol was added to make up to 100 ml after which 1 g magnesium oxide added. The mixture was digested in a boiling water bath for 1.5 hrs under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small bucher funnel. The residue was returned to the flask, re-digested for 30 min and evaporated with 50 ml alcohol after which, 3 drops of 10% HCl was added. The whole solution was later transferred into a 250 ml volumetric flask 5ml of zinc acetate solution and 5ml of potassium ferrocyanide solution was added and mixed thoroughly to give a homogenous solution. The flask was allowed to stand for a few minutes after which 10ml of the filtrate was transferred into a separatory funnel. The alkaloids

present were extracted by shaking the filtrate vigorously with five successive portions of chloroform. The residue obtained was dissolved in 10ml hot distilled water and transferred into a Kjeldahl tube with addition of 0.20 g sucrose and 10 ml Conc. H_2SO_4 and 0.02g selenium for digestion to be a colourless solution after which % N was determined using Kjeldahl distillation method. % Nitrogen got was converted to % total alkaloid by multiplying it with a factor of 3.26 i.e

% Total alkaloid = %N X 3.26

% alkaloids = %N X 3.26

Flavonoids: About 0.50 g of finely ground sample was weighed into a 100 ml beaker and 80 ml of 95% ethanol was added, stirred with a glass rod to prevent lumping, filtered into a 100 ml volumetric flask and made up to mark with ethanol. Also, 1 ml of the extract was pipetted into 50 ml volumetric flask and four drops of Conc. Hydrochloric acid was added via a dropping pipette after which 0.5 g of magnesium turnings was added to develop a magenta red colouration. Standard flavonoid solution which ranged 0-5ppm were prepared from 100ppm stock solution and treated in a similar way with HCl and magnesium turnings like sample. The absorbance of magenta red colouration of sample and standard solutions were read on a digital Jenway V6300 Spectrophotometer at a wavelength of 520 nm. The percentage flavonoid was calculated using the formula **(6)**.

Saponins: One (1 g) of finely ground sample was weighed into a 250 ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No.1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated Magnesium carbonate was again filtered to obtain a clear colourless solution. 1 ml of the colourless solution, was pipetted into 50 ml volumetric flask and 2 ml of 5% Iron(III) chloride solution was added and made up to mark with distilled water. The mixture was allowed to stand for 30 min for blood red colour to develop. 0-10 ppm standard Saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% Iron(III) chloride solution as done for 1 ml sample above after which absorbance of the sample and standard saponin solutions were read after colour development in a Jenway V6300 Spectrophotometer (380mm). **(7)**

Steroids: About 0.50g of sample was weighed into a 100ml beaker and 20m1 of Chloroform-Methanol (2:1) mixture was added to dissolve the extract after which the mixture was filtered into another 100ml Conical Flask. The resultant residue was repeatedly treated with Chloroform-Methanol mixture until free of Steroids. One (1ml) of the filterate was pipetted into a 30ml test tube and 5ml of alcoholic potassium hydroxide was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37°C-40°C for 90minutes, cooled to room temperature and 10 ml of petroleum ether was added followed by the addition of 5ml distilled water and later evaporated to dryness on the water bath. 6 ml Liebermann Burchard reagent was added to the residue in dry bottle and absorbance was taken at a wavelength of 620nm on a Spectronic 21D digital Spectrophotometer.

Standard Steroids of concentration of 0-4 mg/mi were prepared from 100 mg/mi stock steroid solution and treated similarly like sample as above. **(8)**

Anthocyanins: Approximately**,** 1g of the sample was blended in a blender with 75m1 (Methanol: Water: Acetic Acid) (25: 24: 1) mixture to extract anthocyanin. The extract was then centrifuged at 12,000rpm for 20mins at 15°C. The residue remaining was mixed thoroughly with the 75m1 of (rnethanol/water/ acetic acid) mixture. The extraction was repeated thrice. The three extracts were pulled together into a 250 ml beaker to evaporate to dryness in a rotary evaporator. The residue obtained above was re dissolved in 10ml of 15ml of 15% methanol and 85% of 5%(w/v) formic acid solution. This extract was diluted to 250 ml with 135 ml of a mixture of methanol/0.1M HCl at ratio of 85:15. Working standard solutions of anthocyanin of range 0-10 mg/mi were prepared from stock 50 mg/mi anthocyanin solution and treated like sample above. Absorbances of sample extracts as well as anthocyanin working standard solutions were read at a wavelength of 535nm on a UV Spectronic 21D Spectrophotometer. **(9)**

Phenol: Approximately**,** 0.20 g of sample was weighed into a 50 ml beaker, 20 ml of acetone was added and

homogenized properly for 1 hr to prevent lumping. The mixture was filtered into a 100 ml Volumetric flask using acetone to rinse and made up to mark with distilled water. 1 ml of sample extract was pipetted into 50 ml Volumetric flask, 20 ml water added, 3 ml of phosphomolybdic acid added followed by the addition of 5 ml of 23% Sodium carbonate and mixed thoroughly, made up to mark with distilled water and allowed to stand for 10 min to develop bluish-green colour. Standard Phenol of concentration range 0-10 mg/ml were prepared from 100 mg/L stock Phenol solution from Sigma-Aldrich chemicals, U.S.A. The absorbance of sample and standard concentrations of Phenol were read on a Digital Spectrophotometer at a wavelength of 5l0 nm. The percentage Phenol is calculated using the formula **(10).**

Polyphenol: About 1 g of dry material of each plant was transferred to a 250 ml Erlenmeyer containing 150 ml of water which was maintained on a water-bath for 30 min at 80 – 90˚C. After cooling, the mixture was transferred into a 250 ml volumetric flask and made up with water and decanted thereafter. This solution was filtered and the first 50 ml of the filtrate then discarded. Then 5 ml of the filtrate above were transferred to a 25 ml volumetric flask and volume made up with water.

Statistical Analysis

Data obtained were analysed using Statistical Analysis System (SAS). One way Analysis of Variance (ANOVA) was conducted to determine significant difference between parameters. Means were separated using Duncan's Multiple Range Test at $p < 0.05$

RESULTS

Proximate composition in leaves, stem-bark and roots in E. camaldulensi, H. sabdariffa* and *M. lucida

Results showed that crude protein (13.83 mg/100g), fat (1.12 mg/100g) and total nitrogen (2.21 mg/100g) were significantly ($p < 0.05$) higher in the leaves of *E. camaldulensis* while ash (7.51 mg/100g), moisture (10.63 mg/100g), carbohydrates (74.43 mg/100g) and crude fibre (12.88 mg/100g) were significantly higher ($p < 0.05$) in *E. camaldulensis* roots (Table 1). In *H. sabdariffa,* crude protein (13.23 mg/100g), fat (1.22 mg/100g), carbohydrate (74.43 mg/100g) and total nitrogen (2.12 mg/100g) were significantly ($p < 0.05$) higher in the leaves, ash (7.66 mg/100g) and crude fibre (15.84 mg/100g) in stems while higher moisture (9.76 mg/100g) was recorded in the roots of the plant (Table 1).

Also, crude protein (12.84 mg/100g), fat (1.48 mg/100g), moisture (11.42 mg/100g) and total nitrogen (2.05 mg/100g) were significantly higher in *M. lucida* leaves, ash content (6.93 mg/100g) and crude fibre (14.82 mg/100g) in the stems while carbohydrate (74.49 mg/100g) was significantly ($p < 0.05$) higher in roots of the plant (Table 1).

Mineral elements in leaves, stem-bark and root of *E. Camaldulensis, H. sabdariffa* and *M. lucida*

Sodium (31.98 mg/kg), potassium (168.46 mg/kg), calcium (76.05 mg/kg), phosphorus (89.92 mg/kg) and magnesium (5.83 mg/kg) were significantly ($p<0.05$) higher in *E. camaldulensis* leaves (Table 2). Also, sodium (51.05 mg/kg) and potassium (232.81 mg/kg) were significantly ($p < 0.05$) higher in *H. sabdariffa* leaves while calcium (116.17 mg/kg) and magnesium (6.73 mg/kg) were significantly higher in stems of the three plants (Table 2). Similarly, results also revealed that sodium (34.46 mg/100g), potassium (277.71 mg/kg), calcium (321.53 mg/kg) and magnesium (6.58 mg/kg) were significantly higher in *M. lucida* leaves (Table 2).

Anti-nutriction in leaves, stem bark and root of *E. Camaldulensis, H. sabdariffa* and *M. lucida*

Anti-nutrients varied across leaves, stems and roots of three plants. Tannin (0.55 mg/100g), oxalate (0.30 mg/100g), phytate (0.34 mg/100g), trypsin inhibitor (0.24 mg/100g) and cardiac glycoside (0.38 mg/100g) were significantly ($p < 0.05$) higher in the root of *E. camaldulensis* compared with stems and leaves (Table 3) while in *H. sabdariffa*, tannin (0.40 mg/100g), oxalate (0.32 mg/100g), phytate (0.32 mg/100g) and trypsin inhibitor (0.15 mg/100g) were significantly higher ($p < 0.05$) in roots than stems and leaves (Table 3). Similar observation was noticed in the amount of oxalate (0.30 mg/100g) and phytate (0.39 mg/100g) determined in the roots of *M. lucida*. (Table 3).

Phytochemical contents in leave, stem-bark and roots in *E. camaldulensi, H.* sabdariffa and *M. lucida*

Table 4 showed quantities of phytochemical in leaves stems and roots of *E. camaldulensis, H. sabdariffa* and *M. lucida.*. It was also observed that *E. camaldulensis* leaves contained higher amount of

alkaloids (7.23 mg/100g) and polyphenols (1.696 mg/100g) while roots of the plant contained higher amount of saponins (0.47 mg/100g), phenols (0.45 mg/100g) and anthocyanins (0.12 mg/100g). Alkaloids (6.89 mg/100g) and polyphenols (1.96 mg/100g) were significantly higher ($p < 0.05$) in *H. sabdariffa* leaves while saponinins (0.38 mg/100g) and steroids 0.23 mg/100g significantly ($p < 0.05$) higher in the stems of the plant (Table 4). In the same trend, alkaloids (6.67 mg/100g), flavonoids (0.86 mg/100g), steroids (0.58 mg/100g) anthocyanins (0.145 mg/100g) and polyphenol (2.96 mg/100g), were significantly ($p < 0.05$) higher in the leaves of *M .lucida* than stems and roots (Table 4).

$$\textbf{Crude fibre}\ (\%) = \frac{\text{weight of spoutless beaker containing crude fibre} - \text{weight of spoutless beaker and crude fibre}}{h}\ 100 \quad \textbf{(1)}$$

$$\text{Crude fat}\ (\%) = \frac{\text{Weight of flask with fat} - \text{weight of empty flask})}{\text{Weight of original sample}} \text{X100} \quad \textbf{(2)}$$

$$\text{Moisture} = \frac{\text{Weight of sample before drying} - \text{weight of sample after drying}}{\text{Weight of sample before drying}} \text{X100} \quad \textbf{(3)}$$

$$\%\ \text{Tannin} = \frac{\text{Absorbance of sample X average gradient factor X dilution factor}}{\text{Wt. of sample X 10,000}} \quad \textbf{(4)}$$

$$\begin{aligned} 1\text{ml of } 0.05\text{N } KMNO_4 &= 0.00225 \text{ anhydrous Oxalic Acid} \\ &= \%\ \text{Oxalic Acid} \\ &= \frac{\text{Titre value x } 0.00225}{2} \text{ x} \frac{100}{1} \\ &= \text{T. V x } 0.1125. \end{aligned} \quad \textbf{(5)}$$

$$\text{Flavonoids} = \frac{\text{Absorbance of sample X average gradient factor X dilution factor}}{\text{Wt. of sample X 10000}} \quad \textbf{(6)}$$

$$\%\ \text{Saponin} = \frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10000}} \quad \textbf{(7)}$$

$$\text{Steroids} = \frac{\text{Absorbance of Sample X Gradient X Dilufion Factor}}{\text{Wt of sample x10000}} \quad \textbf{(8)}$$

$$\%\ \text{Total Anthocyanin} = \frac{\text{Absorbance of sample X gradient X Dilution Factor}}{10{,}000} \quad \textbf{(9)}$$

$$\%\text{Phenol} = \frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10,000}} \quad \textbf{(10)}$$

Table 1: Proximate composition in leaves, stem-bark and roots in *E. camaldulensi, H. sabdariffa* and *M. lucida*

Plants /parts	Proximate composition (mg/100g)						
	Crude protein	Fat	Ash content	Moisture content	Carbohydrate	Crude fibre	Total nitrogen
E.camaldulensis							
Leaves	13.83±0.02^{a}	1.12±0.01^{a}	1.94±0.01^{c}	9.44±0.02^{b}	73.66±0.05^{b}	5.39±0.038^{c}	2.21±0.03^{a}
Stems	8.94±0.02^{b}	0.37±0.02^{c}	6.84±0.13^{b}	10.68±0.09^{a}	73.17±0.17^{c}	11.33±0.33^{b}	1.35±0.10^{b}
Roots	6.95±0.03^{c}	0.48±0.05^{b}	7.51±0.06^{a}	10.63±0.06^{a}	74.43±0.13^{a}	12.88±0.05^{a}	1.11±0.02^{b}
H.sabdariffa							
Leaves	13.23±0.02^{a}	1.22±0.30^{a}	2.47±0.01^{c}	8.65±0.03^{c}	74.43±0.08^{a}	5.65±0.02^{c}	2.12±0.01$_{a}$
Stems	8.35±0.32^{b}	0.16±0.01^{c}	7.66±0.12^{a}	8.79±0.02^{b}	74.87±0.47^{a}	15.84±0.03^{a}	1.29±0.01^{b}
Roots	7.42±0.02^{c}	1.02±0.01^{b}	6.49±0.34^{b}	9.76±0.12^{a}	75.30±0.43^{a}	12.66±0.05^{b}	1.42±0.23^{b}
M.lucida							
Leaves	12.84±0.2^{a}	1.48±0.012^{a}	3.66±0.01^{c}	11.42±0.03^{a}	70.60±0.06^{c}	6.86±0.085^{c}	2.05±0.01^{a}
Stems	8.77±0.012^{c}	1.17±0.01^{b}	6.93±0.17^{a}	9.77±0.18^{b}	73.37±0.17^{b}	14.82±0.04^{b}	1.40±0.04^{c}
Roots	8.95±0.015^{b}	1.14±0.012^{b}	6.57±0.10^{b}	8.85±0.03^{c}	74.49±0.14^{a}	10.74±0.12^{b}	1.44±0.01^{b}

Means (± standard error) followed by different superscript across columns of each plant are significantly different at 5% ($p<0.05$) using Duncan's Multiple Range Test.

Table 2: Mineral elements in leaves, stem-bark and root of *E. Camaldulensis, H. sabdariffa* and *M. lucida*

	Sodium	Potassium	Calcium	Phosphorus	Magnesium
E. camaldulensis					
Leaves	31.98±0.29^{a}	168.46±0.90^{a}	76.05±0.20^{a}	89.92±0.79^{a}	5.83±0.08^{a}
Stems	20.90±0.25^{c}	126.30±0.01^{c}	69.707±0.44^{b}	79.97±0.54^{b}	5.21±0.06^{b}
Roots	27.68±0.10^{b}	143.88±0.50^{b}	54.85±0.02^{c}	5.89±0.08^{c}	4.81±0.08^{c}
H. sabdariffa					
Leaves	51.05±0.18^{a}	232.81±0.65^{a}	81.02±0.29^{c}	85.48±0.61^{a}	5.68±1.63^{b}
Stems	34.91±0.04^{b}	127.84±0.70^{b}	116.17±0.80^{a}	85.66±1.05^{a}	6.73±0.01^{a}
Roots	28.77±0.05^{c}	115.69±0.57^{c}	94.45±0.03^{b}	76.22±1.16^{b}	5.27±0.09^{c}
M. lucida					
Leaves	34.46±0.23^{a}	277.71±1.09^{a}	321.55±0.65^{a}	79.36±0.35^{c}	5.58±015^{a}
Stems	5.84±0.03^{c}	116.48±0.54^{b}	27.50±0.01^{c}	96.18±0.66^{a}	2.90±0.02^{c}
Roots	21.45±0.03^{b}	215.92±0.53^{b}	96.42±0.01^{b}	94.15±0.02^{b}	5.75±0.01^{b}

Means (± standard error) followed by different superscript across columns of each plant are significantly different at 5% ($p<0.05$) using Duncan's Multiple Range Test

Table 3: Anti-nutriction in leaves, stem bark and root of *E. Camaldulensis, H. sabdariffa* and *M. lucida*

	Tannin	Oxalate	Phytate	Cyanogenic glycosides	Trypsin Inhibitor	Steroids	Cardiac glycoside
E. camaldul ensis							
EC Leaves	0.25±0.01^{c}	0.16±0.03^{c}	0.18±0.03^{c}	0.08±0.01^{a}	0.13±0.01^{c}	0.22±0.02^{a}	0.25±0.03^{b}
EC Stems	0.49±0.05^{b}	0.26±0.07^{b}	0.26±0.01^{b}	0.22±0.09^{a}	0.23±0.05^{b}	0.17±0.01^{b}	0.21±0.02^{c}
EC Roots	0.55±0.01^{a}	0.30±0.02^{a}	0.34±0.02^{a}	0.12±0.01^{a}	0.24±0.03^{a}	0.15±0.01^{b}	0.38±0.01^{a}
H. sabdariffa							
HS Leaves	0.22±0.02^{c}	0.14±0.07^{b}	0.23±0.01^{c}	0.02±0.01^{a}	0.14±0.02^{b}	0.22±0.02^{a}	0.20±0.01^{b}
HS Stems	0.38±0.04^{c}	0.27±0.02^{b}	0.29±0.01^{a}	0.019±0.01^{a}	0.14±0.01^{b}	0.16±0.01$_{b}$	0.21±0.03^{b}
HS Roots	0.40±0.01^{a}	0.32±0.02^{a}	0.320.01^{a}	0.12±0.01^{b}	0.16±0.01^{a}	0.14±0.01^{b}	0.33±0.02^{a}
H. lucida							
ML Leaves	0.426±0.01^{a}	0.18±0.02^{c}	0.19±0.01^{c}	0.02±0.03^{c}	0.33±0.01^{a}	0.09±0.05^{a}	0.10±0.05^{a}
ML Stems	0.43±0.01^{a}	0.27±0.1^{b}	0.28±0.01^{b}	0.25±0.01^{b}	0.29±0.01^{a}	0.09±0.04^{a}	0.15±0.06^{a}
ML Roots	0.43±0.01^{a}	0.30±0.01^{a}	0.39±0.04^{a}	0.11±0.1^{b}	0.25±0.01^{a}	0.11±0.03^{a}	0.03±0.002^{a}

Means (± standard error) followed by different superscript across columns of each plant are significantly different at 5% ($p<0.05$) using Duncan's Multiple Range Test

Table 4: Phytochemical contents in leave, stem-bark and roots in *E. camaldulensi, H.* sabdariffa and M. lucida

	Alkaloids	Flavonoids	Saponins	Steroids	Phenol	Anthocyanins	Polyphenol
E. camaldulensis							
Leaves	7.23±0.02^{a}	0.16±0.03^{b}	0.35±0.01^{b}	0.13±0.01^{b}	0.26±0.02^{b}	0.11±0.002^{c}	1.67±0.24^{a}
Stem-barks	4.66±0.01^{b}	0.50±0.03^{a}	0.38±0.01^{b}	0.36±0.03^{a}	0.22±0.01^{c}	0.113±0.01^{b}	1.20±0.05^{b}
Roots	3.62±0.04^{c}	0.49±0.01^{a}	0.47±0.04^{a}	0.27±0.04^{a}	0.45±0.01^{a}	0.131±0.02^{a}	0.54±0.01^{c}
H. sabdariffa							
Leaves	6.89±0.01^{a}	0.53±0.04^{a}	0.38±0.03^{b}	0.12±0.02^{c}	0.23±0.04^{a}	0.12±0.06^{a}	1.96±0.03^{a}
Stem-barks	4.21±0.03^{b}	0.40±0.01$_{a}$	0.42±0.01^{a}	0.23±0.02^{a}	0.35±0.01^{a}	0.12±0.01^{a}	1.42±00.6^{b}
Roots	3.90±0.04^{c}	0.65±0.17^{a}	0.39±0.02^{b}	0.21±0.02^{b}	1.34±1.01^{a}	0.12±0.01^{a}	0.86±0.02^{c}
M. lucida							
Leaves	6.67±0.01^{a}	0.86±0.02^{a}	3,46±0.002^{a}	0.58±0.01^{a}	0.39±0.04^{b}	0.15±0.02^{a}	3.86±0.01^{a}
Stems	4.57±004^{b}	0.670.01^{b}	0.49±0.01^{b}	0.13±0.01^{c}	0.38±0.01^{b}	0.11`±0.01^{c}	1.44±.02^{b}
Roots	4.72±0.04^{c}	0.54±0.02^{c}	0.36±0.003^{c}	0.15±0.0^{b}	0.40±0.03^{a}	0.12±0.01^{b}	0.48±0.08^{c}

Means (± standard error) followed by different superscript across columns of each plant are significantly different at 5% ($p<0.05$) using Duncan's Multiple Range Test

DISCUSSION

Nutritional and medicinal compositions of *E camaldulensis, H. sabdariffa and M. lucida* varied relatively across leaves, stems and roots of the plants. The variation observed in proximate compositions across the three parts of the three plants could indicate that proximate contents of the plants showed certain level of discrepancy across leaves, stems and roots of

the plants. This submission may help to ascertain sites of production of metabolites and quantities that are responsible for therapeutic and nutritional effects. Amount of crude protein obtained in this study were higher than the protein contents in the leaves of *Occimun gratissimum* (9.10- 9.22 %) reported by Mlitan *et al.* (2014) but lower than those (29.78) and (23.74) in *Mussaenda roxburghii* reported by Pandey *et al.* (2016). Moderate variations in moisture contents determined in leaves, stems and roots of the three plants investigated compared with reports in literature could be due to loss of water by the sample during drying process (Ayuba 2011). This observation suggests that these plants in fresh form can be stored over a period of time without spoilage (Sobowale *et al.,* 2011). Dehydration of the plants either as food or medicine may increase their shelf-life and reduce growth of microorganisms so that the plants could be used at any season of the year even in dry form (Esayas *et al*., 2011**).**

Higher amount of ash and fibre recorded in of *H. sabdariffa* and *M. lucida* stems as well as roots of *E camaldulensis* may indicate amount of minerals present in the leaves, stems and roots of the plants (Fagbohun *et al.,* 2012). Also, it may be a justification for hypoglycemic potential of the plants (Ojewumi and Kadiri, 2014; Adesuyi *et al.,* 2012; Odutuga *et al*., 2010), therefore, food rich in fibre content is usually recommended for diabetic patients. The significant amount of carbohydrate recorded and its variations across the plants investigated suggest that the plants contain appreciable amount of carbohydrates which serve as a source of energy and fuel for physical performance and regulation of nerve tissue of human body (Adesuyi *et al.,* 2012; Mensah *et al.,* 2008; Tanumihardjo *et al.*, 2011; Olayiwola, 2013; and Kadiri *et al.,* 2014; Achikanu *et al.,* 2013; Nkafamiya *et al.*, 2016).

Highest amount of nitrogen content reported in leaves of the plants investigated suggests that leaves are sites where nitrogen is synthesized and translocate to other plant parts (Farooq *et al.,* 2012; Mingzhu *et al*., 2015).

Mineral elements investigated significantly varied across leaves, stems and roots of the three plants. The mineral elements in leaves of the plants may be a clear justification of the nutritional relevance of leaves of the three plants or site of synthesis or storage of the minerals. On the other hand, high level of these minerals in *H. sabdariffa* may be a scientific proof why leaves of the plants are consumed as vegetables and prepared as local drink called ‘zobo (Asaolu and Asaolu. 2010). High amount of macro elements observed in *M. lucida* roots and stems are used for physiological processes and as co-factors for various metabolic processes (Balogun and Olatidoye, 2012; Fakankun *et al*., 2013; Khan *et al.,* 2015; Ojewumi *et al.,* 2016).

Anti-nutrients were found to be significantly higher in the roots of *E. camaldulensis, H. sabdariffa* and *M. lucida,* the observation may suggest that anti-nutrients are produced or stored more in the roots before translocating to aerial parts of plant via adhesion-cohesion theory through xylem cells (Igwilo *et al*., 2014). Studies have shown that consumption of anti-nutrients beyond acceptable ranges (4 to 9 mg/L) could be nutritionally dangerous because the metabolites have ability to form complexes with mineral during physiological processes such as calcium absorption and nutrient mineralization. This association always make mineral unavailable for utilization or results into formation of kidney stone (Khan *et al*., 2015). Despite the effects of anti-nutrients on nutritional status of plants, Kadiri *et al.* (2015) and Osunbitan *et al*. (2015) revealed that moderate consumption of anti-nutrients is useful for management of kidney and liver disorders. Also, anti-nutrients found in leaves, stems and roots of the three plants investigated are relatively lower than established toxic level (6 %) Sobowale *et al.* (2011) and Kadiri *et al.* (2015) reported. The observation that phytochemicals such as alkaloids, flavonoids, saponins, steroids, anthocyanins and polyphenols were significantly higher in the leaves of *M. lucida* could contribute to medicinal potential of leaves of the plant. This result is in line with findings of Harry and Samson (2014); Atamgba *et al.* (2015); Akinmoladun *et al* (2010) and Okeke *et al.* (2015) who observed high concentration of secondary metabolites in the leaves of Neem and *Jatropha* than other parts of the plants. Variations of phytochemical noticed in different parts of the plants may explain scientific basis for multi-purpose uses of the three plants in the trado-medical system (Ojewumi and Kadiri., 2014;

Kadiri *et al.,* 2013; Kadiri *et al.,* 2014; Taiye and Pass 2014).

CONCLUSION

The study showcased quantities and variations of nutritional and medicinal compositions in leaves, stems and roots of *Eucalyptus camaldulensis, Hibiscuss sabdariffa* and *Morinda lucida*. Out of the three plants under investigation, *Morinda lucida* leaves contained highest proportions of mineral and phytochemical contents.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this research.

DESIGNATION

OAW conceived the experiment. OAW and DGA designed the work, performed experiment and analyzed the data statistically. OAW drafted the article and critically revised by DGA, MK and OWA. All authors gave final approval.

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