

# Phytochemical screening and mineral composition of the bark of some medicinal trees in Ondo State, Nigeria

Richard Odunayo Akinyeye\* • Ayomadewa Mercy Olatunya

Department of Chemistry (Analytical Unit), Ekiti State University, P. M. B. 5363, Ado Ekiti, Nigeria.

\*Corresponding author. E-mail: richardakinyeye@gmail.com. Tel: +2347032770985.

Accepted 5<sup>th</sup> November, 2014

**Abstract.** The bark of some medicinal trees (*Alstonia boonei*, *Pycnanthus angolensis*, *Anacardium occidentale*, *Mangifera indica*, *Khaya iroresis*, *Nauclea diderichii*, *Morinda lucida* and *Bridelia micrantha*) found in Nigeria were analysed for mineral composition and phytochemical screening. The mineral composition were determined and found to contain some elements such as sodium, potassium, calcium, magnesium, zinc, iron, copper, manganese, lead and phosphorous in high concentrations. The result of the phytochemical study revealed presence of alkaloids, saponins, tannins, steroids, phlobatanin, terpenoids, flavonoids and cardiac glycoside in most of the samples. The percentages of tannin (1.75 to 3.32); saponin (2.58 to 4.11) and flavonoid (1.86 to 4.13) were generally high in all the samples studied with *M. lucida* having the highest value for tannin (3.32%) and flavonoid (4.13%). The presence of these phytochemicals in the result is an attestation that the trees are potentially medicinal. The data for the relative mineral concentration from each tree is compared and appraised as potential sources of good health support for humans.

**Keywords:** Phytochemical screening, mineral analysis, bark, medicinal trees.

## INTRODUCTION

Phytochemicals are compounds that occur naturally in plants. They contribute to the colour, flavour and smell of plants. They form part of a plant's natural defence mechanism against diseases. Their therapeutic values to human health and disease prevention have been reported (Okwu, 2004). Some examples of phytochemicals include tannins, saponin, alkaloids, and flavonoids to mention but a few. Tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks (Chikezie et al., 2008). Their presence can cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in the plants implies they may have astringent properties and in addition, could quicken the healing of wounds and burns (Chikezie et al., 2008). Phytic acid is a major phosphorus storage compound of most seeds and cereal grains. Phytic acid has the strong ability to chelate multivalent metal ions especially zinc, calcium and iron. Phytic acid is considered to be a natural antioxidant and is suggested to have potential functions of reducing lipid

peroxidation and also as a preservative in foods (Zhou and Erdman, 2009). Oxalic acid (also referred to as oxalates) is found in many foods. Oxalic acid is a naturally occurring chemical in plants and animals and is also consumed in a variety of different foods such as leafy greens, nuts, seeds, most berries, certain fruits, soy and soy products, meat and dairy products. It has anticancer ability and this it does without affecting or harming normal cells (Tracy, 2009).

Alkaloids are basic natural products occurring primarily in plants. They occur as one or more heterocyclic nitrogen atoms and are generally found in the form of salts with organic acids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antiparasitic and antibacterial properties (Eleazu and Eleazu, 2012). Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis (Okwu, 2004).

Flavonoids in the intestine lower the risk of heart diseases. The antioxidant potentials of plants have been linked with their flavonoids contents (Okwu, 2004).

*Alstonia boonei* (Apocynaceae) is a medicinal plant that is widely used across Africa for various ailments. It is an important anti-malarial herb which consists of about 50 species widely distributed in the continents of Africa, Asia, and America. *A. boonei* is known as 'Ahun' in Yoruba and widely distributed in lowlands and rainforest area of Nigeria (Olajide et al., 2000). *A. boonei* has a wide coverage of primary health care delivery applications in Africa and the rest of the world (Elujoba et al., 2005). When cooked as herbal medicine, it is used for anthelmintic and the latex is used for snakebites (Olajide et al., 2000).

*Pycnanthus angolensis* is known as wild African nutmeg, it is known as 'Akomu' in Yoruba. It is a low tree native to West, Central, Southern and East Africa. It is used for analgesic, carminative, anthelmintic anti-inflammatory haemostatic and antimicrobial action in Africa ethnomedicine (Burkill, 2000). *P. angolensis* are also useful in the treatment of female sterility, gonorrhoeal infertility, rheumatism, sore throat and bronchopneumonia. *Anacardium occidentale* is a native of tropical American, naturalized and cultivated throughout India especially near the coastal area like Kerala for which the plant of interests is collected (Choudri, 1999). It is commonly called cashew. It is used in India for centuries as an important therapeutic source for ailment and found to be of immense global importance. The extracts are utilized for treatment of diarrhoea, dysentery and colonic pain (Bilcalho and Akinpelu, 2001). It possesses anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic properties (Bilcalho and Akinpelu, 2001). The bark of cashew tree secrete a gum or resin of yellowish colour, soluble in water, it has emulsifier, adhesive and stabilizer properties, the major cations in *Anacardium occidentale* are K, Na, Ca and Mg (Bilcalho and Akinpelu, 2001).

*Mangifera indica* called mango is originally from India related to pistachio, it is a widely cultivated fruits of the tropical world and rich in vitamin A, C and D. Mango is a tropical Asian called evergreen trees cultivated for its edible fruits, the ovoid fruits of this tree having a smooth rind, sweet juicy and a flat one seeded stone. It is eaten ripe. It has slightly acidic and spicy taste; mango skin can cause allergic cutaneous reactions and irritates the skin and the mouth (Jedele et al., 2003). *Khaya irensis* is popularly known as 'Ogao' in Yoruba. It is a medicinal malicious plant used in traditional Africa remedies and is commonly used in bitter tonic in folk as a popular medicine for malaria; fever, mucous and venereal disease as well as anthelmintic and a taeniicide remedy (Iwu, 1993).

*Nauclea diderichii* is a species of plant in *Rubiaceae* family widely found in Angola, Cameroon, Central African and Nigeria. It is an evergreen tree that reaches a height

of 30 to 40 m and a diameter of 0.9 to 1.5 m; bole cylindrical, slender, straight and branchless, rising to 20 to 30 m and a broad spherical crown with thick foliage.

*Morinda lucida* is a genus of flowering plants in the madder family *Rubiaceae*. It consists of some species distributed in tropical region of the world. It bears aggregate or multiple fruits, which are used for medicinal purposes (Quattrocchi, 2000). *M. lucida* (Benth) has been recommended for the prevention and treatment of hypertension and its cerebral complications (Iwu, 1993).

*Bridelia micrantha* is a coastal golden-leaf in the *phyllanthaceae* family and native to Africa with dense widely spreading crown, the trees are deciduous or evergreen found in coastal forests. It serve as food for butterflies, for treatment of diverse condition of the central nervous system, also useful hygienically as a mouth wash, it fight against cancer, a *bridelia micrantha* is a source of cytotoxic aryltetraen lignin glycoside (Pooley, 1993).

This work presents a preliminary investigation of the presence and concentrations of the phytochemicals and the mineral concentrations of the extract from the bark of trees of some Nigerian medicinal plants.

## MATERIALS AND METHODS

All the reagents used for this analysis are of analytical grade. The major experimental materials used are the bark of trees which are about eight samples namely: *Alstonia boonei*, *Pycnanthus angolensis*, *Anacardium occidentale*, *Mangifera indica*, *Khaya irensis*, *Nauclea diderichii*, *Morinda lucida* and *Bridelia micrantha*. They were collected from a farmland in the south western part of Nigeria specifically from Araromi-obu in Odigbo Local Government Area of Ondo State and identified by a Plant Scientist in the Department of Plant Science, Ekiti State University, Ado Ekiti, Nigeria.

### Preparation of samples

The plant samples (bark of trees) were collected washed several times with distilled water, then oven dried at about 40°C for one week and ground into powder using a medium kitchen blender. 100 g of each powder sample was exhaustively extracted by soaking in 200 ml of distilled water for 12 h; the extracts were filtered using Whatman Filter Paper No 42 (125 mm). Phytochemical screening was done (as described below) to trace the chemical constituents present in the samples.

### Phyto-chemical screening

The chemical tests were carried out on the aqueous extract and on the powdered specimens using standard

procedures to identify the constituents as described by (Asuzu and Anaga, 1991).

**Test for alkaloid:** 5 g of the powdered sample was boiled with water on a steam bath for 30 min, after filtration the filtrate obtained was tested for the presence of alkaloids using alkaloid reagent like Mayer's, Wager's and Hager's, the procedure was repeated using 10% tetraoxosulphate (VI) acid as extracting solvent, methanol and chloroform were separately used as extracting solvent. The filtrate obtained from each was evaporated to dryness on a water bath, after which the residues were dissolved in 1% tetraoxosulphate (VI) acid and the filtrate tested for the presence of alkaloid (Harbone, 1973).

**Test for saponin:** About 2 g of the powdered samples were boiled with 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water vigorously for a stable persistent froth; this was mixed with olive oil and shaken vigorously then observed for the formation of emulsion indicating the presence of saponin (Sofowora, 1993).

**Test for tannin:** About 0.5 g of the dried powdered samples were boiled in 20 ml of water in a test-tube and then filtered, a few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration which indicate the presence of tannin (Okwu, 2004).

**Test for steroid:** 2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2 ml of tetraoxosulphate(VI) acid, the colour change; violet to blue or green in some samples indicate the presence of steroid (Sofowora, 1993).

**Test for phlobatanin:** The deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatanin (Manikandan et al., 2006).

**Test for terpenoid (Salkowski test):** 5 ml of each extract was mixed in 2 ml of chloroform and concentrated tetraoxosulphate (VI) acid (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface was formed to show positive result for the presence of terpenoids.

**Test for flavonoid:** 5 ml of dilute ammonia solution was added to a portion in the aqueous filtrate of each plant extract followed by addition of concentrated tetraoxosulphate (VI) acid, a yellow colouration was observed indicating the presence of flavonoid (Sofowora, 1993).

**Test for cardiac glycoside (Killer-killani test):** 5 ml of

each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, this was under layered with 1 ml of concentrated tetraoxosulphate(VI) acid, a brown ring at the interface indicates a de-oxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just gradually throughout thin layer.

#### ***Determination of tannin content***

Tannin content was determined using Van-burden and Robinson (1981) method: 500 mg of the sample was weighed into a 50 ml plastic bottle; 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test-tube and mixed with 2 ml of 0.1 M ferric chloride in 0.1 M hydrochloric acid and 0.008 M potassium ferrocyanide, the absorbance was measured at 220 nm within 10min. The tannin content for each sample was determined from a standard calibration plot.

#### ***Determination of phytic acid content***

Samples were extracted with 3.5% (w/v) HCl and the extract was further purified by AGI-X8 chloride anion exchange column. A calorimetric determination of phytic acid was carried out at 500 nm by using wager's reagent (Vaintaub and Lapteva, 1988).

#### ***Determination of oxalate content***

1 g of the sample was weighed into 100 ml conical flask, 75 ml of 15 M tetraoxosulphate (VI) acid was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman Filter Paper No 42 (125 mm). 25 ml of the sample filtrate (extract) was collected and titrated hot (80 to 90°C) against 0.1 M  $\text{KMnO}_4$  solution to the point when a faint pink colour appeared that persisted for at least 30 s.

#### ***Determination of saponin content***

Saponin determination was done using the method of AOAC (1990). Saponin extraction was done using two different solvents. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponin proper. Two gram of the sample was folded into a thimble and put in a soxhlet extractor and a reflux condenser fitted on top. Extraction was done with acetone

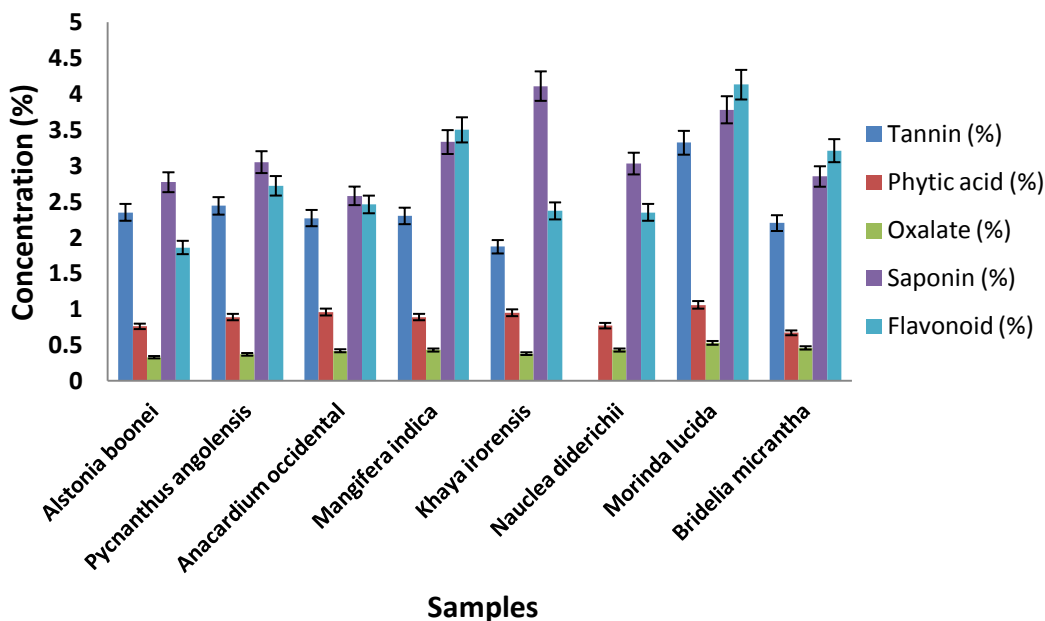


Figure 1. Quantitative phytochemical screening of extracts from the bark of the selected Nigerian plants.

in a 250 cm<sup>3</sup> capacity round bottomed flask for 3 h, after which the apparatus was dismantled and another 150 cm<sup>3</sup> capacity round bottomed flask containing 100 cm<sup>3</sup> of methanol was fitted to the extractor and extraction was carried on for another 3 h. The weight of the flask was taken before and after the second extraction in order to note the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken. The saponin content of the sample was calculated thus:

$$\% \text{ Saponin} = \frac{\text{weight of saponin} \times 100}{\text{weight of sample}}$$

#### Determination of flavonoid content

The flavonoid content was determined by the method of Boham and Kocipai (1994). 10 g of the plant sample was extracted repeatedly with portions from 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman Filter Paper No 42 (125 mm). The filtrate was later evaporated in a water bath and weighed to a constant weight.

#### Determination of mineral composition

The mineral composition was determined using standard analytical methods (AOAC, 1990).

## RESULTS AND DISCUSSION

Phytochemically, all the plants studied showed the presence of alkaloids, saponin, tannin, terpenoid, flavonoid and cardiac glycoside. Steroid was not detected in *Pycnanthus angolensis*, *Khaya irorensis* and *Bridelia micrantha* so also was phlobatanin not found in *Alstonia boonei*, *Anacardium occidentale* and *Nauclea diderichii*. The percentage composition of these phytochemicals is given in Figure 1. All the plant has high percentage of saponin, flavonoid and tannin. This justifies their use for medicinal purposes.

The presence of saponins can control human cardiovascular disease and reduce blood cholesterol. Tannins may provide protection against microbial degradation of dietary proteins in the rumen (Aletor, 1993; Erukainure et al., 2011). *Khaya irorensis* has the highest percentage of saponin, this is in agreement with the work done by Falodun et al. (2009). *Morinda lucida* has the highest percentage of all the phytochemicals studied. The flavonoid content is also relatively high; the best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species (ROS) (Erukainure et al., 2011). The presence of flavonoids in *Morinda lucida* may be the reason for its antioxidant activities and healing effects (Ogunlana et al., 2008). Phytic acid and Oxalate are present in low concentration in all the samples studied and this also makes them safe for consumption. Oxalate should be consumed in small quantity because oxalic acid binds with other mineral such as calcium to form oxalate salt

**Table 1.** The concentration of minerals (mg/100 g) in the bark of some selected Nigerian plants.

Trees	Minerals (mg/100 g)									
	Na	K	Ca	Mg	P	Zn	Fe	Cu	Mn	Pb
<i>Alstonia boonei</i>	235.6	625.4	78.9	187.7	2314.5	23.5	43.7	3.5	13.5	0.3
<i>Pycnanthus angolensis</i>	214.2	734.2	94.3	236.4	3744.5	34.1	38.7	4.2	22.1	0.1
<i>Anacardium occidentale</i>	197.8	653.9	108.5	214.8	3061.1	28.8	45.5	5.0	12.7	0.2
<i>Mangifera Indica</i>	195.4	748.8	116.6	304.1	3272.5	35.0	37.5	3.6	20.8	0.1
<i>Khaya irorensis</i>	285.5	677.3	94.8	238.8	2485.1	43.7	46.2	3.3	23.3	0.1
<i>Nauclea diderichii</i>	248.2	715.8	213.4	159.5	2393.3	35.4	37.7	2.7	34.2	0.2
<i>Morinda lucida</i>	226.7	658.9	127.8	195.4	2401.6	29.5	45.8	4.3	27.2	0.1
<i>Bridelia micrantha</i>	254.8	87.94	87.7	223.3	2545.4	34.8	45.5	2.2	37.7	0.3
Mean	232.3	612.8	115.3	220.0	2777.3	33.1	42.6	3.6	23.9	0.18
Standard deviation	30.5	216.3	42.7	43.2	521.4	5.96	3.90	0.90	23.9	0.18

which has been postulated to be the cause of kidney stone (Bridget, 2010).

Table 1 shows the result for the mineral concentrations (micro and macro) from the samples of plant barks investigated. All the macro minerals were highly concentrated. Phosphorus, potassium, magnesium and calcium were highly concentrated with means (mg/100 g) of  $2777.3 \pm 521.4$ ,  $612.8 \pm 216.3$ ,  $220.0 \pm 43.2$ ,  $115.3 \pm 42.7$ , respectively. The ratio of sodium to potassium in the body is of great concern for prevention of high blood pressure, a Na/K ratio less than one is recommended (Fleck, 1976). All the Na/K ratios for these samples are less than one. This is an indication that consumption of these plants extracts would reduce high blood pressure disease (Nieman et al., 1992). The mineral values reported in this work for *Anacardium occidentale* and *Mangifera indica* are lower compare to the values reported by Abulude (2007). The discrepancy in the result may be due to the differences in the parts of plant used for the studies. Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and protein are all involved in bone formation (Mann et al., 2007). Calcium is also important in blood clotting, muscle contraction and in certain enzymes in metabolic processes (Abulude, 2007). Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves (Abulude, 2007). Zinc is important for behavioural and mental function, the immune and antioxidant system functioning (Shankar and Prasad, 1998; WHO, 1996), and bone metabolism (WHO, 1996). It is necessary for protection against oxidative cell damage, enhances DNA repair, and controls cell proliferation in diseased states (Shankar and Prasad, 1998). African diets for the poor, elderly, children and women, especially the pregnant and lactating mothers are generally deficient in zinc (Steyn et al., 2008; Oldewage-Theron et al., 2008). Iron is chiefly important to the human body because it is the main constituent of haemoglobin, cytochrome, and other components of respiratory enzyme systems. A constant although small

intake of iron in food is needed to replace erythrocytes that are destroyed in the body processes. Most iron reaches the body in food, where it occurs naturally in the form of iron compounds. Iron deficiencies may cause serious disorders and anaemia (Miller-Keane Encyclopaedia and Dictionary of Medicine, 2003). Shortage in iron intake can lead to different sicknesses and human deficiencies. The Recommended Dietary Intake (RDA) of iron for the adult male is an average of 8 mg/day, while that for the adult woman is an average of 13 mg/day. For a pregnant woman, an average of 27 mg/day is recommended but during lactation it reduced to an average of 9 mg/day. The RDA for infants of 6 months and above is 11 mg/day, while that for children 4 to 8 years of age is 10 mg/day (Dietary Reference intakes (DRIs), 2001). All the samples studied are high in Zn and Fe, so these can serve as supplement for Zn and Fe especially for pregnant and lactating mothers since these minerals are good for growth and development of infants.

## CONCLUSION

The phytochemical compositions of the bark of trees studied showed their therapeutic ability and suitability for medicinal applications. The abundance of these plants in Nigeria makes the plants readily available as potential raw materials for different medical applications. The samples have high concentration of mineral elements and this also shows that they can serve as sources of minerals when included in diets.

## ACKNOWLEDGEMENTS

The contributions of Mr Omotayo FO and Adesanya AV of Ekiti State University, Ado Ekiti, Nigeria and Mr Oguntokun MO of the Federal University of Technology, Akure, Nigeria to the success of this manuscript are gratefully acknowledged.

## REFERENCES

- Abulude FO (2007).** Phytochemical screening and mineral contents of leaves of some Nigerian woody plants. *Res J. Phytochem.* 1:33-39.
- Aletor VA (1993).** Allelochemicals in plant food and feeding stuffs: 1. Nutritional, biochemical and physio-pathological aspects in animal production. *Vet. Hum. Toxicol.* 35:57-67.
- Association of Official Analytical Chemists (1990).** Official Methods of Analysis (15 Ed.) The Association of Official Analytical Chemists, Washington DC, USA.
- Asuzu IU, Anaga AO (1991).** Pharmacological screening of the aqueous extract of *Alstonia boonei* bark. *fitoterapia* 63:411-417.
- Bilcalho B, Akinpelu DA (2001).** Antimicrobial activity of *Anacardium occidentale* bark. *fitoterapia* 72(3):286-287.
- Boham AB, Kocipai AC (1994).** Flavonoid and condensed tannins from leaves of Hawaiian vaccinium vaticulum and vicalycinium. *Pacific Sci.* 48:458-463.
- Bridget C (2010).** Oxalates and Diets. Available on [www.livestrong.com](http://www.livestrong.com) last modified Oct 13, 2010. Retrieved on 12/06/2013.
- Burkill HM (2000).** The useful plants of West Tropical Africa. Royal botanic Gardens. 2:144-150.
- Chikezie PC, Agomuo EN, Amadi BA (2008).** Biochemistry, Practical/Research Method, A fundamental approach., Mega soft publishers 2:51-53.
- Choudri RD (1999).** Herbal drug industrial pharmacy, 3<sup>rd</sup> edition Lea and ferbiger Philadelphia pp. 297-303.
- Dietary Reference intakes (DRIs) (2001).** Recommended dietary allowances & adequate intakes elements: Food & Nutrition Board institute of Medicine National Academies 2001. Available on [www.nap.edu](http://www.nap.edu). Retrieved 12/06/2013.
- Eleazu CO, Eleazu KC (2012).** Physico-chemical properties and antioxidant potentials of 6 new varieties of ginger (*Zingiber officinale*). *Am. J. Food Technol.* 7(4):214-221.
- Elujoba AA, Ogunyemi CM, Odeleye OM (2005).** Traditional medicinal development for medical and dental primary health care delivery system in Africa. *Afri. J. Trad. compl. Alter. med.* 2(1):46-61.
- Erukainure OL, Oke OV, Owolabi FO, Adenekan OS (2011).** Chemical composition and antioxidant activities of *Aframomum sceptrum*. *Trends Appl. Sci. Res.* 6:190-197.
- Falodun A, Poh CF, Adelusi SA, Emmanuel O (2009).** Phytochemical and antiinflammatory evaluation of khaya grandifoliola stem bark extract. *Inter J. Pharm. Tech. Res.* 1(4):1061-1064.
- Fleck H (1976).** Introduction to Nutrition, 3<sup>rd</sup> edition Macmillan, New York, USA pp. 207-219.
- Harbone JB (1973).** Phytochemical methods .Chapman and Hall Ltd London pp. 49-188.
- Iwu MM (1993).** Handbook of African medicinal plants. CRC press Boca Raton Ann Arbor, London Tokyo pp. 110-190.
- Jedele S, Hau AM, Von O (2003).** An analysis of the world market for mangos and importance for developing countries conference on international Agricultural research development.
- Manikandan L, Senthilkumar GP, Rajesh OS (2006).** Cancer chemopreventive agents, from medicinal plants. In: Trivedi P.C(ed) Medicinal plants ethnobotanical approach agrobios, India p. 410.
- Mann A, Yahaya AY, Suleiman ID (2007).** Physicochemical studies of some fruits of Nigerian medicinal plants used for treatment of respiratory diseases. *Chem. Class J.* 4:91-94.
- Miller-Keane Encyclopaedia and Dictionary of Medicine (2003).** Nursing and Allied Health, Seventh Edition. © 2003 by Saunders, an imprint of Elsevier
- Nieman DC, Butter Worth DE, Nieman CN (1992).** *Nutrition:* Wm.C. Brown Publisher Dubugue 9:540.
- Ogunlana OE, Ogunlana O, Farombi OE (2008).** Morinda lucida: antioxidant and reducing activities of crude methanolic stem bark extract. *Adv. Nat. appl. Sci.* ISSN: 1995-0772.
- Okwu DE (2004).** Phytochemicals and vitamin content of indigenous species of South Eastern Nig. *J. Sustain. Agric. Environ.* 6:30-34.
- Olajide OA, Awe SO, Makinde M, Morebise O, Okpako DT (2000).** Studies on the anti-inflammatory, antipyretic and Analgesic properties of *Alstonia boonei* bark. *J. Ethnopharmacol.* 71:179-186.
- Oldewage-Theron WH, Samuel FO, Venter CS (2008).** Zinc deficiency among the elderly attending a care centre in Sharpeville, South Africa. *J. Hum. Nutr. Diet.* 26(1):566-74.
- Pooley E (1993).** The complete field guide to trees of Natal Zululand and Transkei. Available on [en.wikipedia.org/wiki/special:bookstores](http://en.wikipedia.org/wiki/special:bookstores). ISBN 0-620-17697-0 Retrieved 12/06/2013.
- Quattrocchi U (2000).** Traditional herbal medicines for Japanese, Korean and Chinese for antioxidant and antibacterial activities of three stem bark against ovine nematodes. *Filaterapia* 72:12-21.
- Shankar AH, Prasad AS (1998).** Zinc and immune function: the biological basis for altered resistance to infection. *Am. J Clinical Nutr.* 68(2): 447S-464S.
- Sofowora A (1993).** Medicinal plants and traditional medicines in Africa. 2<sup>nd</sup> edition spectrum books, Ibadan.
- Steyn NP, Wolmarans P, Nel JH, Bourne LT (2008).** National fortification of staple foods can make a significant contribution to micronutrient intake of South African adults. *Public Hlth Nutr.* 11(3):307-313.
- Russel T (2009).** Oxalates in spinach. Is oxalic acid a green smoothie health concern. Available on [www.incrediblesmoothies.com](http://www.incrediblesmoothies.com). Retrieved 12/06/13.
- World Health Organisation (1996).** Trace elements in human nutrition and health. WHO, Geneva.
- Vaintaub IA, Lapteva NA (1988).** Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Ann. Biochem.* 175(1):227-230.
- Van-burden JP, Robinson WB (1981).** Formation of complexes between protein and tannic acid. *J. Agric. food chem.* 17:772-777.
- Zhou JR, Erdman JW (2009).** Phytic acid in health and disease. *Crit. Rev. Food Sci. Nutr.* 35(6):495-508.