COMPOSITIONAL INVESTIGATION OF PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF VARIOUS PARTS OF *MORINGA OLEIFERA* PLANT

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ABSTRACT

In view of the nutritional and the medicinal importance of *Moringa oleifera* Plant (Horseradish or Drumstick tree popularly called Ewe-igbale in Yoruba Language). The present study was carried out to investigate the phytochemical and antioxidant properties of the various parts of the *Moringa oleifera* plant. The *Moringa oleifera* plant used in this work was obtained from Afao- Ekiti, Ekiti state, Nigeria. The results of phytochemical screening revealed that saponin, terpenoid, steroid and flavonoid were present in all the parts of the plant. Phlobatamin was observed to be absent in all parts of the plant. Alkaloid and tannin were observed in the moringa root as tannin was also observed in the leaf and cardiac glycosides were present in the seed and leaf of the plant. The antioxidant properties showed that total flavonoid, total phenol, iron reducing antioxidant property (FRAP), vitamin C and the free radical scavenging ability against 1,1-diphenyl-2 picrylhydrazyl (DPPH) were evidenced in all the parts of the moringa plant. The results however showed that the various parts of the plant contained varying amounts of phytochemicals and antioxidant properties of medicinal importance.

Keywords: Phytochemical contents, Antioxidant properties, Distribution, *Moringa oleifera.*

INTRODUCTION

Moringa oleifera, locally known as Awe - Igbale, belongs to the monogeneric family Moringaceae and is widely distributed in the Indo-Bangla subcontinent and cultivated throughout the tropical belt (Nikkon,et al.,2003) Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human aliments. Ethanolic leaves extract of *Moringa oleifera* used as hypotensive (Nikkon,et al.,2003;Siddiqui and Khan,1968;[8]. The leaves of *Moringa oleifera* are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent (Dangi, et al., 2002; Ghasi, et al., 2000; Siddiqui and khan., 1968).

Moringa Oleifera is the best known of the thirteen species of the genus Moringacae. Moringa was highly valued in the ancient world. The Romans, Greeks and Egyptians extracted edible oil from the seeds and used it for perfume and skin lotion.In 19th century, plantations of Moringa in the West Indies exported the oil to Europe for perfumes and lubricants for machinery. People in the Indian sub-continent have long used Moringa pods for food. The edible leaves are eaten throughout West Africa and parts of Asia. For centuries, people in many countries have used Moringa leaves as traditional medicine for common ailments. Clinical studies have begun to suggest that at least some of these claims are valid. With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed. India: Traditionally used for anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye & ear infections, fever, glandular swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat,

sprain, tuberculosis. Malaysia: Traditionally used for intestinal worms Guatemala: Traditionally used for skin infections and sores Puerto Rico: Traditionally used for intestinal worms.

Philippines: Traditionally used for anemia, glandular swelling and lactating. Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular — Trees for Life, Church World Service, and Educational Concerns for Hunger Organization — have advocated Moringa as "natural nutrition for the tropics." Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. A large number of reports on the nutritional qualities of Moringa now exist in both the scientific and the popular literature. It is commonly said that Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of Moringa leaves rivals that of milk and eggs. However, the leaves and stem of *M. oleifera* are known to have large amounts of their calcium bound in calcium oxalate crystals, which is not a form of calcium available to the body. Infact, the nutritional properties of Moringa are now so well-known that there seems to be little doubt of the substantial health benefit to be realized by consumption of Moringa leaf powder in situations where starvation is imminent.

MATERIALS AND METHODS

Sample collection and preparation

Fresh samples of the plant *Moringa oleifera* was collected from Afao- Ekiti, Ekiti-State, Nigeria.The identification and authentication was carried out at the Department of plant science, Ekiti State University, Ekiti state, Nigeria. All the chemicals used were of analytical grade, while the water was glass distilled. The samples collected were separated into leaf, seed, root, bark which were air dried, crushed orpounded to reduce the size for blending and subsequently blended to powder separately with Marlex Excella laboratory blender.

PHYTOCHEMICAL SCREENING

Chemical tests were carried out on each extract to screen for phytochemical constituents as described by Sofowora (2006),[19] and Harborne (1984).

Test for Tannins

0.5 g of the dried powdered samples was 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.

Test for Saponin

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Alkaloids

5.0 mL of 1% aqueous hydrochloric acid was added to 2mg of the extract in a test tube, heated in a steam bath and filtered; 1mL of the filtrate was treated with 6-10 drops of Dragendoff's reagent. The presence of creamish precipitate or turbidity after addition was taken for the presence of alkaloid.

Anthraquinone Determination

5g of each plants extract was shaken with 10ml benzene, filtered and 5ml of 10 percent ammonia solution added to the filterate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicate the presence of free anthraquinones.

Test for Phlobatannins

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 phlobatinins.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowora, 2006; Harborne, 1984). 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration was observed indicating a positive test for
flavonoids.

Salkowski Test for Cardiac Glycosides (Steroidal Ring or Terpenoids)

5.0 mL of each extract was mixed in 2 mL of chloroform, and concentrated H_2SO_4 (3 mL) was carefully added to form a lower layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Legal's test for Cardiac-glycoside (cardenolide)

1.0 ml extract was dissolved in 5.0 ml pyridine, 2 drops 2% Sodium Nitroprusside and 2 drops 20% NaOH were added. A deep red color faded to brown indicates presence of cardenolide.

Keller-Killani Test For Cardiac Glycosides (deoxysugar)

50mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1.0 mL of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar 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.

Lieberman's Test for Steroidal Nucleus

2.0mL of acetic anhydride was added to 0.5 g of each solvent extract of sample with 2.0 mL H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Aqueous extract preparation for antioxidant properties

The aqueous extract of the various parts were prepared by homogenizing them in distilled water (1:20 w\v) and left for 20 minutes and the respective supernatants were used for the assays on:

Determination of total phenol content

The total phenol content was determined on the extracts using the method reported by[16]. Appropriate dilutions of the extracts were oxidized with 2.5mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The totalphenol content was subsequently calculated using Tannic acid as standard.

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl3 solution as described by Oyaizu (1986). A 2.5mL aliquot was mixed with 2.5mL of 200 mmol l−1 sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5mL of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5mL of the supernatant was mixed with an equal volume of water and 1mL of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing antioxidant property was subsequently calculated using ascorbic acidas standard.

DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Lin, et al., (2010). 1.0mL of the extract was mixed with 1mL of 0.4 mmol l−1 methanolic solution containing DPPH radicals. The mixture was left in the dark for 30mins and the absorbance was measured at 516nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Results

Table 1: Phytochemical screening of *Moringa oleifera*

Extracts	Moringa root	Moringa bark	Moringa seed	Moringa leaf
Saponin	+	+	+	+
Tannin	+	-	-	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+
Alkaloid	+	-	-	-
Steroid	+	+	+	+
Terpenoid	+	+	+	+
Keller-kiliani test for Cardiac Glycosides (deoxysugar)	-	-	+	+
Legal test for Cardiac Glycosides (cardenolides)	-	+	+	+
Salkowski test for Cardiac Glycosides (Steroidal Ring or Terpenoids)	+	+	+	+
Liebaman test for Steroidal Nucleus	+	+	+	+

(+) Sign indicate the presence of the constituents.
(-) Sign indicate the absence of the constituents.

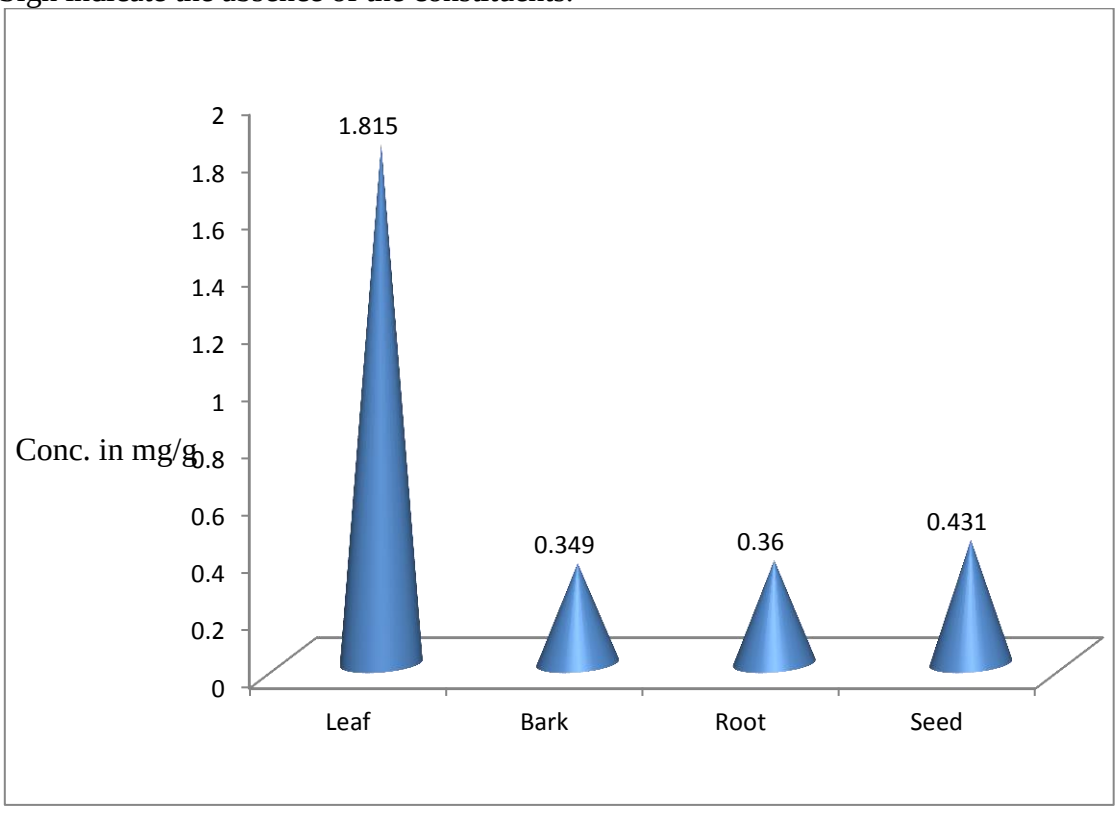

Figure 1: Total phenol content for various parts of Moringa oleifera

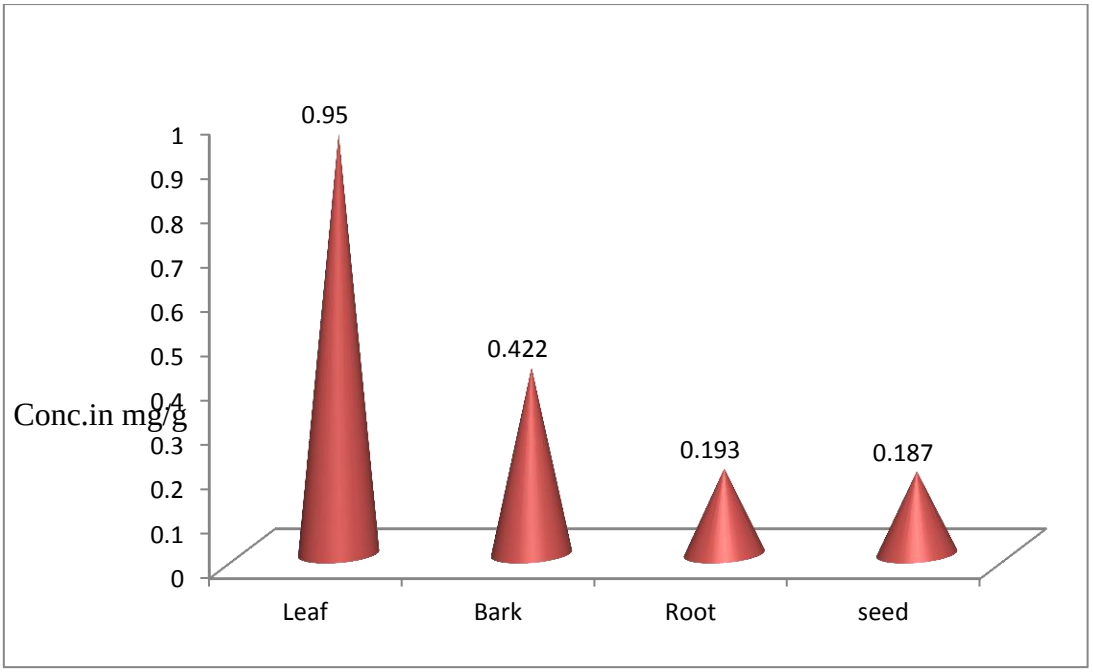

Figure 2: Reducing property for various parts of *Moringa oleifera*

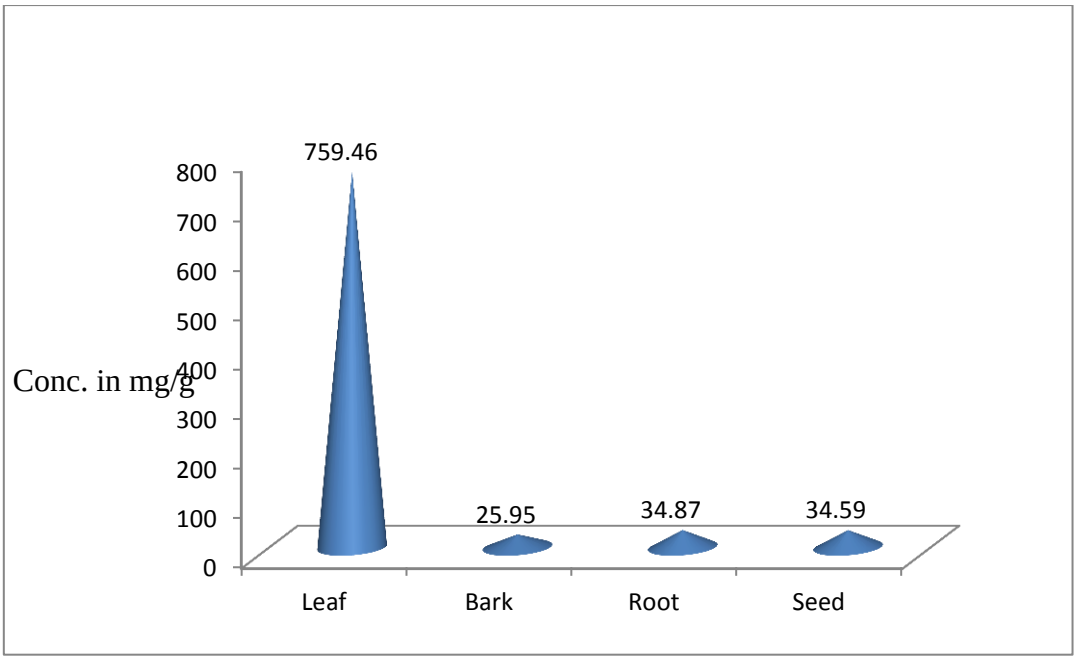

Figure3: DPPH free radical scavenging ability for various parts of Moringa oleifera

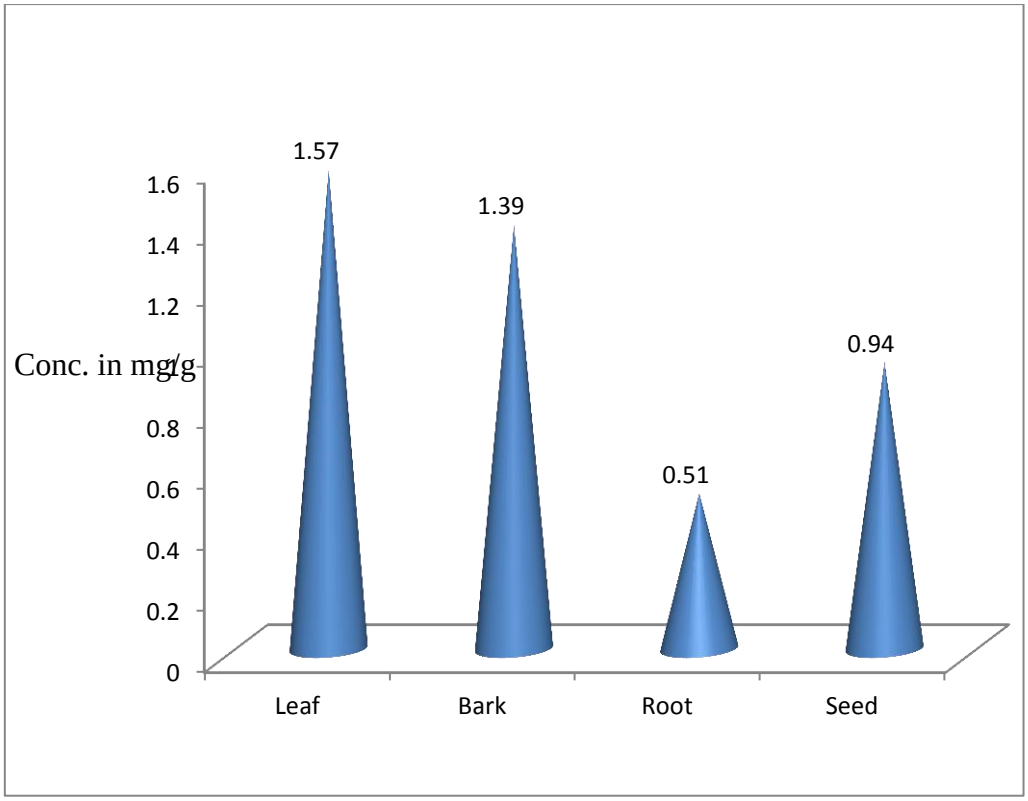

Figure4: Vitamin C Content for various parts of Moringa oleifera

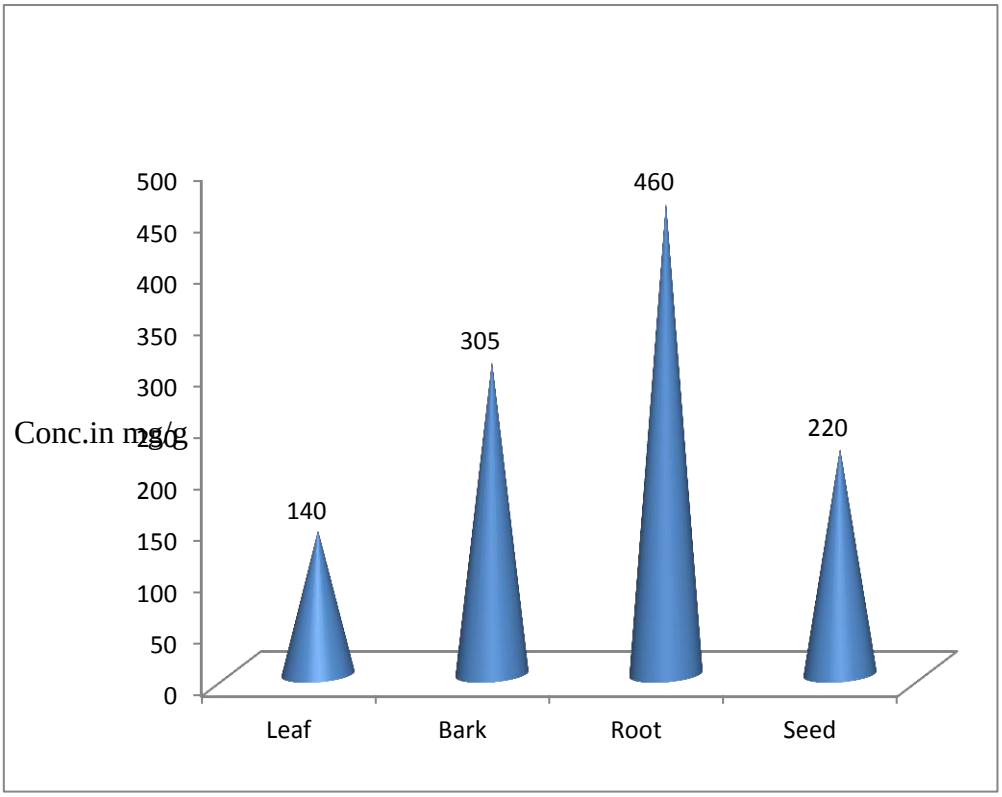

Figure5: Flavonoid content for various parts of Moringa oleifera

DISCUSSION

Table 1 shows the results of phytochemical screening of the aqueous extract of *Moringa oleifera.* This reveals the presence of tannin which may be responsible for bitterness, pungency, astringent properties, hasten the healing of wounds and inflamed mucous membrane [13] in the root and leaf of the plant extract. While Alkaloid was found to be present in only the root extracts as saponin was found in all the parts of the plants, this however agrees with the observations of [10]; Mudzwiri, 2007) that saponin was found to be present in many plants and it is often referred to as "natural detergents" as it causes red blood cell haemolysis and as such toxic. Steroid, flavonoid and terpenoid were found present in all parts of the plant while phlobatamin was not detected in any of the plant extract. The findings in this study agree with earlier studies which also found that, not all phytochemicals are present in all plant parts and that those present differ according to the type of the extracting solvent used [18] [2]. Cardiac glycosides were as well detected in various parts of the plant.

According to [4] [6] [9] [7] [11] as well as [20], however, phytochemical components of the medicinal plants have therefore been found to be responsible for both pharmacological and toxic properties in plants. These metabolites are said to be useful to a plant itself but can be toxic to animals, including man. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance. This is better supported by the fact that members of the family of this plant have been known to be involved in ethnomedicine in the management of various ailments [1][2] Ezeamuzie *et al.,* 1996. Tannins adversely affect protein digestibility but its minimum level required to elicit a negative growth response has not been fully established, hence it is still unclear as to whether it could be harmful [5].

The results of the distribution of total phenol, reducing property, Vitamin C content and DPPH free radical scavenging ability in the leaf was however found to be higher than that obtained from the other parts of the plant though total flavonoid content was observed higher in the root than other parts of the plant. Though the antioxidant properties were found to be present in the various parts of the plant studied, but the leaf of the plant was however found to contain higher values than others. This observation was in accordance with Nikkon,*et al.*, 2003; Siddiqui and Khan,1968; [8] that leaf extract of *Moringa oleifera* was used as hypotensive and that of Dangi, *et al.*, 2002; Ghasi, *et al.*, 2000; Siddiqui and khan, 1968 that the leaves of *Moringa oleifera* are reported to be used as a hypocholesterolemic and hypoglycemic agents.

CONCLUSION

Moringa oleifera has proved to be very rich in both useful phytochemicals and antioxidant components which can be harnessed for both human and animal uses in the areas of food, medicine and pharmaceutical purposes. The various parts of the plants have been studied to contain varying amounts of these phytochemicals which form the basis for utilizations and further investigations. Based on the results obtained in this study, it could be said that M. oleifera leaf, bark, seed and root powders contains chemical constituents of pharmacological and nutritional significances.

However, further works can still be carried out to isolate and purify the bioactive constituents as applicable in all the parts of *Moringa oleifera* plant using various extraction solvents with

a view to characterizing and elucidating their molecular structures, as well as evaluating their safety or otherwise for human and other animal uses.

REFERENCES

1. Aliyu, B.S. (2006): Common ethnomedicinal plants of the semi-arid regions of West Africa – Their descriptions and phytochemicals. Triumph publishing company limited, Gidan Sa'adu Zungur, Kano, Nigeria. 1:93-94.
2. Ayinde BA, D.N. Onwukaeme and E.K.I. Omogbai, 2007. Isolation and characterization of two phenolic compounds from the stem bark of Musanga
3.Caceres, A., Cabrera, O., Morales, O., Mollinedo, P. and Mendia, P. (1991): Pharmacological properties of M. oleifera I: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 133(3):213-216.
4. Duke, J.A. (1992): Handbook of biologically active phytochemicals and their activities. CRC Press, Boca Raton, FL. Pp 22-25.
5. Elemo, B.O., Elemo, G.N., Agboola, O.O. and Oyedun, A.B. (2001): Studies on some anti-nutritive factors and in-vitro protein digestibility of Thaumatococcus danielli (Benth) waste. Nigerian Journal of Biochemistry and
6. Evans, C.W. (1996): Trease and Evans pharmacognosy. 14th edition. WB Saunders
7. Ibrahim, G., Mahmud, N., Yaro, A.H. and Ahmed, A. (2006): Phytochemical and toxicity evaluation of the stem bark of Ficus sycomprus LINN (Moraceae). Biological and Environmental Sciences Journal for the Tropics 3(3):37-40.
8.Kirtikar, K.R. and B.D. Basu, 1987. Indian Medicinal Plants. 2nd Edn. Vol. 3, Dehra Dun: International Book Distributors, India, pp: 2061-2062.
9. Lawal, M., Wasagu, R.S.U. and Ladan, M.J. (2005): Hepatotoxicity risk assessment of neem (Azadirachta indica) seed extract using albino rats. Biological and Environmental Sciences Journal for the Tropics 2(2):36-38.
10. MacDonald, R. S., Guo JuYuan, Copeland, J., Browning, J.D., Sleper, D., Rottinghaus, G.E.,Berhow, M. A. (2005): Environmental Influences on Isoflavones and Saponins in Soybeans and Their Role in Colon Cancer. J. Nutr. 135:1239-1242.
11. Magaji, R.A. and Yaro, A.H. (2006): Acute toxicity evaluation of the aqueous extract of Syzygium aromaticum in mice. Biological and Environmental Sciences Journal for the Tropics 3(1):1-3.
12. McDonald, P., Edwards, R.A., Greenhill, F.D. and Morgan, C.A. (1995): Animal nutrition. Prentices Hall, London.
13. Okwu, D. E., Okwu, M. E. (2004): Chemical composition of Spondias mombia linn plant parts. J. Sustain. Agric. Environ., 6: 140-147.
14. Okwu, D.E.(2001): Evaluation of the Chemical Composition of indigenous Spices and Flavouring Agents. Global Journal of Pure and Applied Science, 7 (3): 455-459
15. Pharmacological properties of M. oleifera I: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 133(3):213-216. Cecropioides
16. Singleton, V.L, Orthofer, R., Lamuela-Raventos, R.M (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Cioalteau
17. Sofowora, A. (1993): Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria. Pp220-224.
18. Tijjani, M., I. Bello, A. Aluyu, T. Olurishe, S. Maidawa, J. Habila and E. Balogun, 2009. Phytochemical and antibacterial studies of root extract of Cochlospermum tinctorium. Res. J. Med. Plants., 3: 16-22.
19. Trease GE, Evans WC (1989). Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.

20. Wasagu, R.S.U., Hassan, S.W. and Lawal, M. (2005): Evaluation of phytochemical and anti-oxidant levels and antimicrobial screening of aqueous leaf extract of Aloe vera (Aloe barbadensis MILLER). Biological and Environmental Sciences Journal for the Tropics 2(2):21-25.
21. Oyaizu M (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Japan J. Nutr. 44:307–315.
22. Lin En-Shyh, Chou Hung-Ju, Kuo Po-Lin, Huang Yi-Chun (2010): Antioxidant and antiproliferative activities of methanolic extracts of *Perilla frutescens* J. of Med. Plants Res. Vol. 4(6), pp. 477-483.
23. Mudzwiri, M. (2007): Evaluation of traditional South African leafy plants for their safety in human consumption: Thesis (M.Tech.: Biotechnology)-Dept. of Biotechnology, Durban University of Technology, pp.64.
24. Harborne J B (1984): Phytochemical method 2nd edition London. Chapman andHall, Ltd.ELBS page 12—56
25. Sofowora, A. (2006): Medicinal plant and traditional medicine in Africa Spectrum Book Limited Ibadan Nigeria.