COMPARATIVE PROXIMATE, MINERALS COMPOSITION AND ANTINUTRITIONAL FACTORS OF *WALTHERIA INDICA* LEAVE, ROOT AND STEM

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Abstract
Different parts of Waltheria indica plant (leave, root and stem) were assessed for their proximate, mineral composition and antinutritional factors content. The result showed that there is significant difference (P<0.001) when the crude protein (18.68±0.06%), crude fat (2.90±0.02%), ash (7.39±0.02%), carbohydrates (89.99±0.02%) and gross energy (3.46±0.00 Kcal/g) of the leave, root and stem were compared against one another. The leave also recorded the highest quantity of all the minerals analyzed. The result of the mineral analysis showed that there is significant difference (P<0.001) when the P (0.33±0.00%), Fe (196.63±0.07mg/kg), Zn (46.37±0.0882 mg/kg), Cu (4.90±0.12 mg/kg) and Mn (24.93±0.20) content of the leaf, root and stem were compared against one another. The Se (0.07±0.01 mg/kg) content of the root showed significant difference (P<0.05) when compared to the stem. The antinutritional factor composition showed that there is significant difference (P<0.001) in the protease inhibitors (trypsin inhibitors (12.30±0.02 TIU/mg), haemaglutinin (37.81±0.05 HU/mg) and chymotrypsin inhibitors (18.64±0.03 CIU/mg)) when the values for the leave, root and stem were compared against one another. The saponin (0.46±0.00%), tannin (0.03±0.00%), anthraquinones (0.00±0.00%), phenolics (0.13±0.00%), glycosides (0.22±0.00%), phytates (0.18±0.00%) and oxalates (0.12±0.00%) content of the leaf showed significant (P<0.001) difference when compared to the root and stem only. The study concluded that the leave part of Waltheria indica plant is most nutritious and least in antinutritional factors. However, further work is necessary on the bioavailability of these nutrients, for optimum benefit.

Key words: *Waltheria indica*, proximate analysis, minerals, antinutritional factors

Submitted: 08.09.2016 Reviewed: 28.10.2016 Accepted: 16.12.2016

INTRODUCTION

Waltheria indica L. commonly called Sleepy morning belongs to the family Sterculiaceae (Burkill 2000). It is widespread in West Africa (Akobundu and Agyakwa, 1998). In Nigeria the plant is locally known as 'hankufah' or 'hankubah' in Hausa, 'kafafi' in Fulfulde, 'korikodi' in Yoruba and 'efu-abe in Nupe (Irvine, 1961).

It is an erect short-lived herb or shrub, usually 20–150 cm tall, and up to 2 cm in stem diameter (Van Wyk and Malan, 1998). The roots are weak, brown and flexible. This plant usually has a single strong stem emerging from the ground, but frequently branches near the ground (Liogier, 1994).

The uses of the plant are diverse. It is used in Nigeria for the treatment of skin diseases, impotence, as an aphrodisiac, aches, pains, syphilis, anaemia and internal haemorrhage (Mohammed *et al.* 2007, Gbadamosi *et al.* 2012). Many of these folkloric uses have been scientifically validated; for example, the antioxidant effects (Sahidu *et al.* 2012), antibacterial effects (Olajuyigbe *et al.* 2011), antimalarial (Clarkson *et al.* 2004), anti-inflammatory effects (Yerra *et al.* 2005), anticonvulsants effects (Hamidu *et al.* 2008) and trypanocidal effects (Bala *et al.* 2010) of *Waltheria indica* have been reported. Despite the numerous uses of this plant, comprehensive information on the proximate, minerals composition and antinutritional factors of *Waltheria indica* plant is lacking. Hence, this research work compares the proximate, minerals and antinutritional factors of *Waltheria indica* leave, root and stem.

MATERIAL AND METHODS

Collection and Preparation of Plant Materials:

The *Waltheria indica* plants were obtained from a farm land in Moniya in Akinyele area Council of Ibadan, Oyo state, Nigeria and identified at the Herbarium, Department of Botany, University of Ibadan, Ibadan with voucher number **UIH-22371.** The *Waltheria indica* plants were separated into the leave, stem and root and air dried at room temperature under the shade in a room for two weeks. The plant material was sorted to eliminate any dead matter and other unwanted particles and then ground into fine powder using a mechanical blender. The grounded *Waltheria indica* leave, stem and root were then used for analysis.

Determination of Proximate Analysis:

The proximate analysis of the air-dried leaves, root and stem was determined by methods already described in literature (AOAC, 1999). These included the determination of crude protein, crude fat, crude fibre, ash, gross energy, moisture content, dry matter and the nitrogen free extract.

Analyses of the Mineral elements:

The minerals analyzed were Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Phosphorus (P), Copper (Cu), Zinc (Zn), Iron (Fe) and Selenium (Se). The sodium and potassium contents were determined by flamephotometry (Jenway Limited, Donmow Essex, UK) and Phosphorus was Determined by the vanado- molybdate method (AOAC, 1995). Calcium, Magnesium, Iron, Selenium, Copper and Zinc were determined after wet digestion with a mixture of nitric, sulphuric and perchloric acid using atomic absorption spectrophotometer (Buck Scientific, East Norwalk, CT, USA).

Quantification of the Anti-nutritional Factors:

Trypsin inhibitor activity of the samples was done based on the method of Liener (1979).

Hemagglutinin levels were determined according to the method of Jaffe (1979).

Phytates were analysed by the method of Maga (1983). Tannins were according to the method of Dawra *et al* (1988), while saponin levels were done based on the method of Brunner (1984). Total oxalates were quantitated according to the procedure of Fasset (1996). A complete detail of the methodology was reported in Soetan, (2012).

Statistical Analysis

The analyses were done in triplicates. The data obtained were expressed as mean ± standard error of the means (mean ± S.E.M). The data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Bonferroni's Multiple Comparison Test using GraphPad Prism (Version 5.0, San Diego, CA). P values at 5% were regarded as significant.

RESULTS AND DISCUSSION

The proximate analysis of *Waltheria indica* leave, root and stem is presented in Table 1.

Table 1: Proximate Composition of *Waltheria indica* leaf, root and stem

PARAMETERS	LEAF	ROOT	STEM
Crude protein (%)	18.68±0.06[a]	2.88±0. 05	6.41±0.09
Crude fat (%)	2.90±0. 02[a]	0.87±0. 01	1.30±0.02
Crude fibre (%)	15.00±0.02[a]	17.31±0.01	21.13±0.01
Ash (%)	7.39±0.02	3.26±0.01	3.98±0.71
Moisture (%)	8.21±0.02[b]	9.88±0.01	10.01±0.02
Dry matter (%)	91.78±0.02[b]	90.11±0.01	89.98±0.02
Carbohydrates (%)	89.99±0.02[a]	15.00±0.02	57.15±0.64
Gross energy (Kcal/g)	3.46±0.00[a]	1.87±0.00	2.12±0.00
Metabolisable energy (Kcal/g)	2.92±0.00[a]	1.23±0.00	1.47±0.00

Results are shown as mean ± standard error of mean,
[a] Indicates significant differences in mean when the values for the leaf, stem and root were compared against one another at $p<0.001$
[b] Indicates significant differences in mean when the values for the leaf were compared to the stem and root at $p<0.001$

There were significant ($p<0.001$) differences in the composition of crude protein, crude fibre, carbohydrates, gross energy and metabolizable energy of leave, root and stem when compared against each other. While the values for the moisture content and dry matter only showed significant ($p<0.001$) difference when the value for leave was compared to the root and stem. The ash composition of the leave, root and stem did not show any significant difference when compared against each other. The moisture and dry matter composition of *Waltheria indica* plant did not show significant difference when the values for the root were compared to the stem.

The proximate composition of *Waltheria indica* showed that the leave is rich in crude protein, crude fat, Ash and carbohydrates (Table 1). This is in agreement with the finding of Gbadamosi *et al*. (2010). The nutrient rich *Waltheria indica* leaf might be the main factor for the choice of this plant as haematinics among the south western people of Nigeria (Gbadamosi *et al*. 2010). Protein is essential for the synthesis of haemoglobin, the pigment responsible for the oxygen carrying capacity of red blood cell.

Furthermore, the stem has the highest crude fibre content. This might be responsible for the folkloric use of *Waltheria indica* plant in making cords, padding and sandals (Olajuyigbe *et al*. 2011). However, the nutrient laden *Waltheria indica* leaf is seldom considered as food plant. This might be due to the high quantity of its antinutritional factors and toxicity (Zongo *et al*. 2013).

The result of the mineral composition of the *Waltheria indica* leaf, root and stem is presented in Table 2.

Table 2. Mineral Composition of *Waltheria indica* leaf, root and stem

PARAMETERS	LEAF	ROOT	STEM
Na (%)	0.22±0.00[b]	0.09±0.00	0.09±0.00
K (%)	0.83±0.00[b]	0.11±0.00	0.12±0.00
Ca (%)	0.25±0.00[b]	0.11±0.00	0.12±0.00
P (%)	0.33±0.00[a]	0.12±0.00	0.14±0.00
Mg (%)	0.27±0.00[b]	0.14±0.00	0.15±0.00
Fe (mg/kg)	196.63±0.06[a]	89.70±0.12	95.70±0.06
Zn (mg/kg)	46.36±0.08[a]	11.53±0.14	13.53±0.09
Cu (mg/kg)	4.90±0.12[a]	1.93±0.15	3.27±0.09
Mn (mg/kg)	24.93±0.20[a]	9.10±0.12	11.47±0.15
Se (mg/kg)	0.41±0.01[b]	0.07±0.01	0.13±0.01[c]

Results are shown as mean ± standard error of mean,
[a] Indicates significant differences in mean when the values for the leaf, stem and root were compared against each other at $p<0.001$
[b] Indicates significant differences in mean when the values for the leaf were compared to the stem and root only at $p<0.001$
[c] Indicates significant differences in mean when the values for the root were compared to the stem only at $p<0.05$

There are significant differences ($p<0.001$) in P, Fe, Zn, Cu and Mn content of the leaf, root and stem when compared against each other. While the Na, K, Ca, Mg and Se content of the leaf only showed significant ($p<0.001$) difference when compared to the root and stem only. The Se content of the root showed significant ($p<0.05$) when compared to the stem. The Na, K, Ca, P and Mg content of the root did not show significant difference when compared to the stem.

The mineral analysis of the *Waltheria indica* plant showed that the leave is richest in mineral content when compared to the stem and root.

Calcium salts provide rigidity to the skeleton and play a role in many metabolic processes. Many neuromuscular and other cellular functions depend on the maintenance of the calcium concentration in the blood. Calcium also serves as important mediators of hormonal effects on target organs through several intracellular signaling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems (Murray *et al*. 2000).

Iron has several important functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (Malhotra, 1998).

Magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (Al-Ghamdi *et al*. 1994). Magnesium plays a major role in relaxing muscle along the airway to the lungs thus allowing asthma patient to breathe easier. It plays fundamental roles in most reactions involving phosphate transfers and intestinal absorption (Appel, 1999). This might contribute in part to the traditional use of *Waltheria indica* in management of asthma (Zongo *et al*. 2014).

Manganese supports the immune system, regulates blood sugar levels and is involved in cell reproduction. It works with Vitamin K to support blood clotting; working with B complex vitamins manganese help controls the effect of stress (Anhwange *et al*. 2004). This corroborates the folkloric use of *Waltheria indica* plant as restorative medicine after the stress of crop harvesting (Mohammed *et al.* 2007). Zinc is an essential component of many enzymes that are involved in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids that are important for normal body development (Melaku, 2005). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). High amount of potassium in the body was reported to increase Iron utilization (Adeyeye 2002) and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of K through the body fluid (Arinathan *et al*. 2003). Phosphorus serves as constituent of bones, teeth, Adenosine Triphosphate (ATP) and nucleic acids. It also involves in buffering system (Murray *et al.* 2000). Sodium is the main cation in the extracellular fluids. It regulates blood volume, acid-base balance and osmotic pressure of the body fluids (Murray *et al*. 2000). Copper is an essential micro-nutrient necessary for the haematologic and neurologic systems (Tan *et al*. 2006). It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in haemoglobin, assists in the absorption of iron from the gastrointestinal tract (GIT) and in the transfer of iron from tissues to the plasma (Malhotra, 1998). Deficiency of copper has been reported to cause cardiovascular disorders as well as anaemia and disorders of bones and nervous systems. Potassium is the main cation in intracellular fluids and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse and muscle contraction especially smooth muscle. Selenium is the constituent element of the body defense system that protects the living organism against harmful action of free radicals (Soetan *et al*. 2010). The mineral elements such Zn, Cu and Mn have been postulated to have hypoglycemic effect (Al-Awadi *et al*. 2004). This might be responsible for the hypoglycemic effect of *Waltheria indica* root (Povi *et al*. 2015). More so, the presence of Fe and Cu also lend credence to the folkloric use of *Waltheria indica* plant in the management of anaemia (Oladiji *et al*. 2005). Moreover, the presence of mineral elements such as Mn, Zn and Se in *Waltheria indica* plant also supports it use as immune enhancer.

The composition of antinutritional factors in *Waltheria indica* plant is presented in Table 3.

Table 3: Antinutritional factors of *Waltheria indica* leave, root and stem

PARAMETERS	LEAF	ROOT	STEM
Alkaloid (%)	0.68±0.00[c]	0.83±0.00	0.83±0.00
Saponin (%)	0.46±0.00[b]	1.23±0.00	1.23±0.00
Tannin (%)	0.02±0.00[b]	0.04±0.00	0.04±0.00
Flavonoid (%)	0.00±0. 00	0.02±0.02	0.02±0.02
Anthraquinones (%)	0.00±0.00[b]	0.08±0.00	0.08±0.00
Phenolics (%)	0.13±0.00[b]	0.25±0.00	0.25±0.00
Glycosides (%)	0.22±0.00[b]	0.77±0.00	0.77±0.00
Terpenes (%)	0.00±0.00	0.01±0.00	0.01±0.00
Phytate (%)	0.18±0.00[b]	0.11±0.00	0.11±0.00
Oxalate (%)	0.12±0.00[b]	0.08±0.00	0.08±0.00
Steroids (%)	0.01±0. 00[c]	0.02±0.00	0.02±0.00
Trypsin inhibitor (TIU/mg)	12.30±0.02[a]	1.84±0.03	3.47±0.05
Haemagglutinin (HU/mg)	37.81±0.05[a]	9.22±0.03	12.87±0.05
Chymotrypsin inhibitor (CIU/mg)	18.64±0.03[a]	5.75±0.02	7.85±0.02

Results are shown as mean ± standard error of mean,
[a] Indicates significant differences in mean when the values for the leaf, stem and root were compared against each other at $p<0.001$
[b] Indicates significant differences in mean when the values for the leaf were compared to the stem and root only at $p<0.001$
[c] Indicates significant differences in mean when the values for the leaf were compared to the stem and root only at $p<0.05$

The result of the study showed that the leaf has significantly ($p<0.001$) highest content of protease inhibitors (Trypsin inhibitors, haemaglutinin and chymotrypsin inhibitors) when the values for the leaf, root and stem were compared against each other. The saponin, tannin, anthraquinones, phenolics, glycosides, phytates and oxalates content of the leaf showed significant ($p<0.001$) difference when compared to the root and stem only. While the alkaloids and steroids content of the leaf showed significant ($p<0.05$) difference when compared to root and stem. The values of the flavonoids and terpenes for the leave, root and stem did not show any significant difference when compared against each other. While the alkaloids, saponin, tannin, flavonoids, anthraquinnones, phenolics, glycosides, steroids, terpenes, phytates and oxalates content of the root did not show any significant difference when compared to the stem.

Antinutritional factors are secondary metabolites produced by plants as side product of the processes leading to the synthesis of primary metabolites (Habtanu and Negussie, 2014). They have been shown to be biologically active. They exhibit both beneficial and adverse effects.

Alkaloids are synthesized by plant from amino acids. Different alkaloids have been isolated from *Waltheria indica.* For example, anti-trypanosomal quinoline alkaloids have been isolated from *Waltheria indica* root (Cretton *et al*. 2014). Although, some alkaloids has been reported to adversely affect male fertility (Olayemi, 2010). This is corroborated by the findings of Basiru and Olayemi, (2014) that the aqueous extract of *Waltheria indica* leaf adversely affected the sperm parameters of male albino rats. It can also be deduced that this adverse sperm effect will be more in root and stem than the leaf; being of higher alkaloids content (Table III).

Saponin has astringent action and inhibits digestive enzymes. Saponin possesses hypocholesterolaemic, immunostimulatory and anticarcinogenic properties (Sun et al., 2009). The saponin content of *Waltheria indica* might be responsible for it hypocholesterolaemic effect as reported by Bala *et al*. (2011). Also, saponin might also be responsible for the arbotifacient effect of *Waltheria indica* due to its stimulation and release of Luteinizing Hormone (*Francis et al*. 2002).

Tannin binds to proteins through hydrogen binding and hydrophilic interaction and combines with digestive enzymes, thereby making them unavailable for digestion. Tannin has been known to decrease dietary Iron absorption (Felix and Mollo, 2000). Tannin can also inactivate microbial adhesion, enzymes, cell envelope and precipitate microbial proteins (Olajuyigbe *et al*. 2011). The higher content of tannin in the root and stem than the leaf shows that the root and the stem may exhibit better antimicrobial property than the leaf. This is confirmed by the findings of Olajuyigbe *et al*. (2011) who obtained highest antibacterial activity with the root extract, followed by the stem and the least activity by the leaf. Flavonoids are reported to be responsible for the anti-inflammatory, antimicrobial, antiviral and anti-neoplastic properties of many medicinal plants (Ali, 2009). For example, three flavonoids (epicatechin, quercetin, and tiliroside) have been isolated from *Waltheria indica* plant (Yerra *et al*. 2005). These flavonoids dose-dependently inhibit the production of inflammatory mediator NO, cytokines (TNF-a) and interleukin (IL-12) in activated macrophages, without displaying cytotoxicity. This finding corroborates the folkloric use of this plant as anti-inflammatory and or analgesic agent during “sharo” festival among the Fulanis in northern Nigeria (Mohammed *et al*. 2007).

Phenolics compounds and their derivatives act as antiseptic causing denaturation and increasing the permeability of cell membranes in bacteria. The high content of phenolics in the root of *Waltheria indica* plant supported the finding of Olajuyigbe *et al*. (2011) that the root had the highest antibacterial activity against both Gram +ve and Gram –ve bacteria.

Cyanogenic glycosides: many plants produce hydrocyanic acid (HCN) from cyanogenic glycosides when consumed. Their general function in plant is dependent on activation by β-glucosidase to release toxic volatile HCN as well as ketones or aldehydes to fend off herbivores and pathogen (Goldman, 2009). The root and the stem are richer in glycosides than the leaf.

Phytates work in a broad PH region as highly negatively charged ion and therefore, its presence in the diet has negative impact on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+} , Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Khare, 2000). Oxalates interfere with calcium absorption and utilization in animal body (Olomu 1995). The significantly higher content of oxalates and phytates in leaf compare to the root and stem shows that the leaf has more tendency to precipitate mineral deficiency.

CONCLUSION

It is concluded that *Waltheria indica* plant is nutrient and mineral laden. The antinutritional factors contents are equally significant. More so, the leave is richest in nutrient and minerals and contains least antinutritional factors except for protease inhibitors. However, further work should be done on the bioavailability of these nutrient present in *Waltheria indica.*

REFERENCES

[1] Burkill, HM (2000). The useful plants of West Tropical Africa. Royal Botanic Gardens, Kew, UK. 686 p.
[2] Akobundu, IO, Agyaklwa CW (1998). A hand book of West African weeds. 2nd edition, INTEC printers, Ibadan. 1998: 406.
[3] Irvine FR (1961). Woody plants of Ghana, Oxford University Press, London. 1961:185.
[4] Van Wyk B, Malan S (1998). Field guide to the wild flowers of the Highveld. Struik publishers. Capetown, p.178.
[5] Liogier HA (1994). Descriptive flora of Puerto Rico and adjacent Islands. Vol. 3. Editorial de la Universidad de Puerto Rico, Río Piedras, PR. 461 p.
[6] Mohammed Z, Shok M, IIyas N, Musa KY, Yaro AH (2007). Analgesic activity of *Waltheria indica,* European Journal of Scientific Research. 16 (1) 1-6.
[7] Gbadamosi IT, Moody JO, Yekini A O (2012). Nutritional Composition of Ten Ethnobotanicals Used for the Treatment of Anaemia in Southwest Nigeria. *European Journal of Medicinal Plants 2(2): 140-150.*
[8] Sahidu G, Salihu L, Ahmed MU (2012). Antioxidant and anti-microbial activities of ethanol and n-hexane extracts of *Waltheria indica* and *Mucuna pruriens. J. Pharm Sci. Innov.* 1(5): 5-8.
[9] Olajuyigbe OO, Babalola AE, Afolayan AJ (2011). Antibacterial and phytochemical screening of crude ethanolic extracts of *Waltheria indica* Linn. African Journal of Microbiology Research. 5(22): 3760-3764.
[10] Clarkson C, Vinesh JM, Neil RC, Olwen MG, Pamisha P, Motlalepula GM, Niresh B, Peter JS, Peter IF (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of Ethnopharmacology. 92: 177–191.
[11] Yerra KR, Shih-Hua F, Yew-Min T (2005). Inhibitory Effects of the Flavonoids Isolated from *Waltheria indica* on the Production of NO, TNF-a and IL-12 in Activated Macrophages. *Biol. Pharm. Bull.* 28 (5) 912—915.
[12] Hamidu LJ, Ayo JO, Adelaiye AB, Abubakar MS (2008). Sedative and anticonvulsants effects of Ethyl acetate fraction of *Waltheria indica* in Mice. Journal of pharmacology and Toxicology 3(4): 261-266.
[13] AOAC (Association of Official Analytical Chemists) (1999). The Association of Official Analytical Chemists. Official Method of Analysis 4th Edition (129-135), Washington D.C. U.S.A.
[14] AOAC (Association of Official Analytical Chemists) (1995). Official Method of Analysis, 16th Edn, Chapter 4, pp. 17-34, Washington, DC. Journal of the Association of Official Analytical Chemist.
[15] Liener IE (1979). Determination of antitryptic activity of soybean. J. Sci. Agric.Vol 16. pp. 602-609.
[16] Jaffe W (1979). In IE Liener (Ed.): Toxic constituents of plant foodstuffs. Academic Press, Inc. New York. pp. 69.
[17] Maga JA (1983). Phytate: Its chemistry, occurrence, food interaction, nutritional significance and methods of analysis. J Agric Food Chem 30:1-9.
[18] Dawra RK, Makkar HSP, Singh B (1988). Protein binding capacity of microquantities of Tannins. Analytical Biochemistry, 170, pp. 50-53.
[19] Brunner JH (1984). Direct spectrophotometric determination of saponin. Anal.Chem. 42: 1752-1754.
[20] Fasset DW (1996). Oxalates. In: Toxicants occurring naturally in foods.National Academy of Science Research Council, Washington D.C, U.S.A.
[21] Soetan KO (2012). Comparative Evaluation of Phytochemicals in the raw and aqueous crude extracts of three varieties of *Lablab purpureus* seeds. African Journal of Plant Science 6(15): 410-415.
[22] Zongo F, Ribuot C, Boumendjel A, Guissou IJ ((2013). Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*). Journal of Ethnopharmacology. 148:14–26.
[23] Murray RK, Granner DK, Mayes PA, Rodwell VW (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.
[24] Malhotra VK (1998). Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.
[25] Al-Ghamdi SM, Cameron EC, Sutton RA **(**1994). Magnesium deficiency: pathophysiologic and clinical overview. *Am. J. Kidney Dis.*, 24: 737-754.
[26] Appel LJ (1999). Nonpharmacologic therapies that reduce blood pressure. A fresh perspective. Clin. Cardiol. 1111-1115.
[27] Zongo F, Ribuot C, Boumendjel A, Guissou I (2014). Bioguidage search of active compounds from *Waltheria indica* L. (Malvaceae) used for asthma and

inflammation treatment in Burkina Faso. Fundamental and clinical pharmacology. 28, 3: 323-330.
[28] Anhwange BA, Ajibola VO, Oniye SJ (2004). Chemical studies of the seeds of *Moringa oleifera* and *Deuterium micarpum*. Journal of Biol. Sci.4 (6): 711-715.
[29] Melaku U, Clive EW, Habtamon F (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. J. Food Composit. Anal. 18: 803-817.
[30] Shankar AH, Prasad AS (1998). Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr.* 68(suppl.): 447S-463S.
[31] Adeyeye EI (2002). Determination of the chemical composition of the nutritionally valuable parts of male and female common West African fresh water crab (*Sudananoutes africanus*). International Journal of Food Sciences and Nutrition. 3:189-196.
[32] Arinathan V, Mohan VR, Britto AJ (2003).Chemical composition of certain tribal pulses in South India. International Journal of Food Sciences and Nutrition 3: 103-107.
[33] Tan JC, Burns DL, Jones HR (2006). Severe ataxia, myelopathy and peripheral neuropathy due to acquired copper deficiency in a patient with history of gastrectomy. J. Paenteral Nutr. 30: 446-450.
[34] Soetan KO, Olaiya CO, Oyewole O E (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science Vol. 4(5) pp. 200-222.
[35] Al-Awadi FM, Amin JT, Srikumar TS, Mona Al-Rustom (2004). Possible role of trace elements in the hypoglycaemic effect of plant extract in diabetic rats. The journal of Trace elements in Exptl. Medicine. 17, (1) 31-44.
[36] Povi L, Batomayena B, Hodé1 AT, Kwashie1 E, Kodjo1 A, Messanvi G (2015). Phytochemical screening, antioxidant and hypoglycemic activity of *coccoloba uvifera* leaves and *Waltheria indica* roots extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 7(5).
[37] Oladiji AT, Abdullahi SA, Yakubu MT (2005). Evaluation of haematinic potentials of aqueous extract of Waltheria indica (L) root on rats reared on iron sufficient and iron deficient diets feeds. Nigerian Journal of Biochemistry and Molecular Biology, 20(2): 115 – 122.
[38] Habtamu FG, Negussie R (2014). Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects. *International Journal of Nutrition and Food Sciences.* Vol. 3, No. 4, pp. 284-289.
[39] Cretton S, Breant L, Pourrez L, Ambuehl C, Marcourt L, Ebrahimi SN, Hamburger M, Perozzo R, Karimou S, Kaiser M, Cuendet M, Christen PJ (2014). Antitrypanosomal Quinoline Alkaloids from the Roots of *Waltheria Indica.* Nat. Prod. 77, 2304−2311.
[40] Olayemi FO (2010). A review on some causes of male infertility**.** *Afr. J. Biotech*, 9(20): 2834-2842.
[41] Basiru A, Olayemi FO (2014). Effects of aqueous extract of *Waltheria indica* leaves on reproductive indices of male albino rats. *Afr. J. Biotech*. 13(32): 3307-3312.
[42] Sun HX, Xie Y, Ye YP (2009). Advances in saponin-based adjuvants. *Vaccine*. 27(12):1787– 1796.
[43] Francis G, Kerem Z, Makkar HP, Becker K (2002). The biological action of saponins in animal systems: a review. Br. J. Nutr. 88 (6):587–605.
[44] Felix JP, Mello D (2000). Farm Animal Metabolism and Nutrition. United Kingdom: CABI
[45] Ali SYR (2009). Determination of the chemical composition of Senna-siamea (cassia leaves). Pak. J. Nutr., 8: 119-121
[46] Golden M (2009). Nutrient requirements of moderately malnourished populations of children. Food Nutr Bull 30(3).
[47] Khare SK (2000). Application of immobilized enzymes in soybean processing. The Third International Soybean Processing and Utilization Conference (ISPCRC III): 2000 of the Innovation Era for Soybeans. 15-20, October, 2000, Tsukuba, Ibaraka, Japan, pp: 381-382.
[48] Olomu, JM (1995). Monogastric animal nutrition, principles and practice. Jachem publication, pp:320.
[49] Bala AY, Adamu T, Abubakar U, Ladan MJ (2010). Inhibition of *Trypanosomal brucei brucei* by extract from *Waltheria indica* L. Sleeping morning: Research journal of parasitology, ISSN 1816-4943 / DOI:10.3923/jP 2010