ISSN No:-2456-2165

Evaluation of the Phytochemical, Proximate and Elemental Constituents of *Justicia secunda* M. Vahl Leaf

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Abstract:- This study investigated the medicinal and nutritional significance of *Justicia secunda* M. Vahl through the determinations of its phytochemicals, proximate compositions and elements by standard scientific methods, Association of Official Analytical Chemists (AOAC) and atomic absorption spectrometry (AAS) respectively. Phytochemical result (ppm) revealed the presence of terpenoids (14.81 ± 0.04), steroids (2.65 ± 0.02), saponins (2.33 ± 0.30), glycosides (1.58 ± 0.05), tannins (0.55 ± 0.01), flavonoids (0.48 ± 0.01) and absence of alkaloids and phlobatannin. The proximate composition followed the order: carbohydrate > total ash > crude protein > moisture > crude fat > crude fibre. The richness of this plant in phytochemicals, proximate constituents, beneficial elements and the result of heavy metals below AAS machine detectable limit (an indication of the safety of *J. secunda*) might have orchestrated the attractiveness and unbeatable qualities of this plant that endeared it to the traditionalists and its archived ethno-pharmacological relevance. The findings from this study have unsnarled some essential scientific basis justifying its prophylaxis and therapeutic utility in folkloric medicine.

Keywords:- *Phytochemicals, nutrients, ethno-medicine, Justicia secunda***.**

I. INTRODUCTION

Innumerable role of medicinal plants in relieving symptoms and treatment of diseases is indisputable, because a vast percentage of the world population directly or indirectly depends on ethno-medicine for their well being. *Justicia secunda* Vahl (Family: Acanthaceae, Order: Scruphulariales, Sub-class: Asteridae, Class: Dicots) is a medicinal plant that originated from South America[1, 2] and ranked largest in Acanthaceae genus with about 600 species[3]. It is a creeping perennial plant with the ability to grow up to the height of about 1 to 1.5 M. The plant is known as "bloodroot" in Barbados[4] "sanguinaria" in Venezuela, "hounsiman" in Benin, meaning a plant that gives blood and also referred to as "blood leaf" or "blood tonic" in some parts of Nigeria[5]. The plant has become famous majorly in the tropical and sub tropical zones of Madagascar, Asia, the West Indians and Africa[6] but rarely found in the temperate zones[7]. The aesthetic/ornamental value of the plant was held in high esteem and planted for the same until its medicinal potentials rose from obscurity to the limelight. The leaf of *Justicia secunda* is very potent in the treatment of diabetes[8, 9], anaemia, hypertension[2, 10] and gastro-enteritis[11]. The leaf of the plant is purposely consumed with its characteristic red colour decoction for packed cell volume improvement in certain parts of Nigeria, Congo and Cote-d'Ivoire[12]. It has been recognized among the species of Justicia endowed with anti-sickling, anti-inflammatory, immunomodulatory, nephroptotective, antitumoral, hepatoprotective, anti-platelet aggregation, antimicrobial, antiviral, superoxide anion radical scavenging, and hematinic potentials [2,12,13,14,15,16,17]. With the divulged conglomerate of health related benefits of *Justicia secunda* M. Vahl, the evaluation of the phytochemical, proximate and elemental constituents of its leaves therefore becomes pertinent in the justification of its use in fokloric medicine. The study will also keep the teeming multitude of inquirers abreast of beneficial information that were once be-darkened about the plant. The fulfilment of this purpose triggered the designing and implementation of the study.

II. MATERIALS AND METHODS

➢ *Plant collection and preparation*

Fresh leaves of *Justicia secunda* were sourced in November, 2019 from Oshokoti Atanlusi Community, off Aule GRA, Akure, Ondo State, Nigeria (Latitude: $7^0 16'6.708''$ N and Longitude: $5^0 8'54.804''$E). The plant was identified[18] and the leaves, rinsed under a running tap water and properly drained. Well drained leaves were weighed and spread in flat plastic trays under shade to be air-dried. The leaves were pulverized into powder using electric blender and thereafter stored in a sterile plastic container with a firm lid until the commencement of laboratory analyses. The plant is pictorially represented in Figures 1a to d.

➢ *Phytochemical screening*

The presence or absence of saponins, tannins, flavonoids, steroids, glycosides, and phlobatannins were confirmed by standard methods [19] while terpenoids and alkaloids were done as described by [20, 21] respectively.

ISSN No:-2456-2165

➢ *Quantitative Tests*

➢ *Tannins determination*

Tannins determination was carried out using the methods of Folin and Ciocalteu as described[22], saponins[23], flavonoids[24], alkaloids[25], glycosides, terpenoids and steroids according to[26].

➢ *Proximate analysis*

The outlined standard methods[27] were employed in the proximate analyses. Moisture content and total ash were determined by weight difference, crude fat by direct solvent (petroleum ether) extraction using Soxhlet apparatus and crude protein (micro Kjeldahl method) by multiplying the nitrogen fraction by the factor of 6.25[28]. Carbohydrate was calculated by subtracting the percentage crude protein + crude fibre + total ash + moisture + crude fat from one hundred. The method of[29] was adopted to determine crude fibre while metabolizable energy (Kcal/100g) was obtained using food energy-methods of analysis and conversion factors[30].

➢ *Elemental analysis*

Exactly 5 g of the air-dried sample of *J. secunda* was weighed, oven-dried, ashed, digested and filtered[31]. The elements were quantified with the aid of Atomic Absorption Spectrometer (AAS) machine LAMOTTE Smart Spectro 2 RMN 26625 and FP 902 (Nanometer: 510NM) in a reputable Commercial Analytical Laboratory in Akure, Ondo State, Nigeria.

➢ *Statistical analysis*

Collated data from the proximate, mineral and phytochemical determinations were statistically analyzed through compare means of statistical software[32] and the results presented as means ± standard deviation (SD).

Fig 1a:- *Justicia secunda* M. Vahl plant in natural habitat

Fig 1b:- Aerial view of *Justicia secunda* M. Vahl

ISSN No:-2456-2165

Fig 1c:- Flower budding stage of *Justicia secunda* M. Vahl

Fig 1d:- Flowering stage of *Justicia secunda* M. Vahl

III. RESULTS AND DISCUSSION

The results of the phytochemical, proximate and elemental constituents of air-dried leaf of *Justicia secunda* M. Vahl are as shown in Table 1 to 4. Table 1 and Table 2 presented the qualitative and quantitative phytochemicals while Table 3 and 4 showed the proximate and mineral constituents, respectively.

Phytochemicals	Qualitative	Interpretation
Saponins	+	Present
Tannins	+	Present
Glycosides	+	Present
Steroids	+	Present
Terpenoids	+	Present
Alkaloids	--	Absent
Flavonoids	+	Present
Phlobatannin	--	Absent

Where Positive (+) sign = Present and negative (--) = absent

Table 1:- Qualitative phytochemical screening of air-dried leaf of *J. secunda* Vahl

ISSN No:-2456-2165

Phytochemicals	Quantities (mg/g) Mean ± SD
Saponins	2.33 ± 0.30
Tannins	0.55 ± 0.01
Glycosides	1.58 ± 0.05
Steroids	2.65 ± 0.02
Terpenoids	14.81 ± 0.04
Alkaloids	ND
Flavonoids	0.48 ± 0.01
Phlobatannin	ND

Values are means ± standard deviation (SD) of duplicate determinations. ND= Not detected

Table 2:- Quantitative phytochemical screening of air-dried leaf of *J. secunda* Vahl

Parameters	Mean ± SD
Moisture (%)	12.80 ± 0.25
Ash (%)	19.59 ± 0.76
Crude fat (%)	8.10 ± 0.39
Crude protein (%)	18.09 ± 0.18
Crude fibre (%)	0.60 ± 0.14
Dry matter (%)	87.20 ± 0.25
Carbohydrate (%)	40.81 ± 0.94
Energy (Kcal/100g)	308.50 ± 6.36

Values are means ± standard deviation (SD) of duplicate determinations

Table 3:- Proximate composition of air-dried leaf of *J. secunda* Vahl

Minerals	Values (ppm)
Sodium (Na)	24.7
Potassium (K)	58.5
Calcium (Ca)	9.47
Magnesium (Mg)	10.84
Iron (Fe)	0.51
Copper (Cu)	0.26
Manganese (Mn)	0.048
Lead (Pb)	BDL
Zinc (Zn)	0.736
Phosphorus (P)	1.775
Cobalt (Co)	BDL
Cadmium (Cd)	BDL
Selenium (Se)	BDL

Values are means ± standard deviation (SD) of duplicate determinations

Where BDL= below detection limit (< 0.001 ppm) and ppm= part per million.

Table 4:- Elemental constituents of air-dried leaf of *J. secunda* Vahl

The importance of phytochemicals in medicinal plants cannot be over emphasized as they form the ready source from which protective mechanisms are elicited by them against incessant assaults from intruders and infectious agents. The results of the phytochemical screening of air-dried leaf *J. secunda* showed the presence of terpenoids, steroids, saponins, glycosides, tannins, and flavonoids while alkaloids and phlobatannin were not detected (Table 1). The presence of flavonoids, tannins, glycosides and saponins in the present study agrees with earlier reports[33, 34, 35] while the six phytochemicals present compared well with other reports on *J. secunda* leaves as well as *J. carnea* leaves[16, 36]. It was observed that the plant has the highest quantity (mg/g) of phytochemical recorded in terpenoids (14.81 ± 0.04), followed by steroids (2.65 ± 0.02), saponins (2.33 ± 0.30), glycosides (1.58 ± 0.05), tannins (0.55 ± 0.01) and least in flavonoids (0.48 ± 0.01).

The proximate compositions of *J. secunda* presented carbohydrate >total ash >crude protein >moisture >fat >fibre. Carbohydrate functions as a source of energy. It helps in the protection and lubrication of the respiratory, reproductive and alimentary tracts due to the lined mucopolysaccharides and also important as component part of nerves, connective tissues and hormones because of its presentation as glycoprotein. Fats are a source of stored energy and they are the body vehicle positioned as carriers of vitamins A. D, E, and K. Dietary fibre stimulates feed intake in animals like rabbits and horses through increased caecal-colonic motility. Availability of moderate level of fibre in the diets of monogastric animals increases their feed intake[37,38]. The moisture content of *J. secunda* as shown in Table 3 was 12.80 ± 0.25. This can easily support long shelf life of the product by preventing its susceptibility to microbial infection and mold degradation. Protein is an

essential nutrient needed by all living things and the basic structural material from which all body tissues are formed. There was a highly significant difference in the crude protein and total ash percent, respectively (18.09 ± 0.18 and 19.59 ± 0.76) of *Justicia secunda* leaf in this study (Table 3) compared to some highly relished vegetables in Nigeria viz; *Hibiscus asper* leaf (0.26 ± 0.12 and 8.18 ± 0.17), *Telfaria occidentalis* (0.15 ± 0.01 and 7.36 ± 0.08), *Amaranthus hybridus* (1.07 ± 0.06 and 6.00 ± 0.03), *Occimum gratissimum* (2.43 ± 0.04 and 5.64 ± 0.03), *Talinum triangulare* (1.58 ± 0.01 and 12.53 ± 0.17), *Corchorus olitorus* (0.80 ± 0.01 and 5.00 ± 0.01), *Abelmoschus esculentus* (0.40 ± 0.03 and 7.60 ± 1.23), *Alium cepa* (0.83 ± 0.02 and 8.00 ± 0.01), *Basella alba* (0.71 ± 0.01 and 10.00 ±0.01) and *Vernonia amygdalina* (2.27 ± 0.01 and 8.00 ± 0.01) as earlier reported[39]. However, the crude protein content compared favourably with that of *Basella rubra* leaf meal (18.16 ± 1.40) reported[40].

The result of the elemental constituents of *J. secunda* displayed the plant to be a repertoire of beneficial elements viz: sodium (Na), phosphorus (P), zinc (Zn), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), and manganese (Mn). Sodium (Na) plays tremendous role in acid-base balance and osmotic regulation of the body fluids and as such the chief cation of blood plasma and other extracellular body fluids. Its deficiency in the body is characterized by stunted growth, dehydration, low osmotic pressure, reduction in egg production etc. Potassium (K) is an important mineral in carbohydrate metabolism, osmotic regulation, acid-base balance, and in the excitability of the muscle and nerve. Deficiency symptoms of potassium (K) includes: retarded growth in poultry, weakness and sometimes severe paralysis in calves. Calcium (Ca) is actively involved in blood coagulation, bone formation, co-enzyme for nerve impulse transmission and muscle contraction. Its deficiency do manifests in form of rickets, joints enlargement, osteomalacia, low milk yield in dairy animals, poor egg shell formation and reduction in daily egg production percentage in poultry[37, 38]. Magnesium is highly needed for the activation of various enzymes like phosphatases and also important for bone formation and carbohydrates metabolism. Deficiency of magnesium may cause lethargic disposition. Phosphorus (P) is an essential element in the metabolism of carbohydrates, fats and amino acids as well as normal bone development. Its deficiency will lead to weakness of bones, stiff joints and muscular weakness. Manganese is very necessary for the synthesis of mucopolysaccharides while zinc and manganese are important for enzymes activation. Their deficiencies result in stunted growth and possibly sometimes leg deformity[41]. The level of lead, cadmium, zinc and copper in *J. secunda* under study falls within the permissibe safe limits approved[42, 43] for Pb: 10 ppm, Cd: 0.3 ppm, Cr: 2 ppm and Cu: 2 ppm but at variance with those reported for the same plant[18]. In consonance with the above, the consumers of the leaf or product(s) from this plant will not be exposed to the adverse effects of heavy metals like; bleeding, skin rashes, cancer, central nervous system disorder, brain and kidney damage, convulsion, low sperm count, chronic nephritis, bone and pulmonary damages, stomach ache etc[44, 45, 46]. Iron is part of the configuration of haem; the Fe-porphyric nucleus of haemoglobin. Copper is necessary for effective utilization of iron and copper deficiency may cause bone fracture, decreased reproductive performance, over deposition of iron in the liver; the outcome of which is anaemia. Iron is an important mineral in the diets of infants, pregnant, nursing mothers, convalescent patients, elderly and anaemic individuals[47]. Iron enables the brain to work well by bringing oxygen and dopamine to the brain. Tyrosine is converted to dopamine in the presence of iron and dopamine is an essential chemical needed by the brain for thought process[48]. The iron level in *J. Secunda* was 0.51 ppm as shown in Table 4. This obtained value was within the approved recommended safe limit of 20 ppm[43]. The value obtained for iron in the present study was higher than iron content of *Justicia gendarussa* (0.15), *Centella asiatica* (0.21), *Strobilantes crispa* (0.14), and *Murraya koenigii* (0.15) as reported[2]. The value was also higher than that reported for *Hibiscus sabdariffa* leaf (0.42) but slightly lower than *Hibiscus asper* (0.67) leaf[49]. This level of iron content might justify its use as haemanitics. Consumption of the leaves of *J. secunda* will go a long way in solving anaemia related health challenge as already been practised traditionally and among the Jehovah's Witnesses in South-Eastern Nigeria, South Côte-d'Ivoire and Congo[12]. It will also help in meeting the daily iron recommended dietary allowance of 15 mg/day[50].

IV. CONCLUSION

The richness of *Justicia secunda M. Vahl* in phytochemicals, proximate constituents, macro elements (calcium, phosphorus, potassium, sodium and magnesium) and micro elements (iron, copper, manganese and zinc), coupled with the AAS machine below detectable limit results for lead (Pb), cobalt (Co), cadmium (Cd) and selenium (Se); which are heavy metals might have been the positive and attractive qualities of this plant that endeared it to the traditionalists and its archived numerous ethno-pharmacological relevance.

RECOMMENDATIONS

Based on the immenseness of the compositions and usefulness of *Justicia secunda M. Vahl*, it can be recommended as a candidate medicinal plant to be further explored by phyto-pharmaceutical industries for its yet inconspicuous pharmacological grandness. Haemopoietic potential of this plant could also be experimented in animals for the possibility of its adoption and use as haematinics in animal production.

- *Competing Interest*

The author of this publication has no competing interest to be declared.

REFERENCES

[1]. N'guessan, K., Kouassi, K. H. and Quattara, D. Plants used to treat anaemia, in traditional medicine, by Abbey and Krobou populations, in the South of Côte-d'Ivoire. *Journal of Applied Sciences Research*, 2010, **6**(8): 1291 – 2197.

[2]. Kitadi, J. M., Lengbiye, E. M., Gbolo, B. Z., Inkoto, C. L., Muanyishay, C. L., Lufuluabo, G. L., Tshibangu, D. S. T., Tshilanda, D. D., Mbala, B. M., Ngbolua, K. and Mpiana, P. T. *Justicia secunda* Vahl species: Phytochemistry, Pharmacology and future Directions: A mini-review. *Discovery Phytomedicine*, 2019, **6**(4): 157 – 171.

[3]. Wasshansen, D. C. and Wood, J. R. I. *Acanthaceae* of Bolivia. *Contr U S Natl Herb*. 2004, **49**: 1 – 152.

[4]. Carrington, S., Cohall, D. H., Gossell-Williams, M. and Lindo, J. F. The antimicrobial screening of a barbadian medicinal plant with indications for use in the treatment of diabetic wound infections. *West Indian Med. J*. 2012, **62**(9): 861 – 864.

[5]. Onoja, S. O., Ezeja, M. I. Omeh, Y. N. and Onwukwe, B. C. Antioxidant, anti-inflammatory and antinociceptive activities of methanolic extract of Justicia secunda Vahl leaf. *Alexandria J. Med.* 2017, **53**: 207 – 213.

[6]. Heywood, V., Brummitt, R. and Culham, A. Flowering plant families of the World. Firefly Books, 2007. pp 424.

[7]. Mabberley, D. J. The plant-book: a portable dictionary of the vascular plants. Cambridge: Cambridge University Press, 1997.

[8]. Marles, R. J. and Farnsworth, N. R. Antidiabetic plants and their active constituents. *Phytomedicine*, 1995, **2**: 137 – 189.

[9]. Mea, A., Ekissi, Y. H. R., Abo, K. J. C. and Kahou Bi, G. P. Hypoglycaemiant and anti-hyperglycaemiant effect of *Justicia secunda* M. Vahl (Acanthaceae)on glycaemia in the Wistar rat. *International Journal of development Research*. 2017, **7**(6): 13137 – 13184.

[10]. Abo, K. J. C., Kouakou, K. L. and Yapo, A. Hypotensive and antihypertensive effect of total aqueous extract of *Justicia secunda* Vahl (Acanthaceae) inrabbit. *Intern. J. Sci. Res.* 2016, **5**(5): 1455 – 1462.

[11]. Hernando, H., Alfredo, R. and Oscar, C. V. Biological activity of "Sanguinaria" (*Justicia secunda*) extracts, *Pharmaceutical biology*, 2002, **40**(3): 206 – 212.

[12]. Mpiana, P. T., Ngbolua, K. N., Bokota, M. T., Kasonga, T. K., Atibu, E. K., Tshibangu, D. S. T. and Mudogo, V. *In vitro* effects of anthocyanin extracts from *Justicia secunda* Vahl on the solubility of haemoglobin S and membrane stability of sickle erythrocytes. *Blood Transfus.* 2010, **8**: 248 – 254.

[13]. Herrera-Mata, H., Rosas-Romero, A. and Crescente, V. O. Biological activity of "Sanguinaria" (*Justicia secunda*) extracts. *Pharm. Biol*. 2002, **40**(3): 207 – 212.

[14]. Pierre, M., Danho, P. A., Calixte, B., Djédjé, S. D. and Goueh, G. B. J. Evaluation of the antihypertensive activity of total aqueous extract of *Justicia secunda* Vahl (Acanthaceae). *Afr. J. Pharm. Pharmacol*. 2011, **5**(16): 1838 – 1845.

[15]. Corrêa, G. M., Antônio, F. de and Alcântara, C. Chemical constituents and biological activities of species of *Justicia* – a review. *Braz. J. Pharmacognosy* 2012, **22**(1): 220 – 238.

[16]. Osioma, E. and Hamilton-Amachree, A. Comparative study on the phytochemical and *in vitro* antioxidant properties of methanolic leaf extract of *Justicia secunda* Vahl. *Nigerian Journal of Science and Environment* 2017, **15**(1): 111 – 117.

[17]. Anyasor, G. N., Okanlawon, A. A. and Ogunbiyi, B. Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. Clinical Phytoscience 2019, **5**(49): 1 – 31.

[18]. Maffo-Maffo, N. L., Zapfack, L., youmbi, E., Dibong, S. D., Ntsomboh-Ntsefong, G. and Nanfack, A. D. Heavy metal concentrations in some common medicinal plants from different geographical locations in Douala, Cameroon. *Int. J. Curr. Res. Biosci. Plant Biol*. 2016, **3**(12): 10 – 23.

[19]. Trease, G. E. and Evans, W. C. Pharmacolognosy. 13th Edition. ELBS/ Bailliare Tindall, London, 1989. Pp 346- 536, 772 – 773.

[20]. Ejikeme, C. M., Ezeonu, C. S. and Eboatu, A. N. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta area of Nigeria. *European Scientific Journal*, 2014, **10**(18): 247 – 270.

[21]. Jayaramu, M., Prabhavanti, R. M. and Prasad, M. P. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quandrangularis. Advances in Applied Science Research* 2016, **7**(4): 11 – 17.

[22]. Prabhavanti, R. M., Prasad, M. P. and Jayaramu, M. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quandrangularis. Advances in Applied Science Research* 2016, **7**(4): 11 – 17.

[23]. Omoruyi, B. E., Bradley, G. and Afolayan, A. J. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province *BMC Complementary and Alternative Medicine* 2012, Vol. **12** pp 215.

[24]. Boham, B. A. and Kocipai-Abyazan, R. Flavonoids and condensed tannins from leaves of Hawaiian *Vacinium vaticulatum* and *V. calycinium*. *Pacific Sci.* 1994, **48**: 458 – 463.

[25]. Obadoni, B. O. and Ochuko, P. O. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl Sci.* 2001, Vol. **8** pp 203 – 208.

[26]. Sofowora, L. A. Medicinal plants and traditional medicine in Africa. 2nd Edition, Spectrum Books Limited, Ibadan, Nigeria 1993, Pp 1 – 153.
[27]. AOAC. Association of Official Analytical Chemists, Official Methods of Analysis of the AOAC. In: Horwitiz, W. (Ed.) 2006, 18th Edition. Association of Official Analytical Chemists, Washington D.C., USA.
[28]. Muhammed, A., Javid, H., Muhammed, T. S., Zabta, K. S., Farman, U., Ali., B., Naeem, K., Abdullatif, K. and Takashi, W. Proximate and nutrient composition of medicinal plants of humid and sub-humid region in North-west Pakistan. *J. Med. Plants Res*. 2010, **4**(4): 339 – 345.
[29]. Pearson, J. Determination of phytic acid and phosphorus content of biological materials. Cambridge University Press, London 1976.
[30]. FAO. Food energy-methods of analysis and conversion factors. Food and Nutrition paper 77, *Food and Agriculture Organization of the United Nations, Rome, Italy* 2003, pp 1-93.
[31]. Ali, R., Hussain, U., Rizwan, U. K. and Ijaz, A. Population based study of metals in *Capparis deciduas*, *International Journal of Pharmacy and Pharmaceutical Sciences* 2013, **4**: 108 – 113.
[32]. SPSS. Statistical Package for Social Sciences, SPSS 23 Statistics Premium for Windows. IBM Corporation computer software package 2015, version 23, 32 and 64 bit, Jordan.
[33]. Adams, M., Gmunder, F. and Hamburger, M. Plants traditionally used in age related brain disorders – a survey of ethnobotanical literature. *J. Ethnopharmacol* 2007, **113**: 362 – 381.
[34]. Agra, F. M., Freitas, P. F. and Barbosa-Filho, J. M. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev. Bras. Farmacogn* 2007, **17**: 114 – 140.
[35]. Bedoya, L. M., Alvarez, A., Bermejo, M., Gonzalez, N., Beltran, M., Sanchez-Palomino, S., Cruz, S. M., Gaitan, I., Del, O. E., Escarcena, R., Garcia, P. A., Cáceres, A., Feliciano, A. S. and Alcamí, J. G. Plant Extracts as virucides against HIV-1 infection. *Phytomedicine* 2008, **15**: 520 – 524.
[36]. Onyeabo, C., Achi, N. K., Ekeleme-Egedigwe, C. A., Ebere, C. U. and Okoro, C. K. Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Sci. Pol. Technol. Aliment* 2017, **16**(2): 217 – 230.
[37]. McDonald, P., Edwards, R. A. and Greehalgh, J. F. D. Animal Nutrition. Fifth Edition. Longman Publi 1995, pp 221 – 235.
[38]. Alu, S. E. Primer of Animal Nutrition and Biochemistry. First Edition, Onaivi Printing and Publishing Co. Ltd, Keffi, Nasarawa State, Nigeria 2015, Pp 31- 125.
[39]. Omale, J. and Ugwu, C. E. Comparative studies on the protein and mineral composition of some selected Nigerian vegetables. *African Journal of Food Science* 2011, **5**(1): 22 – 25.
[40]. Alikwe, P. C. N. Proximate, mineral and phytochemical compositions of Basella rubra leaf meal. *International Journal of Tropical Agriculture and Food System* 2012, **6**(1): 34 – 39.
[41]. Rajesh, C. and Rao, V. D. P., Gomez-Villamandos, J. C., Shukla, S. K. and Banerjee, P. S. Diseases of Poultry and Their Control. International Book Distributing Company, Lucknow, U. P., India 2001, Pp 31 – 40.
[42]. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organization 1989., Geneva, Switzerland.
[43]. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva, Switzerland. In: Comparative study of heavy metals in soli and selected medicinal plants by Afzal shah *et al*., 2005. Article ID 621265.
[44]. Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V. and Brandenburg, P. The toxicity of cadmium and resulting hazards for human health. *J. Occp. Med. Toxicol*. 2006, **22**: 1 – 6.
[45]. Khan, S., Rehman, S., Zeb, K. A., Amjad, K. M. and Tahir, S. M. Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan. *Ecotoxicol. Environ. Saf.* 2010, **73**(7): 1820 – 1827.
[46]. Rehman, H., Ullah Khan, R. U. and Ahmad, I. Population based study of heavy metals in medicinal plant. *Capparis deciduas*. *Int. J. Pharm. Pharmaceut. Sci*. 2013, **5**(1): 108 – 113.
[47]. D'Mello, J. P. F. *Food Safety, Contamination and Toxins*, CABI publishing, Wallington, Oxon 2003, UK, Cambridge, MA, Pp 480.
[48]. Getty, T. A. The Importance of Good Nutrition, Herbs and Phytochemicals for Your Health, Good Looks and Longevity. Falcon Press International 1997. P. O. Box 4113 Mountain View, CA 94040. ISBN 1-884459-02-1. Pp 176 – 181.
[49]. Arogbodo, J. O. and Ajayi, O. A. Comparative study of the phytochemical, proximate and mineral constituents of air-dried leaves of *Hibiscus asper* and *Hibiscus sabdariffa.* 45th Annual Conference of Nigerian Society for Animal Production Book of Proceedings, Bauchi, 2020, Pp 398 – 402.
[50]. FAO/WHO. Report of a joint FAO/WHO Expert Consultation Bankok, Thailand. Expert consultation on human vitamin and mineral requirements 2002, Pp 223 – 229.