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Research Article
Phytochemical Studies, Proximate and Mineral Composition of Ethanol Leaf Extract of *Duranta repens* Linn (Verbenaceae)

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Abstract

Background and Objective: *Duranta repens* Linn (Verbenaceae) is a flowering shrub popularly used as ornamental plant to decorate environment and surroundings; it is an exotic species of tropical American descent. Previous studies indicated antimalarial, antiviral and cytotoxic activities. This study was carried out to determine the toxicity level (LD_{50}), phytoconstituents, proximate composition and mineral element contents of the leaves with a view to assess its nutritional potentials in relation to its ethnomedicinal uses and alleviating the problem of malnutrition. **Methodology:** The dry plant material was macerated in 70% aqueous ethanol for 72 h and the dry extract subjected to lethal dose (LD_{50}) test, phytochemical screening, proximate analysis and mineral elements determination using standard protocols. **Results:** In acute toxicity test, LD_{50} of 1224.74 mg kg^{-1} was obtained, while the phytochemical screening indicated the presence of flavonoids, saponins, tannins, polyphenols, terpenes and alkaloids in high concentrations. The proximate analysis indicated high content of carbohydrate (60.48±1.05%), crude protein (14.40±0.75%), crude fibre (13.63±1.37%) and ash (11.49±0.01%) while lipid was low (0.78±0.04%). The minerals assay recorded the following: Sodium (5.774±0.017 mg/100g), zinc (4.606±0.06 mg/100g), potassium (3.259±0.011 mg/100g), iron (1.430±0.069 mg/100g), manganese (1.073±0.105 mg/100g), copper (0.166±0.001 mg/100g), calcium (0.128±0.006 mg/100g) and magnesium (0.045±0.009 mg/100g). The chemical constituents, mineral elements and food contents might have synergized to provide medicinal uses of the plant. **Conclusion:** The leaf plant material is not poisonous and that it contains beneficial nutrients and mineral elements comparable to other leafy vegetables. Thus, the consumption of these plant materials as observed among local people here does not constitute any health hazard but can help to combat malnutrition.

Key words: *Duranta repens*, durantanin, proximate analysis, phytoconstituents, cytotoxic effect, malnutrition, chemical nutrients

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the World Health Organization (WHO), malnutrition is the gravest single threat to global public health and is estimated to contribute to more than one third of the cause of children's deaths all over the world[1]. Malnutrition which can be defined as the insufficient, excessive or imbalanced consumption of nutrients is caused by lack of access to highly nutritious foods, especially in the present context of rising food prices[2]. In Nigeria, not less than 10 million children or 13% of the population of children are at the risk of becoming mentally deformed in adulthood because of nutrient deprivation, particularly, lack of essential minerals in their diet[3]. Poor feeding practices, such as offering the wrong foods and not ensuring that one gets enough nutritious food, contribute to malnutrition as well. If a person does not eat enough food, or if what he eats does not provide him with the nutrients he requires for good health, he suffers from malnutrition. Poor diet may be caused by inability to consume enough of the right nutrients due to complete lack of knowledge of where to obtain them. Good nutrition is critical to overall health and well-being and it is effectively practiced where precise knowledge of the sources and proximate amount of essential nutrients are available[4]. Previous studies have revealed that wild or semi-wild plants are nutritionally significant because of high vitamins, minerals, proteins, essential fatty acids and fibre contents[5-9]. It is our belief that if plant resources which are abundantly available in environment are well harnessed through careful study of their essential food contents and mineral nutrients, the problem of malnutrition would be greatly reduced. In this regard more plant resources have to be investigated for their proximate and mineral element contents especially virgin ones like *Duranta repens* Linn. *Duranta repens* (also referred to as *Duranta erecta*) belongs to the family of Verbenaceae and is commonly known as sky flower. It is an exotic species of flowering shrubs of tropical American descent, characterized by its remarkable adaptation. It can grow in tropical, arid and semi-arid climates with a geographical distribution comprising Nigeria, Latin America, India and Australia[10]. It is popularly used as an ornamental plant to decorate environments and surroundings and usually cultivated in gardens and as hedges. It is used locally as medicinal herb for a variety of ailments and as item of trade. The fruits and leaves are used as vermifuge, diuretic and in treatment of malaria, intestinal worms and abscesses[11]. Previous study done on the plant reported isolation of triterpenes, namely-durantanin I, II, III, IV, V and oleanolic acid; flavonoids namely acacetin, diosmetin, apigenin, luteolin and quercetin from the leaves[12,13]. In spite of its medicinal value, the safe use of this plant has been enmeshed in controversies since a variety of the plant in Australia was reported to be poisonous to pigs; even children who ate the fruits were reported to become ill and subsequently died[10].

The main purpose of this investigation was to determine the proximate and mineral compositions, as well as phytochemical constituents of the plant. This was with the view to provide a documented data of the type and quantity of the chemical nutrients present in Nigerian grown *Duranta repens* and discuss their possible health benefits as part of the solution to the problem of malnutrition. Moreover, the acute toxicity (LD_{50}) of the plant was determined in order to confirm the claims of toxicity or otherwise of the plant. As far as this search was concerned, there has been no report of evaluation of the food and mineral elements composition of this plant.

MATERIALS AND METHODS

Ethanol (95%), perchloric acid, nitric acid, sulphuric acid were obtained from Sigma-Aldrich, (USA). Atomic Absorption Spectrophotometer (Solar 969AA) employing ASTM[14] and APHA[15] methods were used to determine the mineral element contents of the dried leaf of the plant. The study was conducted in the Research Laboratory of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo and in the Instrumentation Laboratory of Akwa Ibom State Ministry of Science and Technology, Uyo, Nigeria, between March, 2014 and December, 2016.

Collection of plant materials: The leaves of *Duranta repens* Linn were collected from a garden in Ibiono Ibom, Akwa Ibom State, Nigeria in March, 2014. The plant was identified by Dr. (Mrs) M E Bassey of the Department of Botany, University of Uyo, Nigeria. The leaves were dried in the shade, ground to fine powder and weighed. All chemicals and reagents used in this research work were of analytical grade obtained from Sigma-Aldrich (USA).

Extraction of plant material: The powdered plant material (500 g) was subjected to maceration at room temperature in an extracting jar using 70% aqueous ethanol for 72 h. The crude liquid extract was separated from the marc by filtration using Whatman filter paper No 2. The filtrate was then concentrated to dryness on water bath at 40°C; weighed and stored in an air tight desiccator prior to tests.

Phytochemical screening: The dry extract was subjected to phytochemical screening using standard protocol[16,17].

Proximate analysis: Moisture, ash, crude protein, crude lipid, dietary fibre and available carbohydrate (by difference) analyses were done using the methods described by AOAC[18]. Energy content was estimated in kcal/100g by the Atwater general factors system-multiplying the percentages of available carbohydrate, crude protein and crude lipid by 4, 4 and 9, respectively[19].

Mineral elements analysis: Analysis of mineral elements was carried out after digestion of 1 g of the dried sample with 12 cm^3 mixture of nitric/perchloric/sulphuric acids in the ratio of 9:2:1, respectively[5]. Sodium (Na), zinc (Zn), potassium (K), iron (Fe), manganese (Mn), copper (Cu), calcium (Ca) magnesium (Mg), lead (Pb), arsenic (As), nickel (Ni) and cadmium (Cd) were analyzed using Atomic Absorption Spectrophotometer with the appropriate lambs.

Acute toxicity and lethality tests: Lorke's method was used to test the acute toxicity of the ethanol extract[20]. Three groups of 3 mice each were given 10, 100 and 1000 mg kg^{-1} of the extract intravenously. The mice were observed for 24 h for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another three groups of 3 mice each were administered 1500, 2500 and 5000 mg kg^{-1} of the extract intravenously. The animals were observed for 48 h for effects of toxicity and the number dying in each group within the period was recorded.

Statistical analysis: For all statistical analyses, MS-excel software (2010) was used. The analyses were performed in triplicate.

RESULTS

Acute toxicity testing: There was no mortality in mice after intravenous administration of the extract at doses as high as 1000 mg kg^{-1}. The LD_{50} was calculated to be 1224.74 mg kg^{-1}.

Proximate composition: The moisture, ash, crude protein, crude lipid, dietary fibre, carbohydrate and the calorific contents of the plant obtained during the proximate analysis are presented in Table 1. The results showed that the leaves contained relatively high percentage of moisture and carbohydrate than other proximate contents.

Table 1: Proximate composition of leaves of *Duranta repens* Linn

Parameters	Proximate composition (%)
Moisture	64.40±2.14
Ash	11.49±0.01
Fibre	13.63±1.37
Protein	14.40±0.75
Lipid	0.78±0.04
Carbohydrate	60.48±1.05
Calorific value (kcal/100g)	303.35±2.06

Values expressed as Mean±SEM, n = 3

Table 2: Mineral element contents of leaves of *Duranta repens* Linn

Parameters	Value (mg/100g)
Zinc	4.606±0.060
Copper	0.166±0.001
Lead	<0.001
Magnesium	0.044±0.003
Iron	1.430±0.069
Nickel	<0.001
Cadmium	0.485±0.002
Arsenic	<0.001
Sodium	5.774±0.017
Potassium	3.259±0.011
Calcium	0.128±0.006
Manganese	1.073±0.001

Value expressed as Mean±SEM, n = 3

Mineral elements composition of the leaves of *Duranta repens* Linn: The result of the analysis of the mineral elements content of leaves of *Duranta repens* obtained in this research work is presented in Table 2. From the result it was apparent that zinc, sodium and magnesium were the most abundant elements in the leaves while lead, arsenic and nickel were recorded in trace amounts.

Phytochemical composition of the leaves of *Duranta repens*: The phytochemical screening detected the presence of alkaloids, flavonoids, saponins and tannins among other vital phytochemicals while phlobatannins were not detected (Table 3).

DISCUSSION

The proximate analysis of *Duranta repens* leaves (Table 2) indicated the presence of carbohydrate, crude protein, ash, crude fibre, lipid and moisture. These proximate contents have important contributions in the maintenance of human health. Food and Agriculture Organization (FAO)/World Health Organization (WHO) have recommended daily allowance of each nutrient for different ages of human population[5]. The chemical composition of a food is of utmost importance from many stand points, including nutrition, health, toxicology and safety; stability to

Table 3: Results of phytochemical screening of the leaves of *Duranta repens* Linn

Phytochemical constituents	Test	Observation	Inference
Alkaloids	Dragendorff's	Orange-red ppt	+
Anthraquinones	Borntrager's	Pink to violet colour change	+
Cardenolides	Keller-Kellian's	Reddish-brown ring	+
Flavonoids	Shinoda's	Pink ppt	+
Phlobatannins	HCl	No colour change	-
Polyphenols	$FeCl_3$	Deep-blue ppt	+
Reducing sugar	Fehling's	Brick red ppt	+
Saponins,	Frothing	Foam formation	+
Steroidal glycosides	Salkawski's	Reddish-brown ring	+
Tannins	Bromine water	Decolourization	+
Terpenes	Liebermann-Burchard's	Violet colouration	+

+: Present -: Absent; ppt: Precipitate

microbiological, chemical or physical changes. Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials[6]. The dietary fibre can lower serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer[7]. The amount of crude lipid in the plant was lower than the 11% in water spinach leaves, 12% in *Senna obtusifolia* but higher when compared to spinach leaves (0.3%), chaya leaves (0.4%) and 1.6% in *Amaranthus hybridus*[8]. Crude lipids are the principal sources of energy which should not be more than 30 calories to guard against obesity and other related diseases. A diet providing 1-2% of its caloric energy as fat is said to be sufficient in human beings as excess consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging[9]. The ash content of 11.49% indicated that the plant has sufficient mineral elements. This value was higher compared to 1.8% reported for sweet potatoes leaves and 5% in *Tribulus terrestris* leaves but lower than some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20%)[8,21,22]. The moisture content is higher than that of *Acalypha hispida* (11.02%), *Acalypha racemosa* (11.91%), *Acalypha marginata*, (10.83%)[23]. The carbohydrate content of the leaves was high compared to some other leafy vegetables like *Tribulus terrestris* (Tsaida) (55.67%) but lower than *Corchorus tridens* (88.50%)[21]. Carbohydrate provides energy for the body; the body may enter into a hyper-metabolic phase when there is an increased demand for carbohydrate. This cellular activity is followed by adenosine triphosphate (ATP) which is driven by glucose. In the case of insufficient carbohydrate, the body breaks down protein to provide glucose for cellular activity[6]. Therefore, in order to correct hypoalbuminemia, carbohydrate is required as well as protein. The result of the mineral analysis (Table 2) revealed that the leaves of *Duranta repens* contain calcium which is good for growth and maintenance of bones and muscles[24,25]. Potassium, a very important mineral for the proper functioning of all cells, tissues and organs of the body was found to be lower in the plant compared to 14.55 mg/100g found in *Indigofera astragalina* leaves[26]. Potassium known to be an electrolyte, (a substance that conducts electricity in the body) is required for vital life processes in the body[27]. Potassium also helps in preventing muscle contraction and in the sending of all nerve impulses in animals through action potential. Epidemiological studies and studies in animals subject to hypertension indicate that diet high in potassium can reduce the risk of hypertension and possible stroke. The guidelines of the institute of medicine specify that the minimum physiological requirement of potassium should be 4,000 mg/day[27]; thus confirmation of the presence of potassium in the plant indicated that a new source of this mineral has been found. Sodium was another micronutrient present in the plant. Sodium is an essential nutrient that regulates blood volume, blood pressure, osmotic equilibrium and pH. Sodium is also important in the neuron function and osmo-regulation between cells and the extra-cellular fluid; its distribution is mediated in all animals by Na/K ATPase[28]. The minimum recommended requirement for sodium was 500 mg/day; hence the plant would be contributing substantially to the daily requirement of this very important element in human body[25]. Magnesium is an important mineral element in connection with circulatory disease such as ischemic heart disease and calcium metabolism in bones[7,29]. The detection of magnesium in the leaves showed a contribution from this source to the magnesium requirements of human body. Copper is a very powerful pro-oxidant; it catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid. The presence of copper in the plant indicated enormous contribution of this mineral to its human body requirements. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates, protein and fats[30]. The iron content of *Duranta repens* was found to be higher than some cultivated vegetables such as

lettuce (0.7 mg/100g) and cabbage (0.3 mg/100g) though slightly lower than (2.8 mg/100g) in *T. territis*[25]. The high concentration of iron in *Duranta repens* indicated that the plant is a good source that can replenish this nutrient in blood in anaemic conditions. Zinc is an essential element in nutrition of man where it functions as an integral part of numerous enzymes or as a stabilizer of molecular structure of sub-cellular constituents and membrane. The concentration level of zinc observed in *Duranta repens* in this study, has proven the plant as a good source of this nutrient. Zinc participates in the synthesis and degradation of carbohydrates, lipids, protein and nucleic acids and has been shown to play an essential role in polynucleotide transcription and translation and hence in the process of genetic expression[19,31,32]. The manganese, another essential microelement in human nutrition (acting as an activator for many enzymes) was also found to be part of mineral elements in *Duranta repens* in this study[33]. The concentrations of toxic elements like lead and arsenic in the plant were negligible. These cannot constitute any health hazard in consumers being lower than the maximum permissible limit of 3 mg/100g for vegetables and thus fall within safe limits[27,34]. This is contrary to some reports that the plant is poisonous[10]. The presence of secondary metabolites like saponins, tannins and flavonoids, (Table 3) might have contributed to the rich medicinal value as well as physiological activities of the plant[16]. Various studies have shown that saponins can generate some physiological responses in animals that consume them. Saponins show cytoxic effect and growth inhibitions against a variety of cells, making them exhibit anti-inflammatory and anticancer properties[22]. They also precipitate/coagulate red blood cells and show cholesterol binding actions[35]. Tannins have astringent properties that can hasten the healing of wounds and inflamed mucous membrane; flavonoids are popular as super oxidant that provides protection against oxidative cell damage, allergies, viruses, ulcers and inflammations[36]. Acute toxicity studies indicated the LD_{50} of the crude leaf extract as 1224.74 mg kg^{-1}. This is lower and safe for human consumption contrary to some reports that the plant is poisonous.

CONCLUSION

These research findings revealed that the plant contained high levels of phytoconstituents, mineral elements and proximate compositions which may provide scientific basis for the local use of the plant to treat fever, wounds, stomach ache and other ailments. It has also established that the consumption of the plant, its extract or decoction may not pose health hazards as the plant is shown to be non-toxic.

SIGNIFICANCE STATEMENTS

This study discovers that *Duranta repens* is rich in essential nutrients and mineral elements necessary for the maintenance of good health and proper functioning of internal body organs for optimum growth and reproduction which can contribute to alleviate the burden of malnutrition.This study will also help researchers to have documented data of the type and quantity of chemical nutrients and mineral elements present in Nigeria grown *Duranta repens* which was hitherto unavailable.

REFERENCES

1. WHO., 2013. Children: Reducing mortality. World Health Organization, Geneva.
2. Nordqvist, C., 2016. Malnutrition: Causes, symptoms and treatments. Medical News Today. https://www.medicalnewstoday.com/articles/179316.php
3. Olawale, G., 2017. Malnutrition: 10 m Nigerian children risk mental deformity in adulthood. Vanguard, Newspaper, Lagos, Nigeria. https://www.vanguardngr.com/2017/08/malnutrition-10m-nigerian-children-risk-mental-deformity-adulthood/
4. Josserand, H., K. Gunjal, A. Gurkan, R. Sibrian and A. Marx *et al*., 2008. The State of Food Insecurity in the World. Food and Agriculture Organization (FAO.), Rome, Italy.
5. Oko, A.O., J.C. Ekigbo, J.N. Idenyi and L.U. Ehihia, 2012. Nutritional and phytochemical compositions of the leaves of Mucuna Poggei. J. Biol. Life Sci., 3: 232-242.
6. Akubugwo, I.E., N.A. Obasi, G.C. Chinyere and A.E. Ugbogu, 2007. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. Afr. J. Biotechnol., 6: 2833-2839.
7. Atoyebi, J.O., O. Osilesi, O. Adebawo and M. Abberton, 2017. Evaluation of nutrient parameters of selected African accessions of Bambara groundnut (*Vigna subterranean* (L.) Verdc.). Am. J. Food Nutr., 5: 83-89.
8. Nwaogu, L.A., C.O. Ujowundu and A.I. Mgbemena, 2006. Studies on the nutritional and phytochemical composition of *Amaranthus hybridus* leaves. Bio-Research, 4: 28-31.
9. Antia, B.S., E.J. Akpan, P.A. Okon and I.U. Umoren, 2006. Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. Pak. J. Nutr., 5: 166-168.
10. Thomson, N., 2007. Poisonous and Invasive Plants in Australia: Enabling Consumers to Buy Safe Plants. WWF-Australia, Australia, pp: 1-15.
11. Khanal, D.P., B. Raut, R.K. Majhi, A. Kaini, R. Basnet and P.K. Mahat, 2014. Phytochemical and biological studies on fruits of *Duranta repens* L. Int. J. Adv. Pharm. Sci., 5: 1958-1962.

12. Ahmed, W.S., M.A. Mohamed, R.A. El-Dib and M.M. Hamed, 2009. New triterpene saponins from *Duranta repens* Linn. and their cytotoxic activity. Molecules, 14: 1952-1965.
13. Nikkon, F., K.A. Salam, T. Yeasmin, A. Mosaddik, P. Khondkar and M.E. Haque, 2010. Mosquitocidal triterpenes from the stem of *Duranta repens*. Pharm. Biol., 48: 264-268.
14. ASTM E1690-08, 2014. Standard test method for determination of ethanol extractives in biomass. American Society for Testing and Materials International, West Conshhocken, PA., USA.
15. APHA., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC., USA., ISBN-13: 9780875532356, Pages: 1220.
16. Sofowora, E.A., 1996. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp: 55-71.
17. Trease, G.E. and W.C. Evans, 2009. Trease and Evans Pharmacognosy. 15th Edn., Bailliere, Tindall, London, pp: 135-149, 430.
18. AOAC., 2007. Association of Official Analytical Chemists Official Methods of Analysis. 18th Edn., AOAC Benjamin Frankin Station, Washington, DC., USA.
19. Hassan, L.G., S.W. Hassan, T. Hashim, K.J. Umar and N.A. Sani, 2011. Determination of nutritive values of garden cress (*Lepidium sativum* L.) leaves. Bayero J. Pure Applied Sci., 4: 18-23.
20. Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.
21. Adinortey, M.B., J.K. Sarfo, E.T. Quayson, A. Weremfo, C.A. Adinortey, W. Ekloh and J. Ocran, 2012. Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. Res. J. Med. Plant, 6: 171-179.
22. Akindahunsi, A.A. and S.O. Salawu, 2005. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. Afr. J. Biotechnol., 4: 497-501.
23. Iniaghe, O.M., S.O. Malomo and J.O. Adebayo, 2009. Proximate composition and phytochemical constituents of leaves of some *Acalypha* species. Pak. J. Nutr., 8: 256-258.
24. Nielsen, S.S., 2010. Food Analysis Laboratory Manual: Food Science Texts Series. Springer Science+Business Media, Germany, pp: 3-177.
25. Turan, M., S. Kordali, H. Zengin, A. Dursun and Y. Sezen, 2003. Macro and micro mineral content of some wild edible leaves consumed in Eastern Anatolia. Acta Agriculturae Scandinavica Sect. B: Plant Soil Sci., 53: 129-137.
26. Gafar, M.K., A.U. Itodo, F.A. Atiku, A.M. Hassan and I.J. Peni, 2011. Proximate and mineral composition of the leaves of Hairy Indigo (*Indigofera astragalina*). Pak. J. Nutr., 10: 168-175.
27. FAO. and WHO., 2011. Codex alimentarius commission food additives and contaminants: Joint FAO/WHO food standards programme, CF/5 INF/1. Food and Agriculture Organization of United Nations, World Health Organization, Geneva, Switzerland, pp: 1-89.
28. Campbell, G.L., F.W. Sosulski, H.L. Classen and G.M. Ballance, 1987. Nutritive value of irradiated and β-glucanase-treated wild oat groats (*Avena fatua* L.) for broiler chickens. Anim. Feed Sci. Technol., 16: 243-252.
29. Hassan, L.G. and K.J. Umar, 2006. Nutritional value of balsam apple (*Momordica balsamina* L.) leaves. Pak. J. Nutr., 5: 522-529.
30. Adeyeye, E.I. and M.K.O. Otokiti, 1999. Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum* L. (Bell and Cherry peppers). Discov. Innov., 11: 75-81.
31. Hunt, J.R., J.M. Beiseigel and L.K. Johnson, 2008. Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability. Am. J. Clin. Nutr., 87: 1336-1345.
32. Heyneman, C.A., 1996. Zinc deficiency and taste disorders. Ann. Pharmacother., 30: 186-187.
33. Auta, R. and I. Ali, 2011. Nutritional and chemical evaluation of *Solanumin canum* (Bitter garden egg). Int. J. Trop. Med. Public Health Suppl., 1: 96-107.
34. Codex Alimentarius Commission, 2012. List of maximum for contaminants and toxins in food part 1. Food and Agriculture Organization of United Nations, USA.
35. Sodipo, O.A., J.A. Akiniyi and J.U. Ogunbamosu, 2000. Studies on certain characteristics of extracts of bark of *Pausinystalia johimbe* and *Pausinystalia macroceras* (K Schum) Pierre ex Beille. Global J. Pure Applied Sci., 6: 83-87.
36. Harborne, J.B., 1984. Phytochemical Methods. Chapman and Hall, London, pp: 113.