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Proximate and Mineral composition of leaves of *Azadirachta indica*

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Abstract

Herbal consumption has been on the increase in recent time, growing concern in its usage with regards to safety in terms of its toxicity cannot be overemphasized. The leaves of Neem (*Azadirachta indica*) commonly known in Nigeria as ''Dogonyaro'' were collected from three major locations in Otukpo Local Government Areas of Benue State, Nigeria. The sites were designated the depicting code of O_{Mr} (Market Road),O_{Ar} (Adoka road) and O_{Ur} (Upu Road). The experiment was carried out in the Department of chemistry Benue State University Makurdi.Reflux extraction was carried out using 70% methanol as extraction solvent standard analytical methods were used to determine the phytochemical content, while their proximate compositions were determined by methods described by A.O.C.A and their heavy metal contamination were determined using Atomic Absorption spectrophotometer (AAS). The results showed that the proximate composition of Neem leaves ranged from (12.10 to 14.30) %, (3.88 to 4.03) %, (1.22 to 4.04) %, (2.89 to 3.18) % and (9.25 to 10.86) % for Moisture, Ash, Protein, Fat and Fibre respectively. The concentrations of analyzed heavy metals along all the collection locations, ranged from (0.09 to 0.12) mg/100g Zn; (0.07 to 0.13) mg/100g Fe; (0.07 to 0.12) mg/100g Mn; (0.12 to 0.42) mg/100g Mg; 0.02 mg/100g Pb for O_{MR}, with O_{MR} recording the highest value across all the collection locations, while Pb for collection cite O_{AR} and O_{UR} were below detectable limit. The phytochemical composition reveals the ranes of values; (0.53 to 1.12) %, (0.92 to 1.94) %, (4.53 to 6.23) %, (1.85 to 3.96) % and (0.18 to 1.21) % for Tannin, Saponin, Flavonoid, Alkaloid and Hydrogen Cyanide respectively. The result from this study reveals that the plant can be used for medicinal purposes as its heavy metal composition falls within acceptable level recommended by WHO, hence will pose no harmful effect to human health when administered.

Keywords: *Metals, Minerals, Composition, Collection site.*

1. Introduction

Drugs synthesized from medicinal plants are used worldwide for the treatment of several diseases and also act as important sources of raw material for pharmaceutical industries (Martins, 2013). In recent time, the use of herbal medicinesmostly in rural areas has increased worldwide, due to limited modern facilities, hence making the health care system completely dependent on such plants/herbs (Trease and Evans, 1996). This therapeutic plants has application in pharmaceutical, horticultural and sustenance industry (Fawzi, 2013).*Azadirachtaindica* belongs to the family Meliaceae and has a long history of utilization as a treatment against different diseases(NRC, 1992). In Nigeria, the plant is known by the name '*Dogonyaro*' (Toluet al., 2007).leaves, bark, natural products, seed, gum and oil are some greatest valuable non-wood items that gives neem leave superiority over some other tree species(Eleazu et al., 2012). These non-wood items are known to have anti-allergenic, antifungal, anti-inflammatory, anti- and several other biological activities (Pallav, 2014). As a result of these numerous applications, neem leaves has discovered huge

applications making it a green fortune. Ancient medicinal findings, suggested that the concentrates from neem leaves are recommended in for diarrhea and intestinal infections, skin ulcers and infections, and malaria(Jennifer and Alasdair, 2000). The medical value of the plants lies in some chemical substances that deliver an unmistakable physiological activity on the human body (Edeoga et al., 2005). Phytochemical compositions such as alkaloids, tannins, flavonoids, and phenolic compounds, form the most important of these bioactive constituents of plants are (Madakiet al., 2016). The knowledge of medicinal plants has been valuable in this generation of medication and production of other substances such as food, spice and perfume (Abayomi, 1993). In this way, medicinal plants are plants that contain therapeutic items as their active ingredients (Si-Yuan et al., 2013).These plants are utilized industrially in present day prescription and pharmacology (Offor, 2014). Compounds that occur naturally in plants, contributing to its color, flavor and smell are known as phytochemicals (W Hu et al., 2012). In addition, they form portion of a plant's normal safeguard component against diseases (Okwu, 2004). Excessive accumulation of heavy metal in agricultural land through traffic emission may result in soil contamination and high heavy metal uptake by crops, and thus influence food quality and safety (Khaled and Muhammad, 2016).

The chemical compositions of the leaves of this plant in Nigeria are not fully harnessed to reflect the immense medicinal properties, hence are poorly administered without precaution to its health hazard. Thus, the aim of the present study was to determine the proximate, heavy metal and phytochemical components present in the leaves of *Azadirachta indica*.

2. Materials and Methods

2.1 Sampling Collection

Dogonyaro (Azadirachta indica) leaves were collected from three (3) Different locations Along each of these routes, Market Road (O_{MR}), Adoka Road (O_{AR}) and Upu Road (O_{UR}). This selection point was based on the activities being carried out within and around the sampling point. Samples collected were wrapped in a black polythene bag and properly labeled before transporting to the laboratory for further analysis.

2.2 Sample Preparation

The fresh samples were dried at room temperature and then grounded into powder using a laboratory Mortar and Pestle to reduce particle size and then kept in plastic containers with properly sealed caps awaiting digestion.

2.3 Analytical Technique

2.3.1 Proximate and Phytochemical Analysis

The proximate analysis of the samples for moisture, total ash, crude fibre, fat, protein were carried out in triplicate using methods described by A.O.A.C (A.O.A.C, 2005). The nitrogen was determined by micro Kjeldahl method and the nitrogen content was converted to protein by multiplying by a factor of 6.25. The phytochemical constituents were quantitatively determined by standard methods**(**Harbone, 1998.,Sofowara, 2006).

2.3.2 Mineral Analysis

Wet digestion method was used and the digestion was carried out in triplicate for each sample by weighing 2.0g of the powdered sample into 100cm^3 Kjeldahl flasks with a mixture of nitric acid, Perchloric acid (60%) and Hydrochloric acid (10:4:1) (Otache et al., 2017.,Sarkiyayi and Agar, 2010).The solution and was left under a fume hood. The mixture was heated gradually to about 120 ^{O}C for 35 minutes. A dense red-orange fumes was observed at the start of the digestion process and then was replaced by white fumes with continuous heating resulting in a clear solution indicating the completion of the digestion process. The digest was diluted with 10cm^3 of distilled water. The resulting solution after cooling were each filtered into a 100cm^3 volumetric flask and diluted to the mark with distilled water. A Model PG990 Atomic Absorption Spectrophotometer (AAS) was employed in this analysis. Reagents employed in this study are of analytical grade.

2.3.3 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the difference between groups considered at significant level of $p < 0.05$ with the aid of an SPSS programme (Version 21).

3. Results and Discussion

The proximate composition (crude fibre, ash, fat and protein), of the leaves of *Azadirachta indica*, across the three collection site in Otukpo Local Government Areas as shown in Table 1, reveals that among the three collection site, Market road *(*O_{Mr}*)* had the lowest crude protein(1.22±0.22) % and fat (2.89±0.34) % content but recorded the highest values in Moisture, Ash and fibre composition (14.30±0.02) %, (4.03±0.10) % and (10.86±0.11) % respectively across the collection site. Results from this finding reveals that the protein, fat, ash and fibre contents are lower than values of 13.42 %, 5.17 %, 11.93 % and 5.17 % respectively recorded in other study (Atangwho et al., 2009). However, O_{Ar} and O_{Ur} collection site did not show significant variation at $p<0.05$, but shows significant different with O_{Mr} collection site.

Table 1. Proximate Composition of fresh leaves of *Azadirachta indica (%)*

Sample site	*n*	Moisture	Ash	Protein	Fat	Fibre
O_{Mr}	3	14.30^{a}±0.02	4.03^{a}±0.10	1.22^{c}±0.22	2.89^{a}±0.34	10.86^{a}±0.11
O_{Ar}	3	13.82^{b}±0.00	3.94^{b}±0.15	4.01^{b}±0.04	3.12^{b}±0.03	9.09^{b}±0.02
O_{Ur}	3	12.10^{c}±0.23	3.88^{b}±0.01	4.04^{b}±0.02	3.18^{b}±0.10	9.25^{b}±0.00

a, b and c indicate the variance of the three cultivars measured at different sites, as determined by ANOVA at P < 0..05.n = sample size.

The results for the concentrations of Heavy metals in fresh leaves of *Azadirachta indica* obtained from the study as shown in tables 2, reveals that the concentration of Zn ranged from 0.09±0.01 to 0.12±0.02, and that of Fe ranges from (0.13 to 0.07) mg/100g respectively, with Market road *(O_{Mr})* collection point having the highest concentration for both metals. The value for Mn content ranged from (0.07 to 0.12) %while, the concentration of Mg ranged from (0.12 to 0.42) % with the highest concentration recorded in Market road *(O_{Mr})* sample and the lowest in Adoka road (O_{Ar}) sample. Result obtained for Pb only showed value of 0.02 mg/100g recorded in Market road *(O_{Mr})* sample, while other sampling points were not detected. There was no detection of Ni and Cd across the three collection sites. Results from this finding were comparable with values obtained in other study (Atangwho et al., 2009).

Table 2. Concentrations of Heavy metals in fresh leaves of *Azadirachta indica* (*mg/100g*)

Sample site	*n*	Zn	Fe	Mn	Mg	Pb	Ni	Cd
O_{MR}	3	0.12^{a}±0.02	0.13^{a}±0.11	0.12^{a}±0.02	0.42^{a}±0.00	0.02±0.01	ND	ND
O_{AR}	3	0.09^{c}±0.01	0.08^{b}±0.00	0.07^{c}±0.10	0.12^{c}±0.01	ND	ND	ND
O_{UR}	3	0.11^{b}±0.10	0.07^{c}±0,03	0.09^{b}±0.00	0.16^{b}±0.03	ND	ND	ND

a, b and c indicate the variance of the three cultivars measured at different sites, as determined by ANOVA at P < 0.05.n = Sample size. ND = Not Detected

Results of the phytochemical composition of *Azadirachta indica* reveals that Tannin, Saponin, Flavonoid, Alkaloid and hydrogen cynide, recorded values ranging from(0.53 to 1.12) %, (0.92 to 1.94) %, 4.53 to 6.23) % , (1.85 to 3.96) %, (0.18 to 1.21) % respectively. Market road (O_{Mr}) recorded the highest values among the various phytochemical composition. Result from this finding are comparable to results obtained in other study (Atangwho et al., 2009) , but lower in other report (Eleazu et al., 2012).

Table 3. Phytochemical composition of fresh leaves of *Azadirachta indica* (%)

Sample site	*n*	Tannin	Saponin	Flavonoid	Alkaloid	HCN
O_{Mr}	3	1.12^{a}±0.34	1.94^{a}±0.22	6.23^{a}±0.01	3.96^{a}±0.33	1.21^{a}±0.16
O_{Ar}	3	0.84^{b}±0.73	0.92^{b}±0.55	4.53^{b}±0.15	1.85^{b}±0.12	0.18^{c}±0.00
O_{Ur}	3	0.53^{b}±0.54	1.24^{b}±0.23	4.94^{b}±0.45	2.01^{b}±0.00	0.78^{b}±0.01

a, b and c indicate the variance of the three cultivars measured at different sites, as determined by ANOVA at P < 0..05. n = sample size.

Conclusion

The results from this finding reveals that the commercialized O_{Mr} collection site shows higher concentration in all the studied chemical compositions, with the exception of protein content which recorded the least value (1.22±0.22) %. But the core heavy metals that could be toxic to health were not detectable with exception of Pb in O_{Mr} which is still below the WHO acceptable limit (FAO/WHO, 2011), indicating that the use of the leaves of this plant obtained from these locations for medicinal purposes will have no negative health impact when administered orally or used for other medicinal purposes. The result also reveals the antioxidant potentials of the plant due to the high quantities of alkaloids and flavonoids recorded, suggesting their uses in drug formulation. Research geared towards the isolation and characterization of bio active components active against malaria parasites and other diseases should be encouraged.

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