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Assessment of Heavy Metals, Antioxidants and Proximate Contents of Some Medicinal Plants with Hypoglycemic Effects used in Jalingo, Taraba state, Nigeria

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Abstract: *Tradomedicine Practioners in Jalingo, Taraba State have been using Vitex doniana, leptadenia hastate, Zingiber officinale, Guiera senegalensisis and Moringa Oleifera to induce hypoglycemic effect to their patients. The aim of the current work is to assess levels of some heavy metals (Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and Mn), antioxidants and to determine the proximate contents of the above aforementioned medicinal plants. Atomic absorption spectrophotometer (AAS) was used to determine the heavy metal contents of the samples. Antioxidants were determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay model while Standard Procedure was used in determining the proximate contents of the samples. From the results of heavy metals Analyzed, it was observed that the value of ten (10) selected heavy metals were found to be below the WHO recommended permissible limit for heavy metals across the five (5) selected medicinal plants. The results for Proximate contents analyzed for moisture contents reveals to have range from 4.71% for V. doniana to 12.6% for M. oleifera, Ash content was observed to range from 5.4 % (G. senegalensis) to 14.45 % (V. doniana), Fibre content was observed to range from 6.98 % to 22.52 % for L. hestata and G. senegalensis. lipids contents were observed to range from 0.917% to 2.63% for Z. officinale, and M. oleifera, Protein content analyzed spread from 3.13 % (Z. officinale) to 6.140 % (L. hestata), The observed values of Carbohydrate determine was recorded as 56.29 %, 40.9 %, 69.59 %, 59.78 % and 61.98 % for V. doniana, Z. officinale, L. hestata, G. senegalensis, and M. oleifera respectively. The results for DPPH antioxidant scavenging activities indicate a concentration dependent increase in antioxidant activity. As the concentration of the plants extract increased from 100 µg/ml to 500 µg/ml, the percentage of DPPH also increased, this suggests that higher concentration of the substance lead to more effective scavenging of free radicals.*

Keywords: *Proximate, Antioxidant, Heavy Metals, Medicinal plants.*

1.0 INTRODUCTION

Any plant which contains substances that can be used for therapeutic purposes, or as precursors for chemo-pharmaceutical semi-synthesis is referred to as a medicinal plant (Yudharaj *et al*., 2016). Herbal medicines are in great demand in both the developed and the developing countries for primary health care because of their wide biological and medicinal activities, lesser costs couple with advent of drug resistant diseases which made people look for alternative medicines. (Yudharaj *et al.*, 2016). The use of medicinal plants for the treatment of diseases dates back to the history of human life (Abdallah *et al*., 2023). The active compounds in the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. (Dar *et al*., 2023). Human is mainly dependent on raw plant materials in order to meet medical needs to maintain health and cure diseases (Jamshidi-Kia *et al.*, 2017). Although, safety of medicinal plants consumption has become one of the global problems, there is a rekindled interest in the use of natural products for improved health in Nigeria. There is positive correlation between certain diets, specific foods, and disease expression (Chen *et al*., 2020). There is therefore, the need to investigate and create awareness of the roles of nutritive values in medicinal plants as means of boosting the immune system and thus managing and controlling the progress of some diseases, Likewise, the profile of the heavy metals in the medicinal plants will determine their safety and quality. *Vitex doniana* is known by the local names: Hausa (dinyar), Fulani (galbihi), Yoruba (ori nla), Ibo (ucha koro). Several literatures have reported its uses in traditional medicine (Wakawa *et al.,* 2017 and Adewole, 2016). The nutritive and medicinal contents of *Leptadenia hastata* were outlined by (Saini *et al.*, 2022; Aslan *et al*., 2020; Mohammed *et al.,* 2014). *Moringa oleifera* is the most widely cultivated species of the family, a native of the sub-Himalayans but has naturalized in the tropics, popularly called horseradish tree, (Mali *et al.,* 2022). Its high nutritive contents have been advocated (Bidura *et al*., 2020) *Zingiber officinale* originates from Southeast Asia, Old Chinese and Indian medical texts has reported its uses in treatment of catarrh, rheumatism, constipation, vomiting and other digestion disorders (Zhang *et al.,* 2021). *Guiera senegalensisis* is one of the medicinal plants used for the treatment and control of many diseases (Dirar and Devkota, 2021). Although a lot of studies on *Vitex doniana, leptadenia hastate, Zingiber officinale, Guiera senegalensisis* and *Moringa Oleifera* have been conducted as extensively cited above, their nutritive and antioxidant contents as well as levels of heavy metals (Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and Mn) have not been documented nor reported in the study area. Hence, this study is aimed at closing the aforementioned gaps in knowledge from the study area.

2.0 MATERIALS AND METHOD

2.1 Sampling and Sample Preparation

The fresh leaves of *Vitex doniana, leptadenia hastate, Moringa Oleifera, Guiera senegalensis* and *Zingiber officinale* were collected from Jalingo local government area of Taraba state. Each of the samples was carefully cleaned to remove dirt and wrapped separately with a polythene bag and was transported to the laboratory for identification and authentication by a botanist. (Kifle *et al.,* 2020). The drying was carried out under the shade away from the sun to avoid losing some qualities of the secondary metabolites for few weeks until the plant become brittle. The samples were ground using wooden mortar and pestle. (Vishnu *et al.,* 2019). Extraction of the ground samples were done in accordance with method outlined by Solikhah *et al.,* (2020). with few modifications in which maceration extraction method was carried out by weighing 250 g of plant samples and immersing in 1500 ml of distilled water for 72 hours. The mixture was agitated intermittently to facilitate the extraction of bioactive compounds from the plant samples, after filtration, the residue was macerated for another 72 hours. This process was repeated three times using the same volume of distilled water to exhaustively extract the plant material. The solvent-to-sample ratio and extraction time were optimized to ensure maximum extraction efficiency. The final extract was concentrated on a rotatory vacuum evaporator at 45°C and under reduced pressure. The dried extract was kept in a refrigerator at −4°C for used in the experiment.

2.2. Sample digestion and Analysis of heavy metals

Digestion of the sample was carried out by adopting a procedure of Adewale *et al.,* (2019). One (1.0) gram of the powdered sample was weighed and transferred into a clean beaker. Ten (10) cm^3 of analytical grade concentrated nitric acid (HNO_3) was added. The mixture was kept in a fume cupboard overnight. The solution obtained was heated carefully with a heating mantle at 60 °C for 45 minutes until the emission of fume ceased. The container was cooled at room temperature and 5 cm^3 of 70 % analytical grade Perchloric acid ($HClO_4$) was added and further heated at 60 °C until the sample almost dried. The residue obtained was cooled and transferred into 50 cm^3 volumetric flask and diluted with deionized water. The solution was filtered and kept in clean sample bottle for atomic absorption spectrophotometer analysis. Usually while using the AAS, the sample solution was nebulized. Elemental ions are then atomized and the atoms formed absorbed radiation of the characteristic wavelength from the hollow-cathode lamp. The absorbance measured, were proportional to the concentration of the analyte in the sample solution (Usman *et al*., 2018).

2.3 Proximate Analysis

The Moisture Content, Ash content, Crude Lipid Content, Crude Fibre content, Crude protein content and Carbohydrate or NFE content were determine according to the procedure outline by Hassan *et al.,* (2019).

2.4 Total Antioxidant Capacity (TAC) Assay

A separate 5 mL Volumetric flasks was taken and aliquots of 1 cm^3, 2 cm^3, 3 cm^3, 4 cm^3 and 5 cm^3 of sample was added respectively to separate volumetric flasks. 0.5 ml of 0.2 mg/ml of DPPH Was added to each of the mentioned 5 volumetric flasks. Volume was made up to the mark with ethanol, the flasks was shaken Vigorously and allowed to stand at room temperature, protected from Light for 30 minutes. Absorbance was measured immediately at 520 nm by using UV spectrophotometer, the experiment was done in triplicate, the percentage of DPPH scavenging activity was calculated by following equation. (Harami *et al.,* 2018).

DPPH Scavenging activity (%)/ % Inhibition = $\frac{A0-A1}{A0}\ X\ 100\ \%$ ---------- (1)

Where A0= the absorbance of control.

A1= the absorbance of standard.

3.0 RESULTS AND DISCUSSION

3.1 Heavy Metals Analysis

The concentration of heavy metals (Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and Mn) determined were compared with the safety limits by World Health Organization (WHO) as presented in Table 1 below and were all found to be below the maximum permissible limit for heavy metals.

Lead value analyzed was observed to range from 0.060 mg/kg *V. doniana* to 0.590 mg/kg *L. hestata*, while other values fell between the two extreme values, this was found to be higher than another result reported by Umar *et al.,* (2016). Mercury from the medicinal plants samples Analyzed ranges from 0.02 mg/kg *(m. oleifera*) to 0.14 mg/kg (*L. Hestata*). Other values fell between the two extreme values, the minimum and maximum value of Cadmium metal obtained was recorded as 0.018 mg/kg (*V. doniana*) and 0.21 mg/kg (*m. oleifera*), The level of the arsenic determined in medicinal plants ranged from 0.090 mg/kg (*G. Senegalensis*) to 0.280 mg/kg (*Z. officinale*) while other observed values were found between the lowest and highest values. The observed concentration of chromium from five different plants spread from 0.040 mg/kg (G. senegalensis) to 0.190 mg/kg (L. hestata), other observed values were found between the least and highest values, the experimental concentration of nickel in samples from five different plants spread from 0.020 mg/kg (*V. doniana*) to 0.110 mg/kg (*Z. officinale*) while other observed values were found between the two spread values. Even though the reported value of nickel from this study was observed to be lower than another report by Adewale *et al.,* (2019). the levels of copper from five different plants ranged from 0.290 mg/kg (*V. doniana*) to 0.619 mg/kg (*M. oleifera*), other values were found between the experimental extreme values. The observed concentration of Zinc from five different plants ranged from 0.530 mg/kg (*M. oleifera*) to 0.950 mg/kg (*Z. officinale*), while other values are between the two extreme observed value. The levels of iron determined in five selected medicinal plants spread from 0.450 mg/kg (*V. doniana*) to 0.940 mg/kg (*M. oleifera*), while the

other values were recorded as 0.48 mg/kg, 0.611 mg/kg and 0.73 mg/kg for *G. Senengesis, L. Hestata,* and *Z. Offinale* respectively. Manganese samples analyzed was recorded as 0.21 mg/kg, 0.87 mg/kg, 0.23mg/kg, 0.81 mg/kg and 0.17 mg/kg for *V. doniana, Z. officinale, L. hestatya, G. Senegalensis and M. oleifera* respectively. The reported value of manganese was seen to be lower compare to a similar report by Shah *et al.,* (2016).

Table 1: Heavy Metals Concentrations (mg/kg) of Five Different Selected Plant

Parameters (mg/kg)	Samples					
	V. doniana	*Z. officinale*	*L. hestata*	*G. senegalensis*	*M. oleifera*	WHO
As	0.170 ± 0.004	0.280 ± 0.007	0.210 ± 0.001	0.090 ± 0.003	0.140 ± 0.004	1.0
Cd	0.030 ± 0.002	0.018 ± 0.003	0.050 ± 0.001	0.040 ± 0.004	0.210 ± 0.002	0.3
Cr	0.080 ± 0.001	0.060 ± 0.002	0.190 ± 0.005	0.040 ± 0.002	0.110 ± 0.003	2.0
Cu	0.290 ± 0.006	0.330 ± 0.003	0.440 ± 0.004	0.610 ± 0.001	0.619 ± 0.002	10
Fe	0.450 ± 0.008	0.730 ± 0.003	0.611 ± 0.001	0.480 ± 0.007	0.940 ± 0.002	1.0
Hg	0.070 ± 0.002	0.040 ± 0.000	0.140 ± 0.006	0.120 ± 0.001	0.020 ± 0.001	1.0
Mn	0.210 ± 0.005	0.870 ± 0.004	0.230 ± 0.001	0.810 ± 0.006	0.170 ± 0.003	5.0
Ni	0.020 ± 0.001	0.110 ± 0.004	0.060 ± 0.001	0.090 ± 0.001	0.030 ± 0.001	10
Pb	0.060 ± 0.002	0.150 ± 0.008	0.590 ± 0.001	0.110 ± 0.003	0.080 ± 0.001	0.3
Zn	0.670 ± 0.004	0.950 ± 0.007	0.840 ± 0.005	0.780 ± 0.005	0.530 ± 0.009	50

Values are mean ± standard deviation (n=3)

3.2 Proximate Analysis

The Proximate content shown in Figure 1 below reveal the percentage of moisture contents analyzed as 4.71 %, 9.45 %, 6.25 %, 5.55 % and 12.6 % for *V. doniana, Z. officinale, L. hestata, G. senegalensis,* and *M. oleifera* respectively with *M. oleifera* having the highest percentage of moisture content while *V. doniana* has the lowest value for moisture content. Ash contents analyzed from the selected medicinal plants were observed to range from 5.4 % (*G. senegalensis*) to 14.45 % (*V. doniana*). The Fibre contents value analyzed range from 6.98 % to

22.52 % for *L. hestata* and *G. senegalensis* while *M. oleifera, Z officinale and V. doniana* recorded 10.8 %, 12.45 % and 19 % respectively. The percentage of lipids analyzed was recorded as 1.48 %, 2.63 %, 1.8 %, 2.187 % and 0.917 % for *V. doniana, Z. officinale, L. hestata, G. senegalensis,* and *M. oleifera* respectively with *M. oleifera* having the lowest value while *Z. officinale* recorded the highest value. The observed value of the Protein content in medicinal plant analyzed spread from 3.13 % (*Z. officinale*) to 6.140 % (*L. hestata*), while other values of the analyzed medicinal plants where found between the lowest and highest values. The observed values of Carbohydrate determine from the five medicinal samples analyzed was recorded as 56.29 %, 40.9 %, 69.59 %, 59.78 % and 61.98 % for *V. doniana, Z. officinale, L. hestata, G. senegalensis,* and *M. oleifera* respectively as shown in Figure 1 below. This result was almost similar to another study reported by Usman *et al*., (2018).

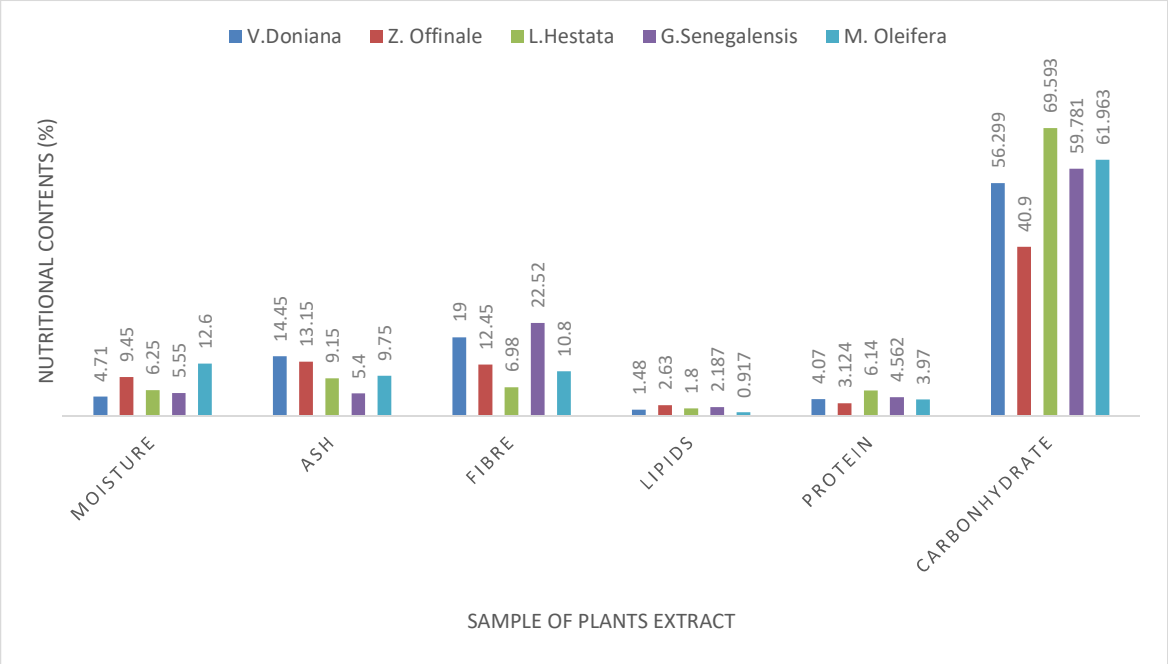

Figure 1: Percent (%) Proximate Contents of Plant Samples Extracts

3.3 DPPH Antioxidant Scavenging Activity

The results for determination of DPPH antioxidant scavenging activities indicate a concentration dependent increases in antioxidant activity. As the concentration of the plants extract increases from 100 µg/ml to 500 µg/ml, the percentage of DPPH radical scavenged also increases, this suggests that higher concentration of the crude drug extracts lead to more

effective scavenging of free radicals. But when the results of the scavenging activities of the five medicinal plants was compare with ascorbic acid across all the concentration, it was discovered that the ascorbic acid has higher antioxidant activities. At lower concentrations of 100 µg/ml, the scavenging activity is relatively low with percentages ranges around 28.816 % (*Z. officinale*) to 39.400 % (*M. oleifera)*. The highest concentrations observed at 500 µg/ml ranges between 58. 110 % (*L. hestata*) to 73.240 % (*M. oleifera*). as seen in Figure 2 below. At higher percentages of DPPH scavenging indicate greater potency and efficacy of the crude drug extract as an antioxidant. Oxidation is an integral part of aerobic processes of life. It involves the transfer of electrons or hydrogen via a chemical reaction from a substance to an oxidizing agent leading to the production of free radicals. These free radicals which are highly reactive in turn initiate a chain of reactions that lead to cellular damage (Nwozo *et al*., 2023).

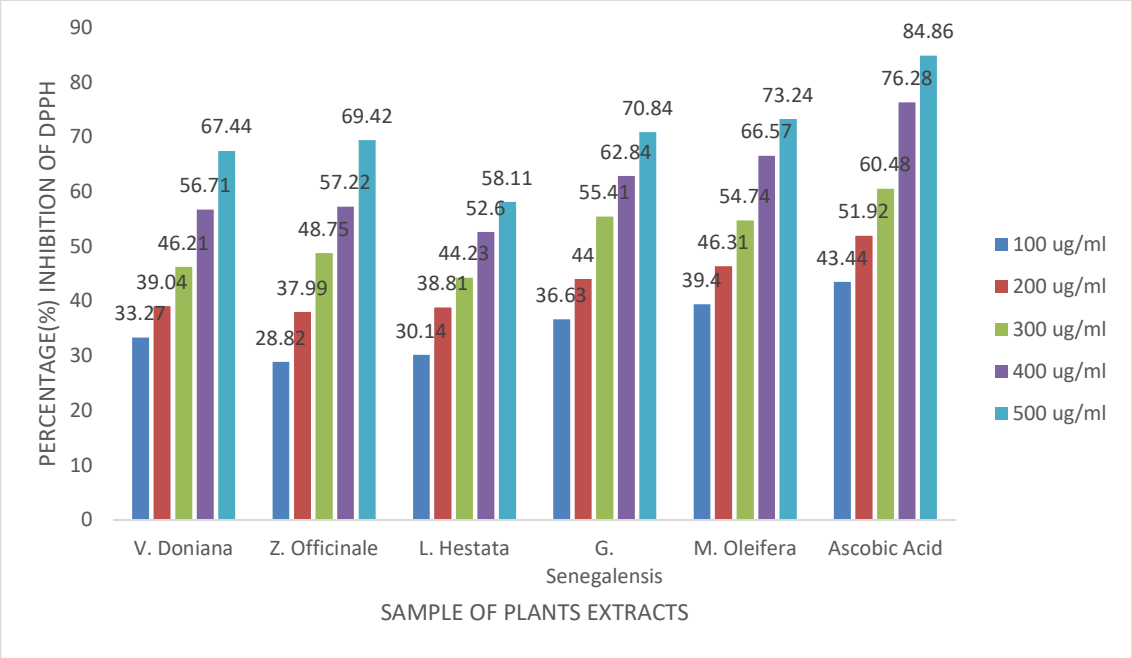

Figure 2: Mean DPPH Antioxidant Scavenging Activity from plant samples extract

4.0 Conclusion

The study analyzed the concentrations of some heavy metals (Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and Mn) from the five different selected medicinal plant samples and they were all dictated at varied concentrations, however all the values determined where below the WHO permissible limits for heavy metals indicating that the selected medicinal plants are safe for consumption. The Proximate composition was recorded at a reasonable percentage for moisture, ash, fibre, protein, lipids and carbohydrate across the five selected medicinal plants, hence the plants are said to be nutritious. The selected plants were also confirmed to have

some antioxidant activities with increase concentration of the plants extract; hence these plants can be said to have free radical scavenging activities.

4.1 Recommendations

It could be recommended that future researchers should implement a regular schedule for testing heavy metals in medicinal plants to ensure levels remain within permissible limits, implement a schedule for regular proximate analysis to ensure consistency in nutritional content and avoid harvesting during adverse weather conditions which may affect the moisture content and other nutritional values.

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