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Assessment of Antioxidant, Heavy Metal and Proximate Contents of some Medicinal Plants with Hypoglycemic Effects used in Jalingo, Taraba state, Nigeria

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***Abstract**: Medicinal plants are in great demands in the developed as well as in the developing countries for primary health care because of their wide biological activities and lesser costs. The medicinal plants such as; Vitex doniana, leptadenia hastate, Zingiber officinale, Guiera senegalensisis and Moringa Oleifera were obtained from Jalingo town, Taraba State Nigeria and were analysed for heavy metals (Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and Mn) using AAS, the plants extracts were prepared using cold aqueous maceration method, the Proximate and free radical scavenging activities of the plant extracts were also assessed using Standard Procedure and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay model respectively. 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

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis (Yudharaj *et al*., 2016). When a plant is designated as medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Herbal medicines are in great demand in the developed as well as in the developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs (Yudharaj *et al.*, 2016). In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings have sought a tool in their environment to recover from a disease, the use of plants was their only choice of treatment (Abdallah *et al*., 2023). The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant.

The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (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). However, the idea about safety of medicinal plants consume has become one of the global problems, the advent of drug resistant diseases has made people look for alternative medicines. There is a rekindled interest in the use of natural products for improved health among the general population in Nigeria. There is a marked increase of packaged herbal medicines and many manufacturing companies are exploiting this interest. Therefore, there is need to create an awareness of the roles of nutritive values in medicinal plant and foods in boosting the immune system and thus managing and controlling the progress of some disease, Likewise, the profile of the heavy metals in the medicinal plant will determine their safety and quality. Reports have shown that approximately two billion people are estimated to suffer from micronutrient deficiencies that make them more susceptible to disease, and this can be a significant obstacle to economic growth (Kiani *et al*., 2022). Nutritive value from plant such as proteins, carbohydrates, fats, vitamins, minerals and water are necessary for sustaining the life and contribute to the caloric content of the body (Bhattacharya, 2016). Fruits and vegetables have always been considered as essential sources of micronutrients and dietary fiber for the body to function properly.

Moreover, regular fruit consumption is recommended for disease prevention and health benefits due to the nutrient composition of fruits, which includes vitamins, minerals, fiber and bioactive compounds (Kaparapu *et al.*, 2020). *Vitex doniana* of the family *Verbenaceae* is a tree crop that grows in open woodland and savannah regions of tropical africa, it is the commonest of the vitex species in west africa. It produces fruits which are plum – like, sweet and edible, the fruit is green when mature and changes to dark brown when fully ripe, with the pulp surrounding a hard stone containing 1–4 seeds. It is a savanna species and can therefore be found in northern, eastern and western Nigeria (Wakawa *et al.,* 2017). *Vitex doniana* is commonly called black plum. Various parts of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders (Adewole, 2016). *Vitex doniana* is known by the local names: Hausa (dinyar), Fulani (galbihi), Yoruba (ori nla), Ibo (ucha koro). The fruits are also referred to as black-plum or African olive (Amah, 2019). It has been reported that syrup similar to honey was produced from the fruit and that physicochemical and sensory results showed that it can be substituted for other syrups as a nutritive sweetener (Gonzalez-Montemayor *et al.,* 2019). *Leptadenia hastata* is a perennial plant of the family *Asclepediaceae*, the plant is edible non-domesticated vegetable and it is collected in wild throughout Africa. It is one of the important medicinal herbs used in Africa by the traditional healers for treatment of disease and ailment and for food by the local people in terms of hunger due to its high content of valuable nutrients rich in various types of amino acids, fatty acids, terpenes, carotenes (Saini *et al.*, 2022). The plant is commonly used in the Northern Nigeria as spices and sauces. Local healers also use the plant for hypertension, catarrh and skin diseases. In certain areas of west africa, breeders claimed the antifertility effect of their animals after consumption of the leaf and stems of *Leptadenia hastata* (Aslan *et al*., 2020).

Additionally, Mohammed *et al.,* (2014) indicated that this extract could prevent the diabetes and insulin resistant-associated effects on spermatogenesis in an experimentally induced diabetes rat model. *Moringa oleifera* is the most widely cultivated species of the family, *Moringaceae* that is native to the sub-Himalayan tracts of India, Pakistan and Bangladesh. This rapidly-growing tree also known as the horseradish tree, was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics (Mali *et al.,* 2022). *Moringa oleifera* has in recent times been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids suitable for utilization in many of the developing regions of the world where undernourishment is a major concern (Alegbeleye, 2018).

Moringa leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, the protein quality of Moringa leaves competes with that of milk and eggs (Bidura *et al*., 2020). *Zingiber officinale* originates from Southeast Asia. It is not known to occur wild. it is a perennial herb, up to 1.5 meter in height, with asymmetric flowers, due to the long period of breeding in different continents, different types of the species have developed, Ginger has been cultivated and used for medicinal purposes since ancient times as described in the old Chinese and Indian texts. It was an important ingredient in herbal medicines for 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 diseases which belongs to the *combretaceae* family, it is distributed in the savannah region of west and central Africa, Senegal, Nigeria, Chad, Gambia, Mali, Guinea, Guinea-Bissau, Niger, Burkina Faso, Mauritania and Ghana. It usually occurs as a shrub or a small tree of 3 to 5 m height relying on the habitat. Its grey to brown spindly bole consists of numerous knots that send out branches. The leaves are grey-green colour and oval shape which are 3 to 5 cm long and 1.5 to 3.0 cm broad. (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, to the best of our knowledge, their proximate and antioxidant contents as well as levels of Pb, Hg, Cd, As, Cr, Ni, Cu, Zn, Fe and

Mn have not been documented nor reported in the study area. Hence, this study aims at assessment of these gaps by taking into cognizance the analysis of heavy metals, antioxidant and proximate composition of the above-mentioned plants.

2.0 MATERIALS AND METHODS

2.1 Materials/Equipment

The materials and equipment used include; Conical flasks, Beaker, Glass rod stirrer, Volumetric flasks, Test tube, Wash bottles, Sample container, measuring cylinder, Pipettes, and 1Liter of plastic container, UV-Spectroscopy and atomic absorption spectroscopy while the reagent use were all of analytical grade.

2.2.1 Plant sampling

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. The collected plant materials were wrapped separately with a polythene bag and were transported to the laboratory for identification and authentication by a botanist. Care was taken to ensure the plant samples are free from physical defects, damage or signs of deterioration. The plants were carefully cleaned to remove any dirt or outer layers. The plants were stored separately at room temperature until the extraction was done (Kifle *et al.,* 2020).

2.3 Sample Preparation

2.3.1 Drying and Grinding

The plant materials were air dried to remove the water content and thus stored after the removal of water. This process was done immediately as soon as the plants were collected which prevented them from spoiling. In this research, natural process of drying under the shade away from the sun to avoid losing some qualities of the secondary metabolites was employed. The drying was carried out for a few weeks until the water content was removed and the plants were ground using blender (Vishnu *et al.,* 2019).

2.3.2 Extraction

Maceration extraction 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 (Jones and Brown, 2019). 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 till used in the experiment.

2.3.3 Heavy Metals Analysis

2.3.3.1 Sample digestion

1.0 g of the sample was weighed and transferred into a clean beaker. 10 cm^3 of analytically 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 (Adewale *et al.,* 2019).

2.4 Proximate Analysis

2.4.1 Determination of Moisture Content

The method described by Hassan *et al.,* (2019) was adopted; a clean crucible was dried to a constant weight in an oven at 110 °C, cooled in a desiccator and weighed (W1). Two grams (2 g) of finely ground sample was accurately weighed into the previously labeled crucible and reweighed (W2). The crucible containing the sample was dried in an oven to constant weight (W3). The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-W1} X\ 100\ \% \qquad \text{-----------------------------------} (1)$$

2.4.2 Determination of Ash Content

The method reported b (Hassan *et al.,* 2019) was used. The porcelain crucible was dried in an oven at 100 °C for 10 mins, cooled in a desiccator and weighed (W1). Two grams (2 g) of the finely ground sample was placed into a previously weighed porcelain crucible and reweighed (W2), it was first ignited and then transferred into a furnace which was set at 550 °C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was removed, cooled in a desiccator and weighed (W3). The percentage ash content was calculated as follows:

$$\% \text{ Ash Content} = \frac{W3-W1}{W2-W1} X\ 100\ \% \qquad \text{---} (2)$$

2.4.3 Determination of Crude Lipid Content

A clean, dried 500 cm^3 round bottom flask containing few anti-bumping granules was weighed (W1) with 30 cm^3 petroleum ether (40-60°C) for extraction and poured into the flask filled with Soxhlet extraction unit. The extractor thimble weighing twenty grams was fixed into the Soxhlet unit. The round bottom flask and a condenser were connected to the Soxhlet extractor and cold water circulation was connected. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out. The solvent was recovered and the oil dried in an oven set at 70 °C for 1 h. The round bottom flask and oil was then weighed (W2). The lipid content was calculated (Hassan *et al.,* 2019). Thus

$$\% \text{ Crude Lipid content} = \frac{W2-W1}{Weight\ of\ sample} X\ 100\ \% \qquad \text{-----------------------} (3)$$

2.4.4 Determination of Crude Fibre

The sample (2 g) was weighed into a round bottom flask, 100 cm^3 0.25 M sulphuric acid solution was added and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it is acid free. It was quantitatively transferred into the flask and 100 cm^3 of hot 0.31 M, Sodium Hydroxide solution was added, the mixture boiled under reflux for 30 min and filtered under suction. The residue was washed with boiling water until it is base free, dried to constant weight in an oven at 100 °C, cooled in a desiccator and weighed (W1). The weighed sample (W1) was then incinerated in a muffle furnace at 550 °C for 2 hours, cooled in a desiccator and reweighed (W2) (Hassan *et al.,* 2019).

Calculation:

The loss in weight on incineration = $\frac{W2-W1}{Weight\ of\ Sample}\ X\ 100\ \%$ ------------------ (4)

2.4.5 Determination of Crude Nitrogen

The determination of total nitrogen was done by the micro-kjeldahl's procedure. 1.0 grams of each sample was weighed and placed in a dry beaker. 1 g of Cu_2SO_4, 4 g of K_2SO_4 and 25 ml of concentrated H_2SO_4 were added. The flask was heated continuously at low heat on the digestion stand. When the water has been removed and frothing has ceased, the heat was increased until the digest is cleared. The mixture was boiled so that the H_2SO_4 condenses about half way up to the neck of the flask. The flask was allowed to cool and 50 ml of water was added to the flask slowly. Then aliquot volume of distilled water was added into the distillation apparatus. The distillation flask was then attached to the distillation. The condenser was kept cool below 30 °C allowing sufficient cold water to flow through and regulate heat to minimize frothing and prevent suck back. Thereafter, 40 ml distillate was collected and the distillation was stopped. Nitrogen was determined in the distillate by titration with 0.01 (M) molar standard HCl using a 25 ml burette graduated at 0.01 ml intervals, the colour change at the end point, was from colorless to pink (Hassan *et al.,* 2019). Then, percentage of nitrogen content in the sample was calculated using the below formula: (Hassan *et al.,* 2019).

$$\%\ Nirtogen \frac{T-DX0.014X25X0.1}{1X50}\ X\ 100$$ ------------------------------------ (5)

2.4.6 Determination of Crude Protein

According to Hassan *et al.* (2019), to estimate crude protein, it involves the determination of total Nitrogen. The amount of crude protein was obtained by multiplying the nitrogen content by a factor of 6.25

2.4.7 Determination of Carbohydrate or NFE

According to Hassan *et al.,* (2019), the Nitrogen Free Extraction (NFE) referred to as soluble carbohydrate was obtained as a difference between crude protein, sum of crude ash, lipid, moisture and crude Fibre.

FORMULA: NFE = 100% - (% crude protein +% Ash + % Crude lipid + % Crude fibre + moisture%).

2.5 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= $\frac{A0-A1}{A0}\ X\ 100\ \%$ --------------------------- (6)

Where A0= the absorbance of control.

A1= the absorbance of standard.

3.0 RESULTS AND DISCUSSION

3.1 Heavy Metals Analysis

In this study, 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 Figures 1, 2, 3, 4,5,6,7,8,9 and 10.

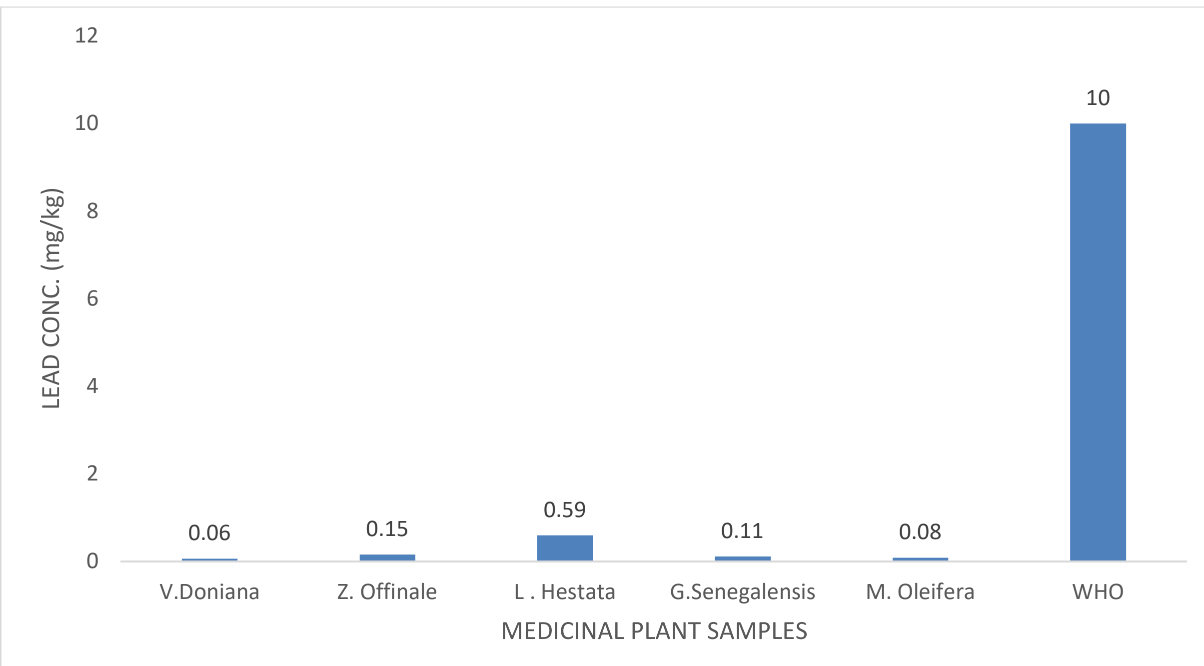

Figure 1: Mean Lead Concentration (mg/kg) From Plants Samples

From the results in Figure 1, It was observed that the value of lead in the current work ranged from 0.060 mg/kg *V. doniana* to 0.590 mg/kg *L. hestata*, while other values fell between the two extreme values. The result obtained from the concentrations of lead in this study was higher than that obtained from the findings of quantitative determination of heavy metals in some commonly consumed herbal medicines in Kano State, Nigeria (0.0927 to 0.0185 mg/kg) (Umar *et al.,* 2016). However, all the five plants sample collected from this study were found to be below the maximum permissible limit for lead (the maximum permissible concentration for lead according to WHO was 10 mg/kg). Therefore, even though lead is very poisonous, the five selected medicinal plant can be said to be lead free for consumption. Lead is a non-essential metal, enters the body in various ways, making it a major public health issue, Traditional and cultural remedies may include dangerous quantities of lead, producing lead poisoning (Amin *et al,* 2023).

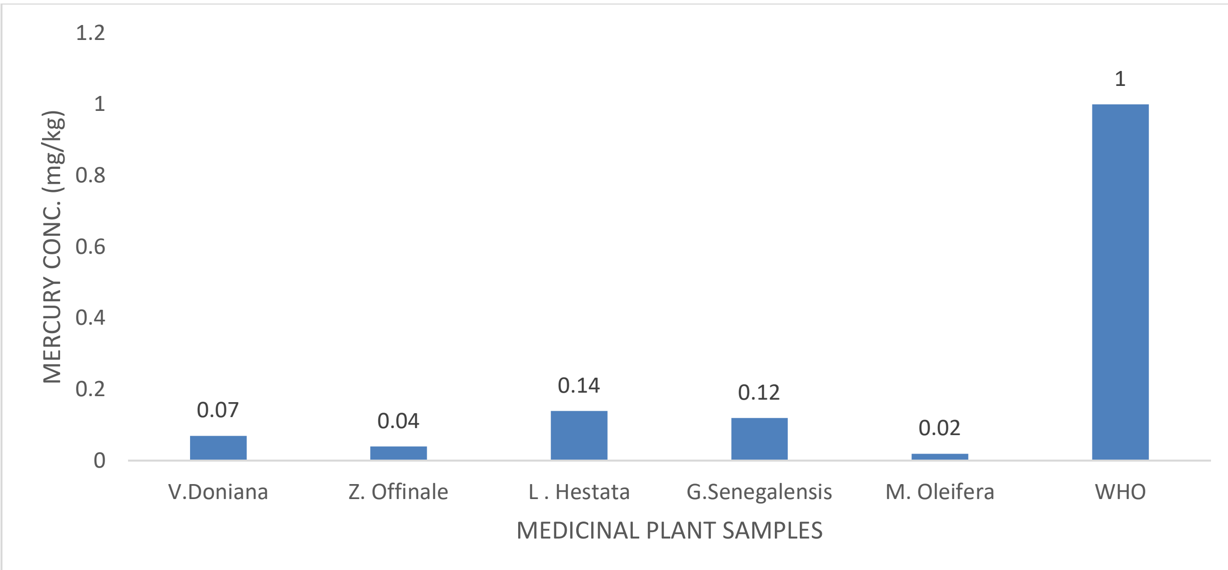

Figure 2: Mean Mercury Concentration (mg/kg) from Plant Samples

From the result in Figure 2, it can be observed that the concentration of 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 WHO standard gives a limit of 1.0 mg/kg. From the result obtained, all the plants sample were within permissible limit for mercury, making them free for consumption. The result of mercury level in this work was well below that obtained by a similar analysis done by Hyder *et al*., (2022) on determination of Heavy metals content, Lead (Pb), Mercury (Hg), Cadmium (Cd), Nickel (Ni), and Copper (Cu) with risk assessment to human consumption as a food and medicine in herbal species through Atomic Absorption Spectroscopy with mercury range from 2.25 mg/kg to 3.75 mg/kg. Mercury is a persistent environmental pollutant of high toxicity, and elemental gaseous mercury can be transported long distances within the soil and plant, Organomercury compounds usually have more toxic effects than their corresponding inorganic forms (Guo *et al*., 2024). Mercury is another toxic metal which is available in the form of white shiny silver and is used for the dental amalgam, thermometer and some batteries. Mercury can exist in the form of gases state which can be inhale and it can be transmitted in human through municipal sewage water discharge, agriculture, incineration and industrial waste water discharge (Hyder *et al*., 2022).

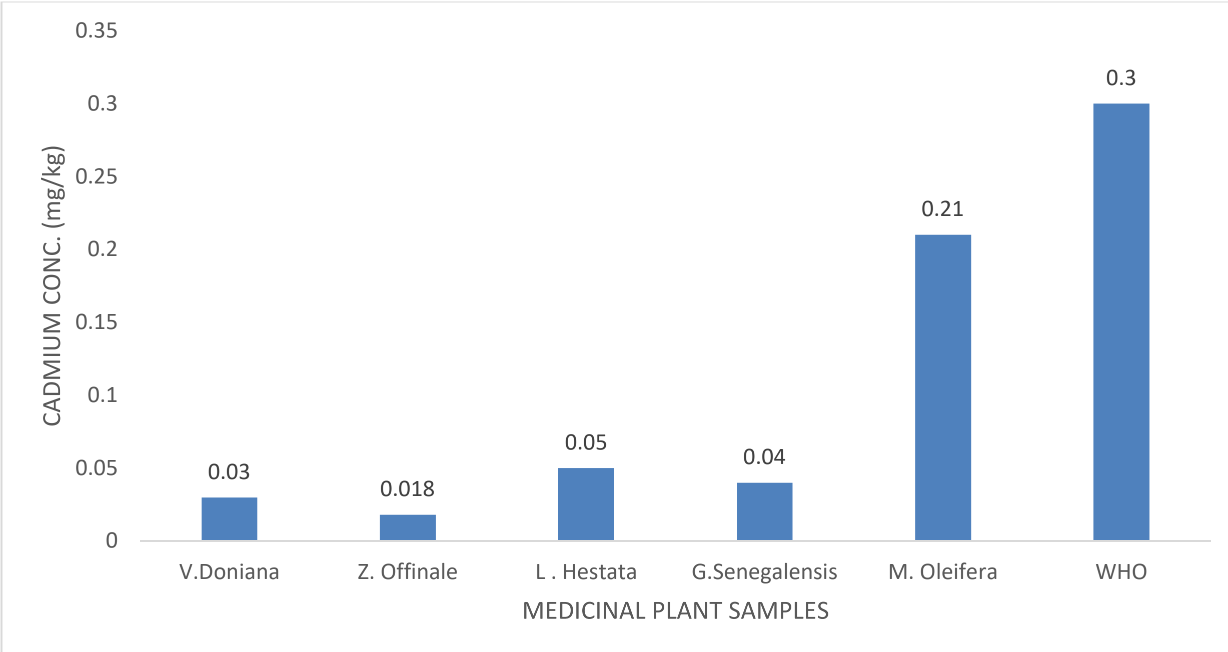

Figure 3: Mean Cadmium Concentration (mg/kg) from Plant Samples

The minimum and maximum concentrations of cadmium metal obtained from the five selected medicinal plants analyzed are shown in Figure 3. The values range from 0.018 mg/kg (*V. doniana*) and 0.21 mg/kg (*m. oleifera*). The maximum permissible concentration of cadmium by WHO is 0.3 mg/kg which is higher than the value of the five medicinal plants analyzed. The value of cadmium obtained from this work is lower than the highest value of cadmium from the finding on heavy metal analysis of some anti-diabetic herbal drugs sold in Ondo State, Nigeria which range between 0.022 mg/kg to 0.2763 mg/kg (Adewale *et al.,* 2019). Cadmium (Cd) has played an important role in industry and brought certain conveniences to mankind, used for electroplating and making rechargeable batteries, etc. Cd is also a harmful pollutant, which will enter the food chain and cause damage to human tissues and organs. (Wang *et al.*, 2023).

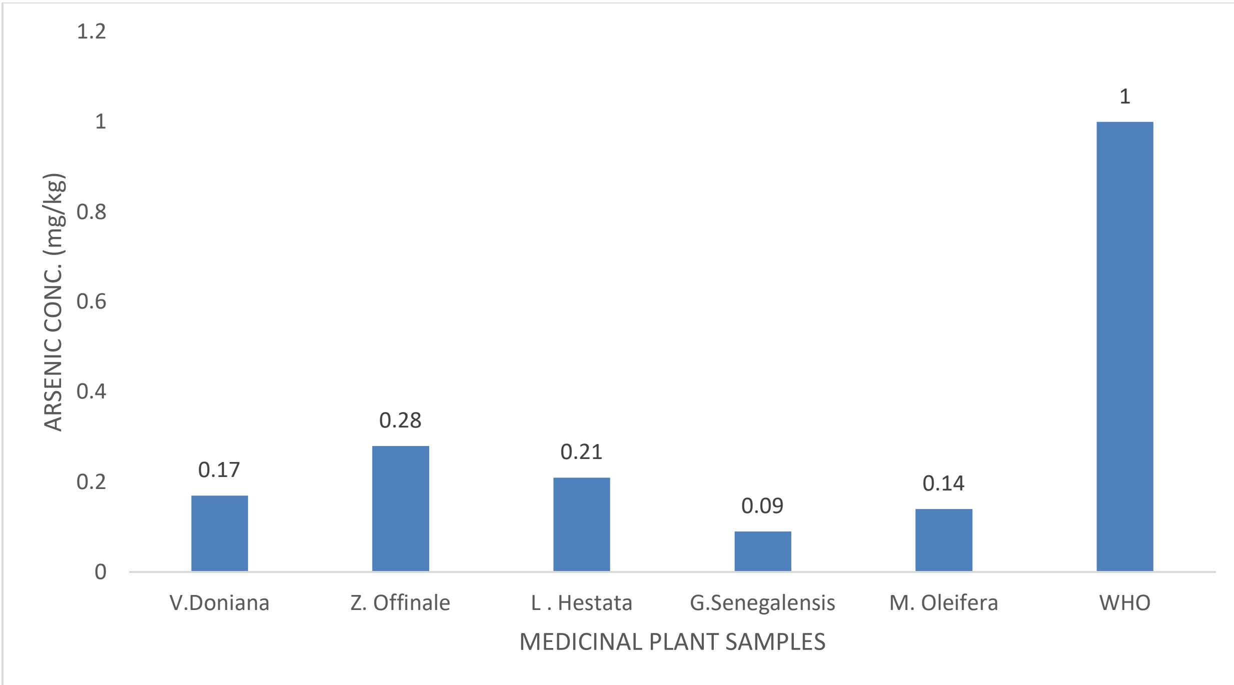

Figure 4: Mean Arsenic Concentration (mg/kg) from Plant Samples

The maximum permissible concentration for Arsenic according to WHO was 1 mg/kg, however, from the results obtained in Figure 4, all the five plants samples collected from the study area were found to be below the WHO maximum permissible limit for Arsenic. The level of the arsenic determined in medicinal plants ranged from 0.090 mg/kg (*G. Senegalensis*) to 0.280 mg/kg (*Z. officinale*). Other observed values were found between the lowest and highest values. The mean value of the five medicinal plant obtained from this study 0.078 mg/kg was observed to be lower than the mean value of 0.422 mg/kg of 10 fish from a study on Human health risk assessment of heavy metals via consumption of fish from Kao Bay (Amqam *et al*., 2020). Arsenic is a highly toxic non-essential metalloid that significantly affects human health leading to gastrointestinal and hepatic disorders, hypotension, polyneuropathy, loss of sight and carcinogenic effects (Marinescu *et al.,* 2020).

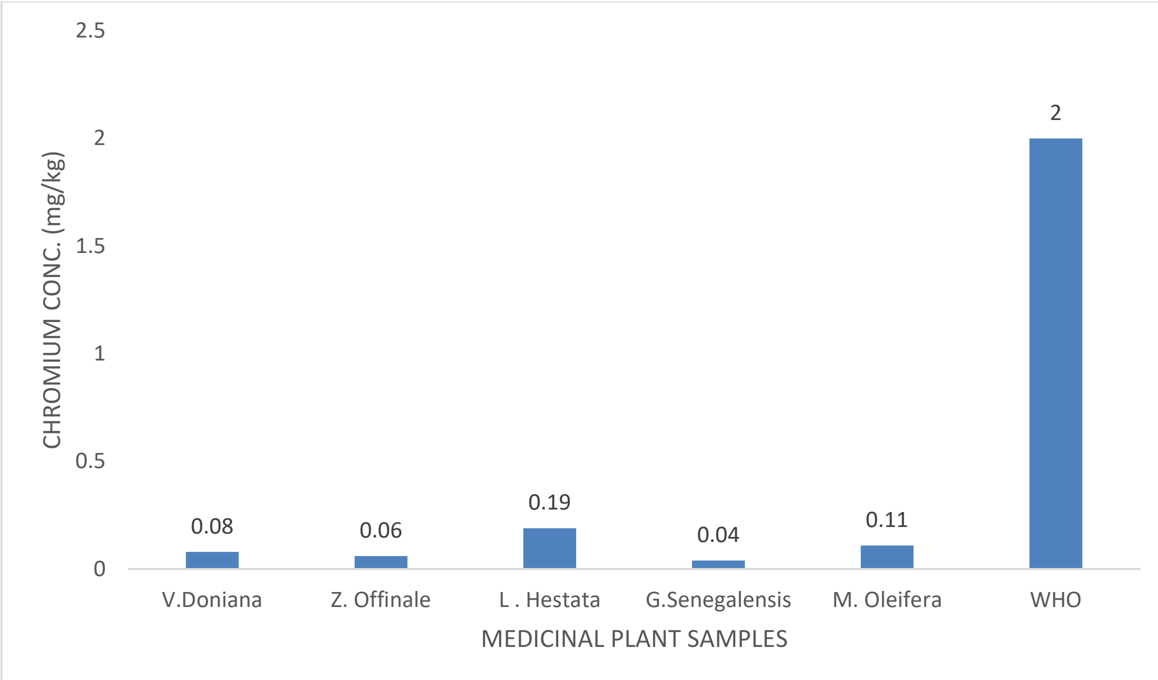

Figure 5: Mean Chromium Concentration (mg/kg) from Plant Samples

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 as shown in Figure 5. Even though the values obtained from this study was found to be higher than the value obtained from a finding determination of heavy metals contamination, risk prediction and antioxidant properties of anti-malarial herbal mixture sold in Kano state, Nigeria with values range between 0.064 mg/kg to 0.089 mg/kg (Babandi *et al.,* 2024), the medicinal plants can be said to be ok for consumption because the values obtained are less than the WHO limit for maximum permissible concentration. Chromium is an important micronutrient that is involved in carbohydrate, lipid, and protein metabolism, Cr is abundant in the Earth's crust, in which it occurs at a level of 100 µg/g, and its toxicity depends on its chemical state, its two most prevalent oxidation forms are trivalent and hexavalent, but whereas trivalent Cr is largely non-toxic, hexavalent Cr is toxic and carcinogenic in living organisms (Shin *et al.,* 2023).

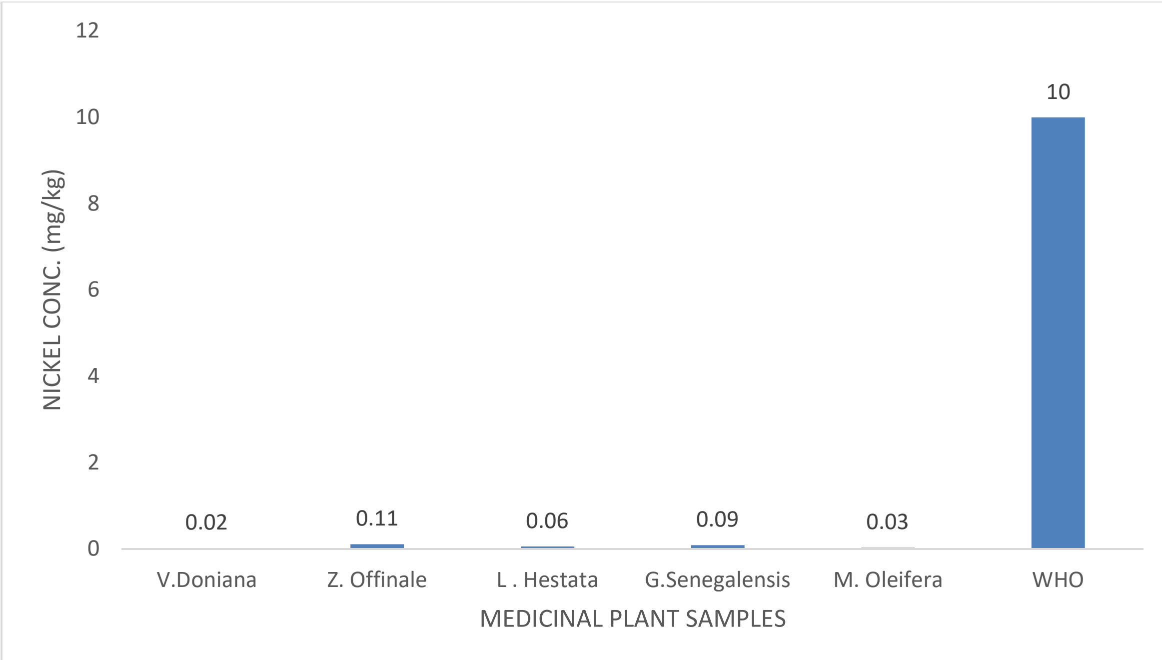

Figure 6: Mean Nickel Concentration (mg/kg) from Plant Samples

From the result in Figure 6, it can be observed that 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*). Other observed values were found between the two spread values as shown in Figure 6. The concentration of nickel from this study is lower than the concentration of nickel reported in the finding of heavy metal analysis of some anti-diabetic herbal drugs sold in Ondo State, Nigeria that range between 0.0551 mg/kg to 0.8740 mg/kg (Adewale *et al.,* 2019). However, the levels of nickel obtained from the selected medicinal plant are lower than the WHO permissible limit of 10 mg/kg. This therefore shows that the medicinal plants analyzed are safe for human consumption. Contact with nickel can have several negative health impacts on people, including allergies, kidney and heart problems, lung fibrosis, and lung and nasal cancer.

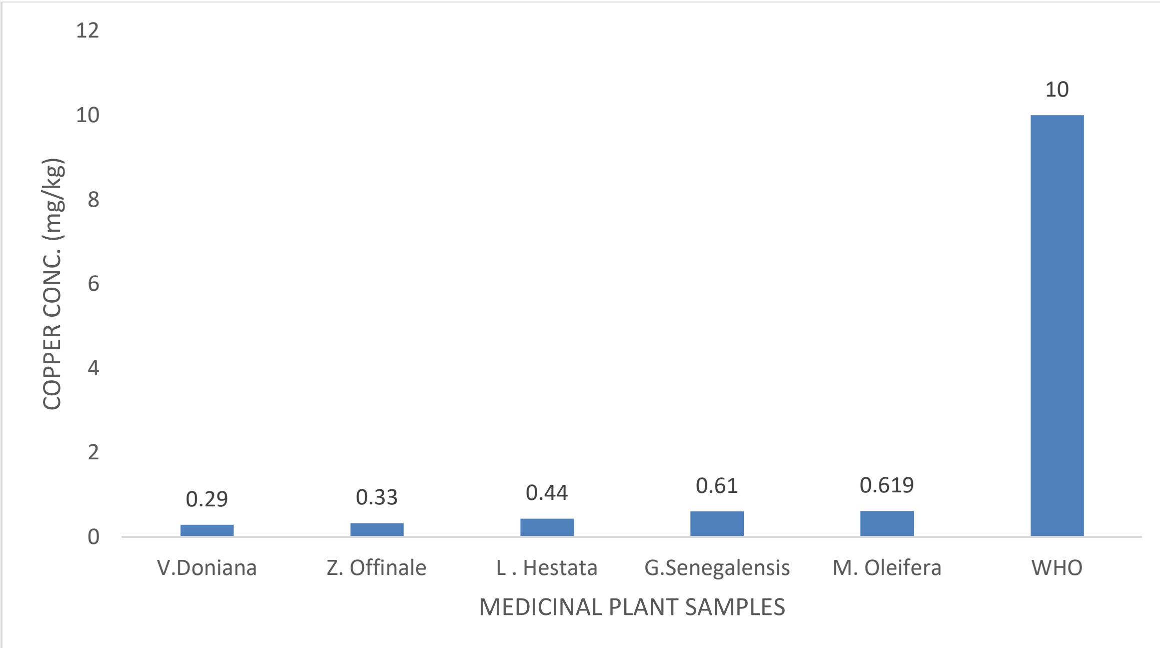

Figure 7: Mean Copper Concentration (mg/kg) from Plant Samples

From the results obtained in Figure 7, it can be concluded that 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 concentration of copper studied in the plant samples was found to be within the WHO maximum permissible limit of 10 mg/kg for copper. The value obtained from this study was observed to be higher than the finding for the quantitative determination of heavy metals in some commonly consumed herbal medicines in Kano State, Nigeria which range from 0.0547 mg/kg to 0.2465 mg/kg (Umar *et al.,* 2016). Copper is an essential nutrient for humans, animals, and plants, it can pose risks to human health with elevated exposure, in humans, acute effects of copper ingestion include gastrointestinal symptoms such as nausea or abdominal pain (Taylor *et al.,* 2020).

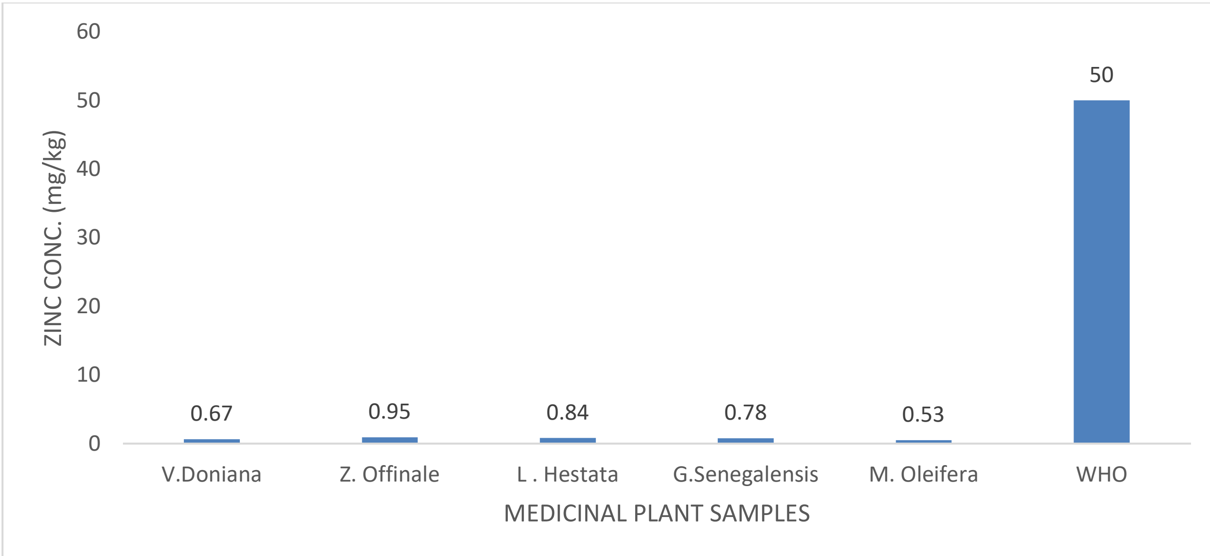

Figure 8: Mean Zinc Concentration (mg/kg) from Plant Samples

It can be concluded from Figure 8, 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 and lower than the WHO maximum permissible level of 50 mg/kg for zinc. From a report tilted the average zinc concentration of the 15 medicinal plant samples analysed were recorded as 0.53 mg/kg less than average concentration of 5 medicinal plants recorded from this study (Nkansah *et al*., 2016). This is therefore showing that plant samples obtained from these are very safe for consumption. Zinc belongs to the group of the most widespread micronutrients. It is considered the most important trace element for human health (Molenda *et al.*, 2023). Zinc is an essential element; it plays an important role in growth and has a recognized action in more than 300 enzymes by participating in their structure or their catalytic and regulatory action. Zinc deficiency causes growth retardation and hypogonadism. Zinc deficiency may also affect the bone metabolism and gonadal function (Nkansah *et al*., 2016).

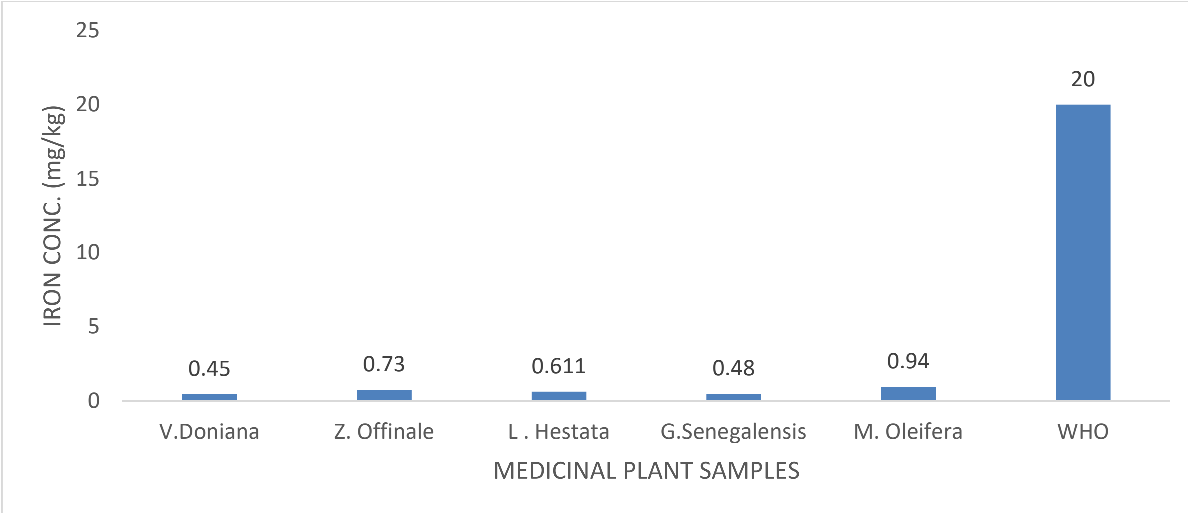

Figure 9: Mean Iron Concentration (mg/kg) from Plant Samples

The results in Figure 9 shows 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. The levels of iron investigated in this study are below the WHO Maximum permissible limit of 20 mg/kg. Therefore, the plant can be said to be free for consumption with respect to the level of iron determined. The value of iron determined from this study was less than the value reported from a finding, which range between 2.51 mg/kg to 7.06 mg/kg (Nkansah *et al*., 2016). Heavy metal contents of some medicinal herbs from Kumasi, Ghana, Iron is an essential trace element, while excess iron can lead to different levels of physical abnormalities or diseases, higher iron intake is positively associated with nine outcomes, including colorectal cancer, type 2 diabetes mellitus, and cardiovascular disease mortality, while dietary total iron intake could decrease the risk of colorectal adenoma, esophageal cancer, coronary heart disease, and depression (Huang *et al.,* 2023).

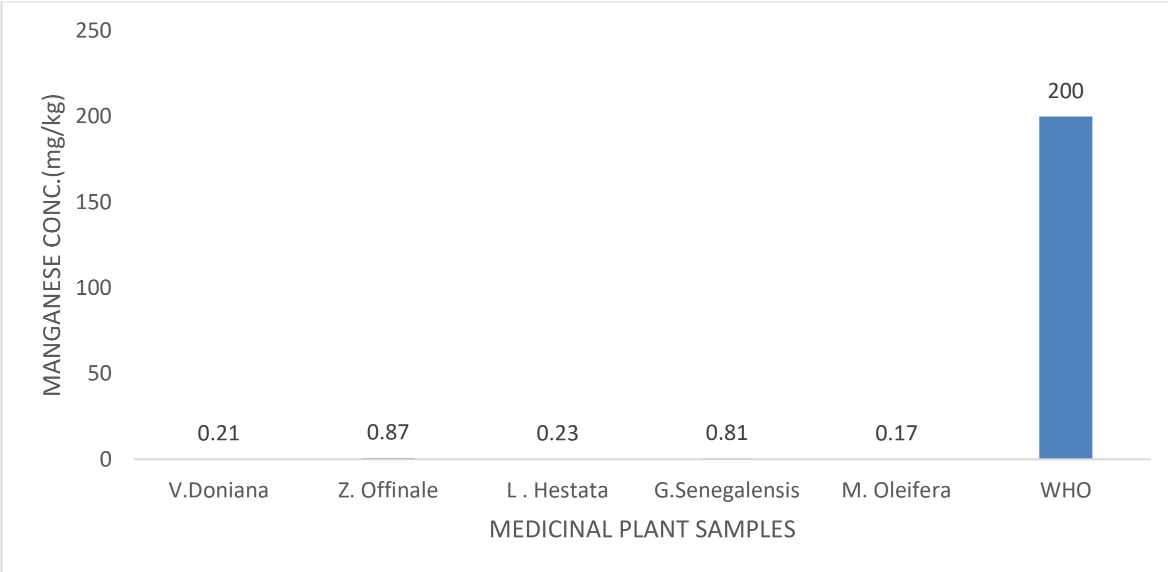

Figure 10: Mean Manganese Concentration (mg/kg) from Plant Samples

The results in Figure 10 shows the value of the plants 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 and this values are very insignificants compare to WHO maximum permissible limit of 200 mg/kg. The value of manganese determined from this study was observed to be less than the manganese value reported from the finding. Comparison of toxic heavy metals concentration in medicinal plants and their respective branded herbal formulations commonly available in Khyber Pakhtunkhwa which range between 12.1 mg/kg to 48.5 mg/kg (Shah *et al.,* 2016). Manganese is an essential metal in the human body, plays a crucial role in regulating protein synthesis, metabolism, neurotransmitter synthesis, and immune function. However, excessive Manganese exposure is toxic to humans and contributes to the development of various health conditions, including neuromuscular diseases, such as Parkinson's disease and dystonias.

3.2 Proximate Analysis

The percentage of moisture contents analyzed was recorded 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 higher percentage of moisture content while *V. doniana* recorded the lowest value for moisture content. Ash content analyzed from the selected medicinal plant was observed to range from 5.4 % (*G. senegalensis*) to 14.45 % (*V. doniana*), other experimental values of the medicinal plants were found to be between the least and highest value. The Fibre content analyzed as depicted in Appendix 4 was observed to 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 11 below. In another study Title Proximate and Phytochemical Analysis and Levels of Heavy Metal (Pb, Cd, Cr, Ni and Cu) of Some Medicinal Plants Collected from Gombe State, Nigeria, it was recorded that percentage of Proximate content range from 5.0 % (S. Singuena) to 9.0% (C. Planchoni), 4.0% (C. Planchoni) to 20.0 % (N. Lotus), 2.1 % (A. Nilotica) to 3.9 % (S. Singuena), 4.0 % (S.singuena) to 42.0% (C. planchoni) and 9.9 % (C. planchoni) to 41.5 % (S. Singuena) for moisture content, Ash content, Crude Protein, Crude Lipid, Crude Fiber and Carbohydrate content respectively (Usman *et al.,* 2018). The body needs six nutrients for proper functioning and overall health. These include carbohydrates, proteins, fats, water, vitamins, and minerals. In many developing countries, starvation and undernourishment are on the rise because of population explosion, scarcity of productive land, and soaring food costs (Shahid *et al*., 2023).

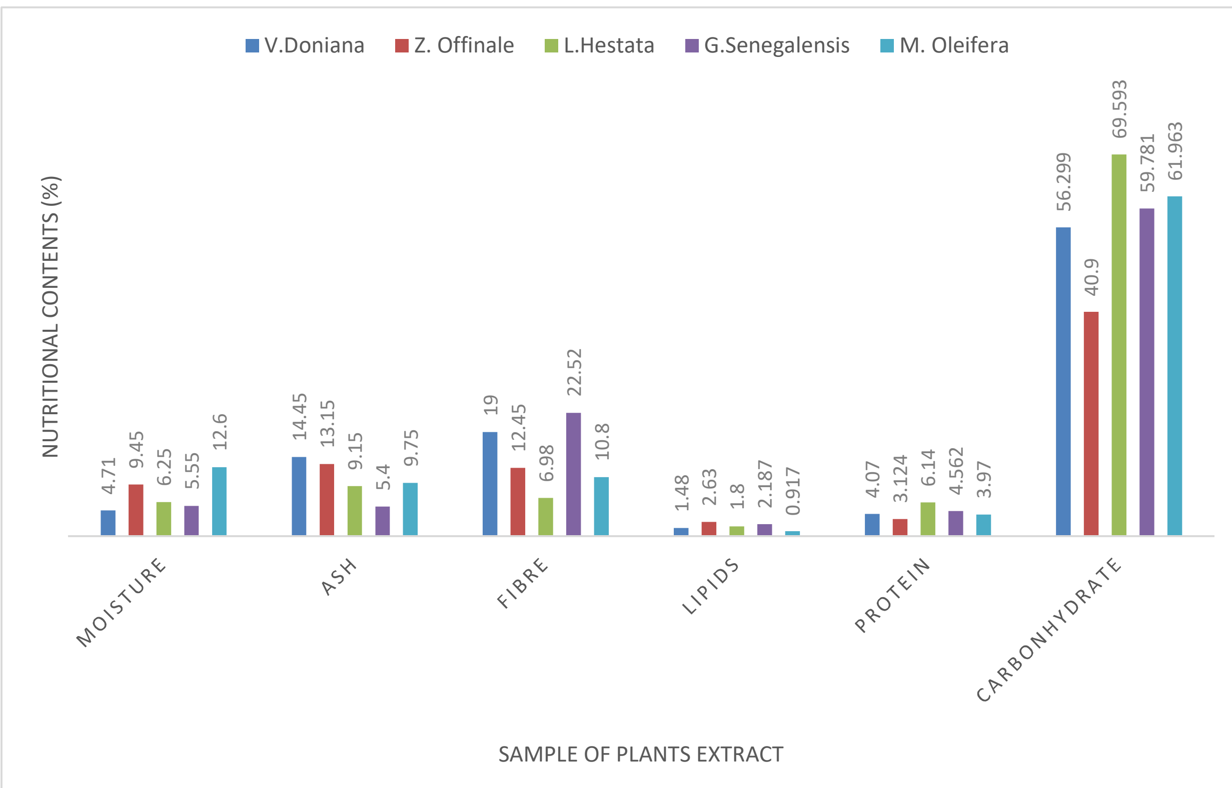

Figure 11: Percent (%) Proximate Contents of Plant Samples Extracts

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 substance 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*) and to 39.400 % (*M. oleifera)*. The highest concentrations observed at 500 µg/ml ranges between 58. 110 % (*L. hestata*) to 73.240 % (*M. oleifera*). (Figure 4.24) By plotting the concentration against the percentage of DPPH radical Scavenged, there is a dose-response curve. This curve typically shows an initial gradual increase in scavenging activity. At higher percentages of DPPH scavenging indicate greater potency and efficacy of the substances as an antioxidant. (Figure 12). In another study on the Evaluation of in vitro and in vivo anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of hagenia abyssinica (rosaceae), it was also observed that antioxidant scavenging activities indicate a concentration dependent increases in antioxidant activity that is as the concentration of the plants extract increases from 15.625 µg/ml to 500 µg/ml, the percentage of DPPH radical scavenged also increases similar to what was obtained from this report (Kifle *et al.,* 2020). 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). Antioxidants protect cells from damage caused by free radicals. Antioxidants have been shown to slow down or prevent the oxidation of other molecules. They possess the ability to terminate chain reactions and inhibit oxidation reactions via the removal of radical intermediates and by becoming oxidized themselves. Plants contain numerous antioxidants such as polyphenols, flavonoids, carotenoids, and vitamins which help to confer protection against free radicals associated diseases. The antioxidant compounds are mostly produced in plants in the form of secondary metabolites (Nwozo *et al*., 2023).

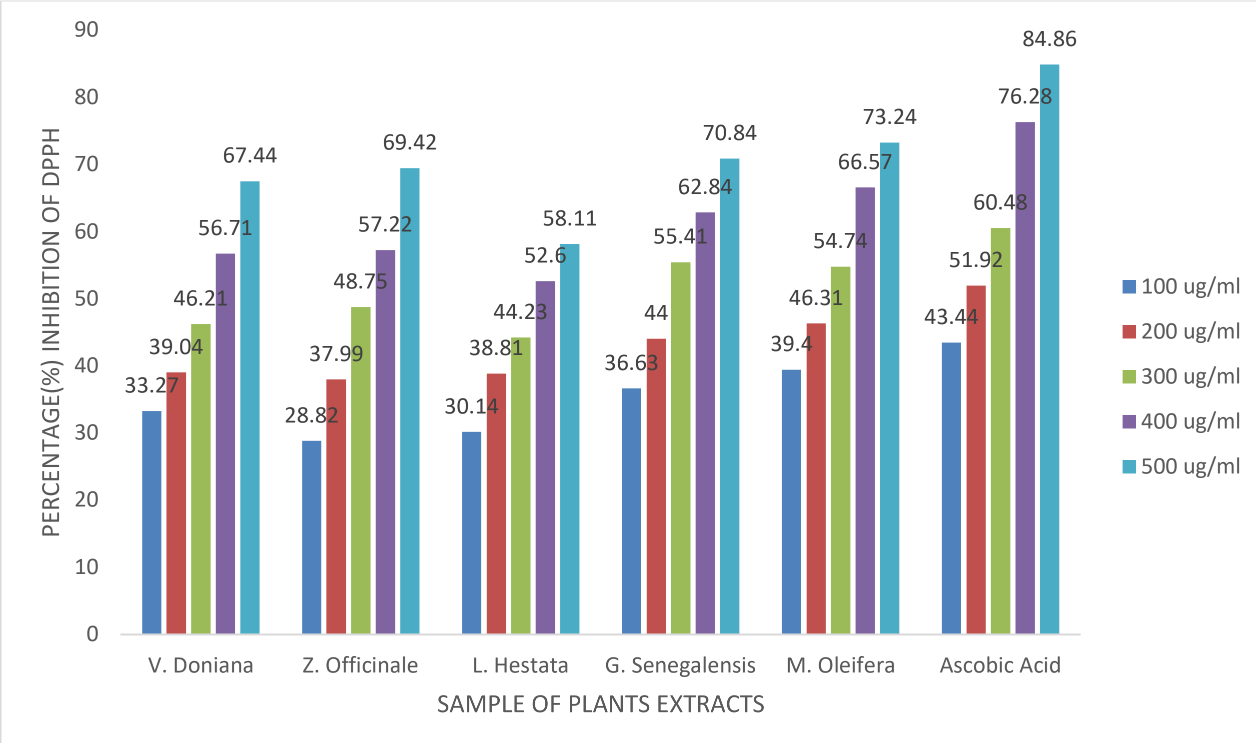

Figure 12: Mean DPPH Antioxidant Scavenging Activity from plant samples extract

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 plants sample and they were all dictated at varied concentration, 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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