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Determination of Macro and Micro Elements in Some Selected Anti-diabetic Medicinal Plants in Adamawa State, Nigeria Using Instrumental Neutron Analysis.

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Abstract
In this study, the concentrations of essential micro and macro elements were investigated in five anti-diabetic medicinal plants collected from part of Adamawa state, Nigeria using instrumental neutron activation analysis (INAA). For each plant, the leaves, stem bark and the root bark samples were analyzed for the essential elements and the concentrations placed in order of preference based on their bioavailability per tissue. The overall order of plants tissues analyzed suggests that the most potent source of macro elements are Ca, K and Mg. As the order of preference revealed, the most potent source of Ca is in the stem bark sample of *Anogeissus leiocarpus* (79780±1277.0 mg/kg), while the leaves of *Daniellia oliveri* was observed to be the potent source of Mg (6012±333.1 mg/kg). Highest concentrations of K (80250±321.0 mg/kg) were observed in large quantity in the root bark sample of *Jatropha gossypiifolia*. For the micro elements, the concentration of Fe was observed to be higher in the root bark sample of *Jatropha gossypiifolia* (1506±50.0 mg/kg). Similarly, the concentration of Cl was observed to be higher in the stem bark sample of *Jathropha gossypiifolia* (4780.0±48.0 mg/kg). These information as regards to the distribution of essential elements in the respective plant tissues will help influence the choice of plant and plant tissues when considering plant-based substrate for the treatment and management of diabetes mellitus as these elements are found to play an active role in potentiating the pharmacological properties of anti-diabetic medicinal plants.

Keywords: Anti-diabetes, Diabetes mellitus, INNA, Medicinal plants, Macro elements, Micro elements

1. Introduction
Plants relative to its spread and varying physiochemistry seems to have the most essential and interesting phytochemicals with great medicinal importance. For decades, these characteristics gingered Nigerians into the search and utilization of herbal medicine to remedy the inaccessible pharmaceutical drugs [1]. For that, quantum of energy are expended globally towards relating phytochemicals and mineral components for various pharmacological applications.
The physiological reaction of phytochemicals is reported to be interrelated, defined by other metabolic constituents in the body systems. These constituents are reported to participate in transforming the phytochemicals into their active forms with specific pharmacological significance [2, 3]. Interestingly, essential elements based on some physiochemistry are reported to play an active role in the formation of these active constituents [2]. However, in parallel with the increasing interest in exploring plants phytochemicals for therapeutic purposes, study into the elemental compositions of medicinal plants will further bring into light their pharmacological significance. Relating the compositions of both micro and macro elements of medicinal plants to specific pharmacological actions is still a virgin area to investigate [3, 4]. Though, the beneficial components of medicinal plants of therapeutic values are mostly in the class of tannins, alkaloids, steroids, polyphenolic acids and etc, the macro and micro elements are generally the essentials mineral nutrients in the medicinal plants [5, 6]. These observations informed the choice of this study to look into the elemental composition of some selected medicinal plants used in Nigeria and relate their physiochemistry towards

suppressing diabetes mellitus. The elements of interest in this study are classified as Macro Elements (Ca, K, Mg; Na) and Micro Elements (Cl, Co, Cr, Fe, Mn; Zn). This classification was made on the basis of plant mineral nutrients requirements to complete the growth cycles such as photosynthesis, enzyme activities and secondary metabolite production [7], although this classification is also very much similar to the mineral nutrient requirements for human health [6]. The macronutrients are consumed in larger quantities and are present in plant tissues in quantities from 0.2% to 4.0% (on a dry matter weight basis), while micro nutrients are present in plant tissues in quantities measured in parts per million, ranging from 5 to 200 ppm, or less than 0.02% dry weight [8, 9].

In this work, INNA method was applied in order to compare the micro and macro nutrient present in five (5) Anti-diabetic medicinal plants collected in Mubi south, Mubi north and Maiha local government of Adamawa state, Nigeria. The choice for INNA methods is due to its selectivity, striking properties, certainty and versatility [10, 11]. Instrumental neutron activation techniques is classic over other methods because of the possibility of simultaneous assaying of a majority of elements in representative mass of substance. And unlike other conventional techniques, this method does not require special sample preparation procedures before analysis [11, 12]. Thus, making INNA method a remarkable sensitive tool for measuring trace elements with low detection limits [13]. The results of this study is therefore, expected to provide information on the potentiating role of essential elements in plants for the treatment and management of diabetes mellitus.

2. Materials and Methods

2.1 Sampling and Sample preparations

The plants samples (leaf, stem bark and root bark) of the selected medicinal plants as described on Table 1 were collected from Mubi North, Mubi South and Maiha Local Government Areas of Adamawa State, Nigeria. Information's on the ethno botanical use by the immediate communities and traditional health practitioners in the study area informed the choice of the selected plants**;** widely utilized for the management of diabetes mellitus. The authentication of the plants was conducted by Mr. Jarafu U. Mamza, from the Department of Botany, Adamawa State University, Mubi and a voucher specimen samples deposited. The dust free samples were disaggregated and shade dried at ambient temperature. The dried samples were then made into powder, sieved using 2 mm mesh and homogenized. At the INAA laboratory, about 250 mg to 300 mg plant samples were weighed onto different polythene films wrapped and heat-sealed following the same protocols described by Magili *et al*, [13].

Table 1: List of the Selected Medicinal Plant used in the investigation

S/No.	Botanical name	Family name	Common name	Local name (Hausa)	Parts used.
1.	*Sclerocarya birrea*	*Anacaardiaceae*	*Spondias birrea*	*Daniya/Lule/Nunu*	Roots,stem bark and leaves
2	*Anogeissus leiocarpus*	*Combretaceae*	*African birch*	*Markee*	Roots,stem bark and leaves
3	*Jatropha gossypiifolia*	*Euphorbiaceae*	*Wild cassada*	*Zugu*	Roots,stem bark and leaves
4.	*Daniellia oliveri*	*Caesalpinioideae*	*Paradaniellia oliveri*	*maje*	Roots,stem bark and leaves
5.	*Sarcocephalus latifolius*	*Rubiaceae*	*Nauclea latifolia*	*tafashiya*	Roots,stem bark and leaves

2.2 Determination of elements concentration in medicinal plants using INAA

The reference material SRM NIST-1547 (Peach leaves) were used for quality control test and quantitative analyses. The analytical values of the reference material obtained from this study were compared with the actual values (in mg/kg) [14].

The plants samples and standards were analyzed using Nigerian Research Reactor-1 (NIRR-1) facility at the centre for energy research and training ABU Zaria. The protocols for sample irradiation were performed in two irradiations stages as described in a work performed by [15-17]. The first irradiation was designed to capture short half-lives radionuclide, the second irradiation was designed to capture long half-life radionuclide in the inner channel of the Miniature Neutron Source Reactor (MNSR) operating at full power of 30 kW thermal with a neutron flux of 2.5 x 10^{11} n/cm^2 s and irradiation period of 600 s. Finally the identification of gamma ray of product radio-nuclides through their energies and quantitative analysis of their concentration were obtained by using the gamma ray spectrum analysis software (WINSPAN 2004) [18], software developed at CIAE, Beijing, China.

2.3 Statistical analysis

The obtained results were presented as mean ± SD (standard deviation). All differences are considered significant at $p<0.05$ using Analyse-it (version 2.3). Significant elemental concentration differences in plants samples were determined by analysis of variance (ANOVA).

3. Results and Discussion

The results of the elemental analysis of the anti-diabetes medicinal plants are graphically presented in Figure 1-5. The distribution of elements in the anti-diabetic medicinal plants were determined and presented in two categories (Macro-elements and Micro-elements). The elements investigated under each category are presented in Table 2

Table 2: Categories of elements determined

S.No	Category	Element
	Macro Element	Ca
		Mg
		Na
		K
	Micro Element	Fe
		Co
		Mn
		Zn
		Cr
		Cl

3.1 Macro Elements in Anti-diabetic Medicinal Plant Samples

The distribution of Ca in the anti-diabetic medicinal plants shows the concentrations of Ca in the leave samples varied from *Sarcocephalus latifolius* (8493±323 mg/kg) to *Sclerocarya birrea* (33850.0±677 mg/kg). As presented in Fig. 1, the variability coefficient was observed to be 41% and the pairwise concentration variation differences were observed to be statistically significant (P<0.05), except for Jathropha *gossypiifolia* vs *Daniellia oliveri*. From the results, the concentration of Ca in the stem bark was observed to varied from *Sarcocephalus latifolius* (14730.0±471.0 mg/kg) to *Anogeissus leiocarpus* (79780.0±1277.0 mg/kg) with a variability coefficient of 65%. The pairwise concentration variation differences were statistically significant (P<0.05) between the respective plants samples. Further analysis of the root bark samples shows the concentration of Ca content ranged from *Jatropha gossypiifolia* (15450.0±603.0 mg/kg) to *Anogeissus leiocarpus* (48970.0±930.0 mg/kg) with a variability coefficient of 69%. Though, Ca was not detected in the root bark of *Daniellia oliveri*, all other pairwise Ca concentration variation differences between the plants samples were observed to be statistically significant (P<0.05). From the study, the order for Ca concentrations distribution were observed to follow the ranking, stem bark>root bark>leaves, on the average.

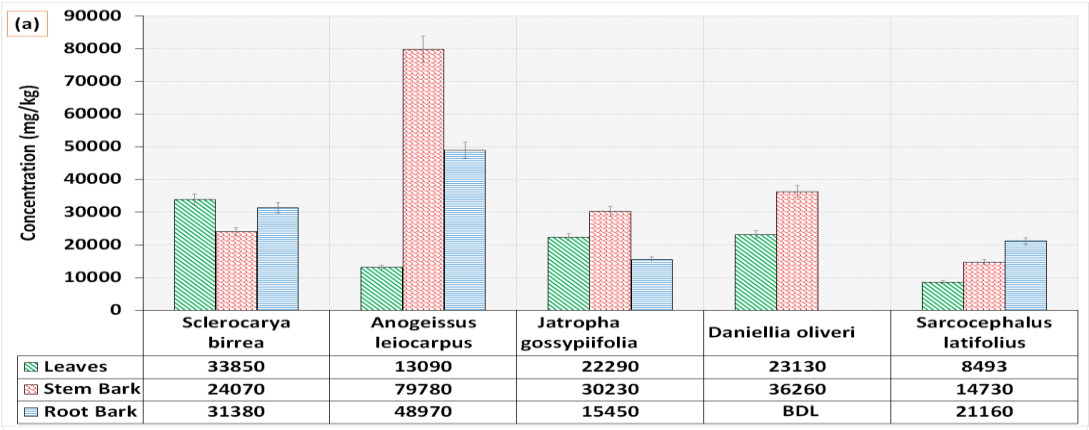
The availability of Ca in the respective medicinal plants further suggest its pharmaceutical importance; especially in regulating insulin related physiology. Studies showed that pancreatic islet responds to alterations in systemic calcium and mediates cell-to-cell communication through local increases in the concentration of extracellular Ca^{2+}, co-released with insulin [19]. Furthermore, insulin release was observed to be positively correlated with the extracellular Ca concentration. This was contained in a study were a defect in cellular glucose metabolic related actions was observed to be as a result of glucose inability to induce calcium influx [20]. Interestingly, glucose-stimulated insulin secretion by sulfonylurea compounds in type 2 diabetic patients were observed to be in part due to their capacity to raise mitochondrial calcium, essential for the generation of metabolic coupling factors [21].

Therefore, it will suffice to say that, the availability of Ca in the medical plants investigated plays a premium role in enhancing the therapeutic efficacy of the plants as remedy for diabetes mellitus. Thus, the results of this finding provided a chart that will help with informed choices of plants part and information for combination therapy. It was observed that, the stem bark tend to be the most potent source of Ca, having about 79780±1277.0 mg/kg in *Anogeissus leiocarpus follow by it root bark* (48970±930 mg/kg). However, when considering the leave part, *Sclerocarya birrea* will be the best choice (33850±677 mg/kg).

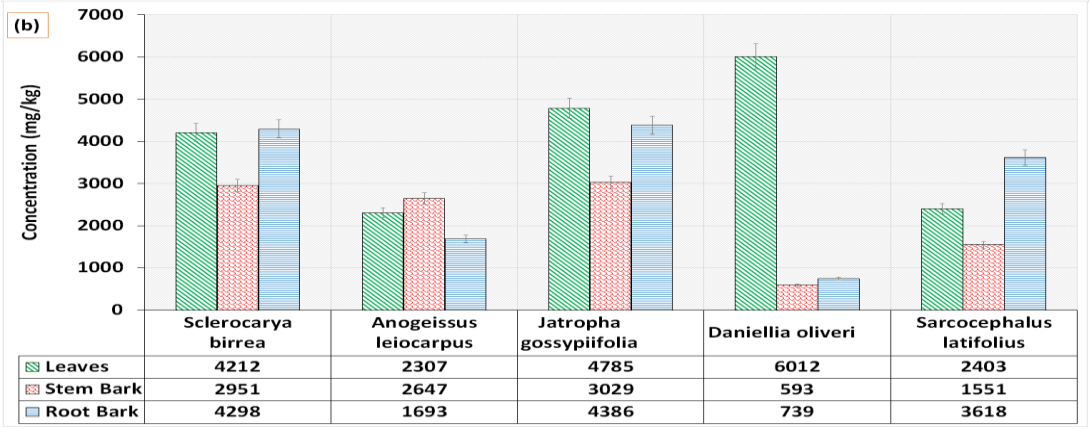
Further study revealed the concentrations of Mg in the leaves to vary from *Anogeissus leiocarpus* (2307.0±164 mg/kg) to *Daniellia oliveri* (6012±331 mg/kg) with a variability coefficient of 40%. From the result as presented in Fig. 1b, more than 60% of all pairwise concentration variation differences were observed to be statistically significant (P<0.05). The Analysis of the stem bark samples shows the Mg content ranged from *Daniellia oliveri* (593±147 mg/kg) to *Jatropha gossypiifolia (*3029±233 mg/kg) with a variability coefficient of 57%. Similarly, more than 60% of all pairwise concentration variation differences were statistically significant (P<0.05). From the root tissues samples evaluated, the concentrations of Mg was observed to vary from *Daniellia oliveri* (739±177 mg/kg) to *Jathropha gossypiifolia* (4386±307 mg/kg) with a variability coefficient of 48%. The results of Mg concentration further showed that more than 60% of all pairwise concentration variation differences in the root bark samples were statistically significant (P<0.05) and followed the distribution order leaves > root bark>stem bark, on the average.

The overall order of the plants tissues analyzed suggests that the most potent source of Mg is the leaves samples, as the order of preference revealed *Daniellia oliveri* (6012±333.1 mg/kg) followed by the leaves of *Jathropha gossypiifolia* (4785±321.0 mg/kg). The results however suggest that, the root bark sample of *Jathropha gossypiifolia* (4386±307.0 mg/kg) is a suitable alternative for a potent source of Mg, and similarly showed the stem bark sample of the same specie as another alternative potent source (3032±233 mg/kg).

Plants as indicated above containing available amount of Mg are well known for potentiating insulin and insulin mediated actions [8, 22]. From the results obtained so far, magnesium appears to be fairly and uniformly distributed within the plants parts analyzed. Study shows a direct relationship between Mg with insulin sensitivity, thus, used an indicator to predict the development of type II diabetes [23]. *In vitro* and *in vivo* studies have demonstrated that insulin may modulate the shift of Mg from extracellular to intracellular space, thus regulating diabetes mediated mechanism [8].



	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	33850	13090	22290	23130	8493
Stem Bark	24070	79780	30230	36260	14730
Root Bark	31380	48970	15450	BDL	21160



	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	4212	2307	4785	6012	2403
Stem Bark	2951	2647	3029	593	1551
Root Bark	4298	1693	4386	739	3618

Fig. 1: Analysis of Tissues Samples of Anti-diabetic Medicinal Plants showing the Concentrations of (a) Calcium, and (b) Magnesium. The results are presented as Mean±SD of three replicate analysis. Where "BDL" signified below detection limit.

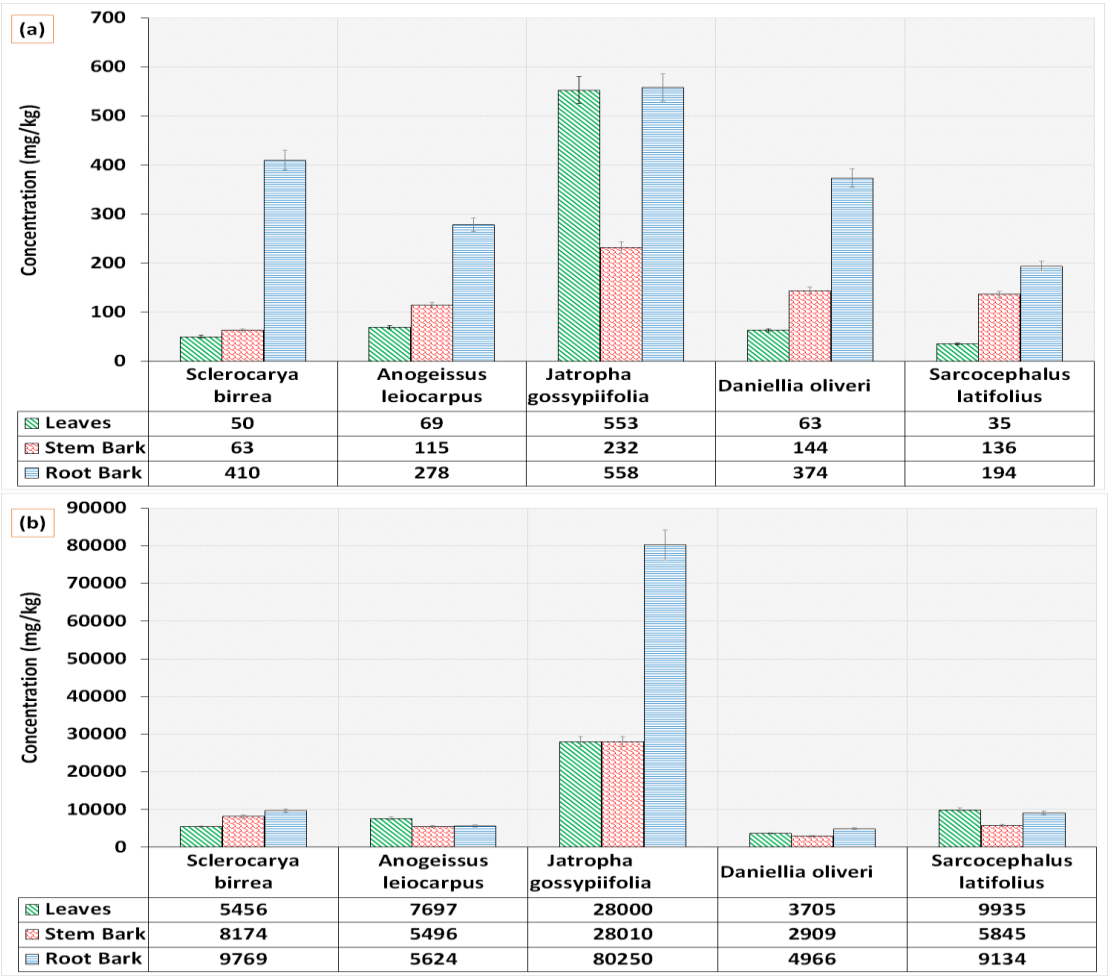
Based on the data presented in Fig. 2a, the concentrations of sodium (Na) in the leave samples was observed to vary from *Sarcocephalus latifolius* (34.5±0.3 mg/kg) to *Jathropha gossypiifolia* (553±1.00 mg/kg), with a variability coefficient of 111%. Sodium was detected in all the leaves samples analyzed and all the pairwise concentration variation differences conducted were observed to be statistically significant (P<0.05). The stem bark samples analyzed shows the concentration of Na varied from *Sclerocarya birrea* (63.3±0.4 mg/kg) to *Jatropha gossypiifolia* (232±1.0 mg/kg) with a variability coefficient of 50%. Sodium was present in all the stem bark sample analyzed with a pairwise concentration variation differences that are statistically significant (P<0.05). Furthermore, studies conducted on the root bark samples give Na concentrations ranged from *Sarcocephalus latifolius* (194±1.0 mg/kg) to *Jatropha gossypiifolia* (558±2.0 mg/kg) with a variability coefficient of 56%. Further, all the pairwise concentration variation differences between the plants samples were observed to be statistically significant (P<.05). From the results, the order of Na concentrations distribution were observed to follow the ranking, root bark>leaves>stem bark, on the average.

Evaluating the content of K in the anti-diabetic medicinal plants shows the concentration in the leaves samples varied from *Daniellia oliveri (*3705±41.0 mg/kg) to *Jathropha gossypiifolia* (28000±112 mg/kg) with a variability coefficient of 84%. As shown in Fig. 2b, all the pairwise concentration variation differences of K conducted on the leaves samples were statistically significant (P<0.05). The stem bark on the other hand, shows concentrations ranged from *Daniellia oliveri* (2909±38 mg/kg) to *Jatropha gossypiifolia* (28010±112 mg/kg) with a variability coefficient of 113%. The pairwise concentration variation differences were also observed to be statistically significant (P<0.05). Furthermore, the concentration of K in the root bark were observed to vary from *Daniellia oliveri* (4966±50 mg/kg) to *Jathropha gossypiifolia* (80250±321 mg/kg), with a variability coefficient of 121%. All the pairwise concentration variation differences of K in the stem bark samples were statistically significant (P<0.05) and followed the concentrations distributions from root bark >leaves>stem bark, on the average.

Insulin is a key defender against exogenous K load by using intracellular buffering to minimize hyperkalemia before renal excretion [24]. In addition to its electrolytic action, K is also one of the principal cation in the extracellular fluids and modulates the maintenance of the intracellular and interstitial volumes. Although, sodium deficiency is rare, its symptoms include decrease of blood pressure, dehydration, fever and dizziness. Results revealed that, Potassium and sodium are the most abundant elements in the plant materials that are interdependent [24]. The regulation of potassium is intimately involved with that of sodium and the two are largely interdependent. Plants absorb Na and K in the form of Na^+ and K^+ from soil. Potassium is the main intra-cellular ion which in association with Na is responsible for the maintenance of membrane potentials and a stable blood pressure. It is also essential for stimulating nerves [24].

Therefore, these medicinal plants investigated, in addition of being helpful therapeutically, could also serve as Na or K supplements. Sodium was present in all the samples analyzed with concentrations of (345±0.3 mg/kg) *sacrcocephalus latifolia* (553±1.00 mg/kg). Potassium is

the second most abundant element in the overall order, the leaves, stem bark and root bark samples of the medicinal plants samples investigated. The results also clearly revealed that *Jathropha gossypiifolia* maintained the lead in high K contents in root bark (80250±321mg/kg) and leaves (28000±112 mg/kg) respectively, while maintaining the same leading position for stem bark samples (28010±112 mg/kg). The root bark of *Sclerocarya birrea* (9768±59.0 mg/kg) was also found to be a good alternative to *Jathropha gossypiifolia* in terms of K content, and as the leaves of *Sarcocephalus latifolius* (9935±60.0 mg/kg).



	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	50	69	553	63	35
Stem Bark	63	115	232	144	136
Root Bark	410	278	558	374	194

	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	5456	7697	28000	3705	9935
Stem Bark	8174	5496	28010	2909	5845
Root Bark	9769	5624	80250	4966	9134

Fig. 2: Analysis of Tissues Samples of Anti-diabetic Medicinal Plants Showing the Concentrations of (a) Sodium, and (b) Potassium. The results are presented as Mean±SD of three replicate analyses

3.2 Micro Elements in Anti-diabetic Medicinal Plant Samples

From the results in Fig. 3a, it was observed that, Fe was present in all plants samples analyzed with the concentrations in the leaves varied from *Daniellia oliveri* (108±27 mg/kg) to *Jatropha gossypiifolia* (419±33 mg/kg). The concentrations in the leaves shows a variability coefficient of 87%, with only about 35% of all pairwise concentration variation differences were statistically significant ($P<0.05$). The Stem bark Fe concentration ranged from *Anogeissus leiocarpus* (0.300±0.04 mg/kg) to *Sarcocephalus latifolius* (426±31 mg/kg) with a variability coefficient of 56%. More than 60% of all the pairwise concentration variation differences of Fe in the stem bark samples were statistically ($P<0.05$) significant. The concentration of Fe in the root bark samples ranged from *Sclerocarya birrea* (222±23 mg/kg) to *Jatropha gossypiifolia* (1506±50 mg/kg) with a variability coefficient of 104%. More than 75% of all the pairwise concentration variation differences of Fe in the root bark samples were statistically significant ($P<0.05$). On the average, the order of Fe concentrations distribution is root bark >stem bark>leaves.

Iron is necessary for red blood cell formation and required for transport of oxygen throughout the body and very important for brain function. The maximum tolerable level for animals was suggested at 1000 mg/kg by the National Research Council [25]. On the other hand, the permissible limit set by FAO/WHO [26] in edible plants was 20 mg/kg. Iron is an important element for human beings and animals because it is an essential component of hemoglobin [27]. It facilitates the oxidation of carbohydrates, protein and fat to control body weight which is a very important factor in diabetes mellitus [28]. When compared with metal limit proposed by FAO/WHO [26], the concentration of Fe in this study is above the proposed permissible limit. High Fe content in these plants could be a possible risk factor for diabetes but could be good for managing anaemia as they are rich in iron (3201±96 mg/kg). Therefore, the choice of the anti-diabetes medicinal plants investigated in this study should base on the plant/or tissues with permissible amount of Fe content.

Figure 4b shows the distribution of Co in the leaves, stem bark and root bark samples of the anti-diabetic medicinal

plants investigated in this study. From the results, measurable amount of Co was detected in all the leaves samples, but was not detected in the stem bark samples of *Anogeissus leiocarpus*, *Daniellia oliveri*, and the root bark samples of *Daniellia oliveri*. The leaves concentrations varied from *Sarcocephalus latifolius* (0.07±0.02 mg/kg) to *Daniellia oliveri* (0.25±0.03 mg/kg) with a variability coefficient of 38%. Only *Jathropha gossypiifolia vs Daniellia oliveri and Daniellia oliveri vs Sarcocephalus latifolius* were statistically significant (P<0.05). Furthermore, the stem bark Co content ranged from *Sclerocarya birrea* (0.050±0.01 mg/kg) to *Sarcocephalus latifolius* (0.180±0.04 mg/kg) with a variability coefficient of 118%. About 50% of all the pairwise concentration variation differences of Co in the stem bark samples are statistically significant (P<0.05). Similarly, the root bark Co content ranged from *Anogeissus leiocarpus* (0.3±0.02 mg/kg) to *Sarcocephalus latifolius* (51.0±8.0 mg/kg) with a variability coefficient of 150%. Also, about 50% of all the pairwise concentration variation differences of Co in the root bark samples were statistically significant (P<0.05). On the average, the order of Co concentrations distribution falls into this ranking, root bark>leaves>stem bark.

Kurtzhals and Ribel [29] and Kurtzhals, *et al*., [30] found that in contrast to the conventional long-acting insulin preparations, Co^{3+}-insulin injected as a neutral, aqueous solution improves the solubility of Co^{3+}-insulin by >600 µmol/l at physiological pH and ionic strength. The results also revealed that the complex did not precipitate in the tissue after injection. Other studies providing evidence of Co-insulin potentiation include Sathianathan *et al*., [31] and Lim *et al.*, [32].

The result of this study revealed that Co concentration in the leaves samples of *Danillia Oliveri* (0.25±0.03 mg/kg) being the most potent, followed by the stem bark samples of *Sacocephalus latifolius* (0.180±0.04 mg/kg) and root bark samples of *Sarcophalus latifolius* (51.0±8.0mg/kg). The result generally suggests that while the root bark samples are the most potent source of Co, the root bark sample of *Jathropha gossypiifolia* (36.0±7.0 mg/kg) is a suitable alternative to *Sarcophalus latifolius* followed by *Sclerocarya birrea* (28.0±4.0 mg/kg).

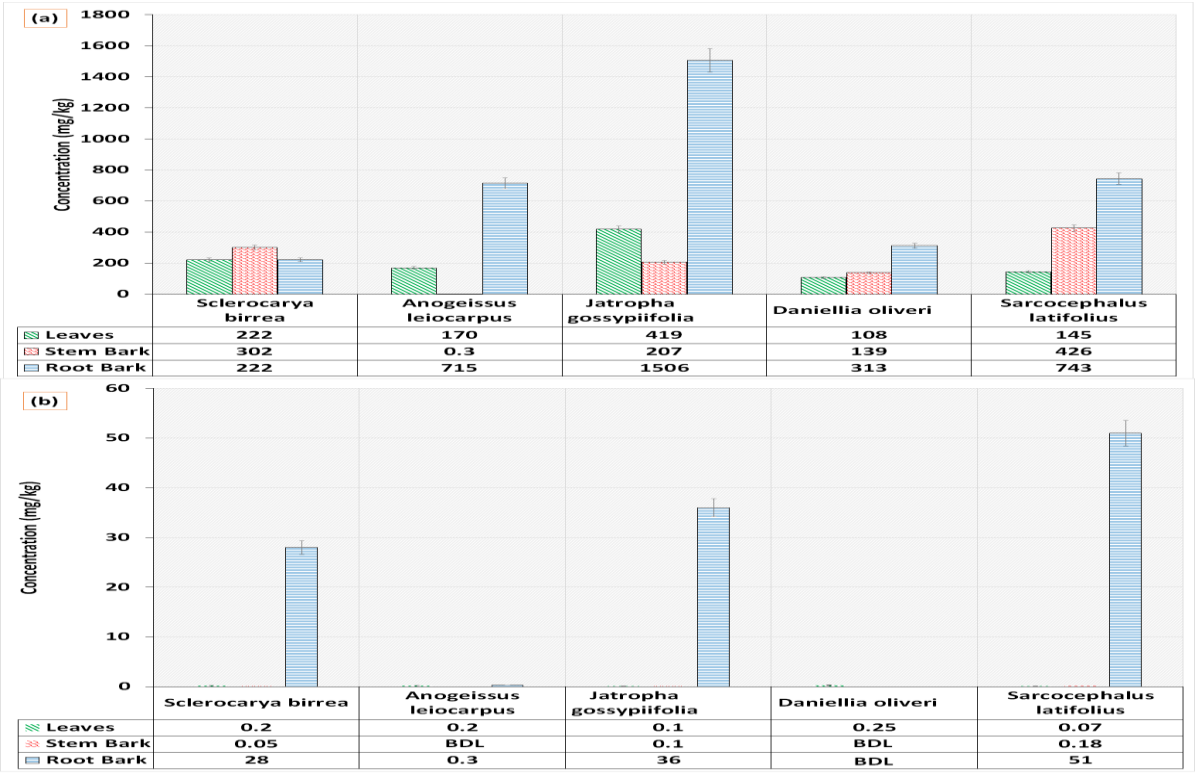


Fig. 3: Analysis of Tissues Samples of Anti-diabetic Medicinal Plants Showing the Concentrations of (a) Iron, and (b) Cobolt. The results are presented as Mean±SD of three replicate analysis. Where "BDL" signified below detection limit.

The distribution of Mn in the leaves, stem bark and root bark of the anti-diabetic medicinal plants are presented on Figure 4a. The analysis revealed the concentration of Mn in the leave samples varied from *Anogeissus leiocarpus* (32.9±0.2 mg/kg) to *Daniellia oliveri* (339.2±.07 mg/kg) with a variability coefficient of 182%. With the exception of *Sclerocarya birrea* vs *Sarcocephalus latifolius* and *Anogeissus leiocarpus* vs *Sarcocephalus latifolius*, all other pairwise concentration variation differences of Mn in the leaves samples were statistically significant (P<0.05). Further studies shows the concentration of Mn in the stem bark to vary from *Sclerocarya birrea* (25.9±0.2 mg/kg) to *Jatropha gossypiifolia* (48.4±0.2 mg/kg) with a variability coefficient of 59%. All the pairwise concentration variation differences of Mn in the stem bark samples are statistically significant (P<0.05). The root bark on the other hand shows the concentration of Mn vary from *Anogeissus leiocarpus* (37.54±0.23 mg/kg) to *Sclerocarya birrea* (114.2±0.5 mg/kg) with a variability coefficient of 79%. With the exception of *Daniellia oliveri* vs *Sarcocephalus latifolius*, all other pairwise concentration variation differences of Mn in the root bark samples were statistically significant (P<0.05) and was observed to follow the distribution order, leaves> root bark>stem bark, on the average.

Manganese deficiency can impair glucose utilization, a known enzyme activator of insulin metabolism [33, 34]. Lee *et al.*, [35] showed that Mn supplementation in normal mice on normal chow, and Mn treatment increased insulin

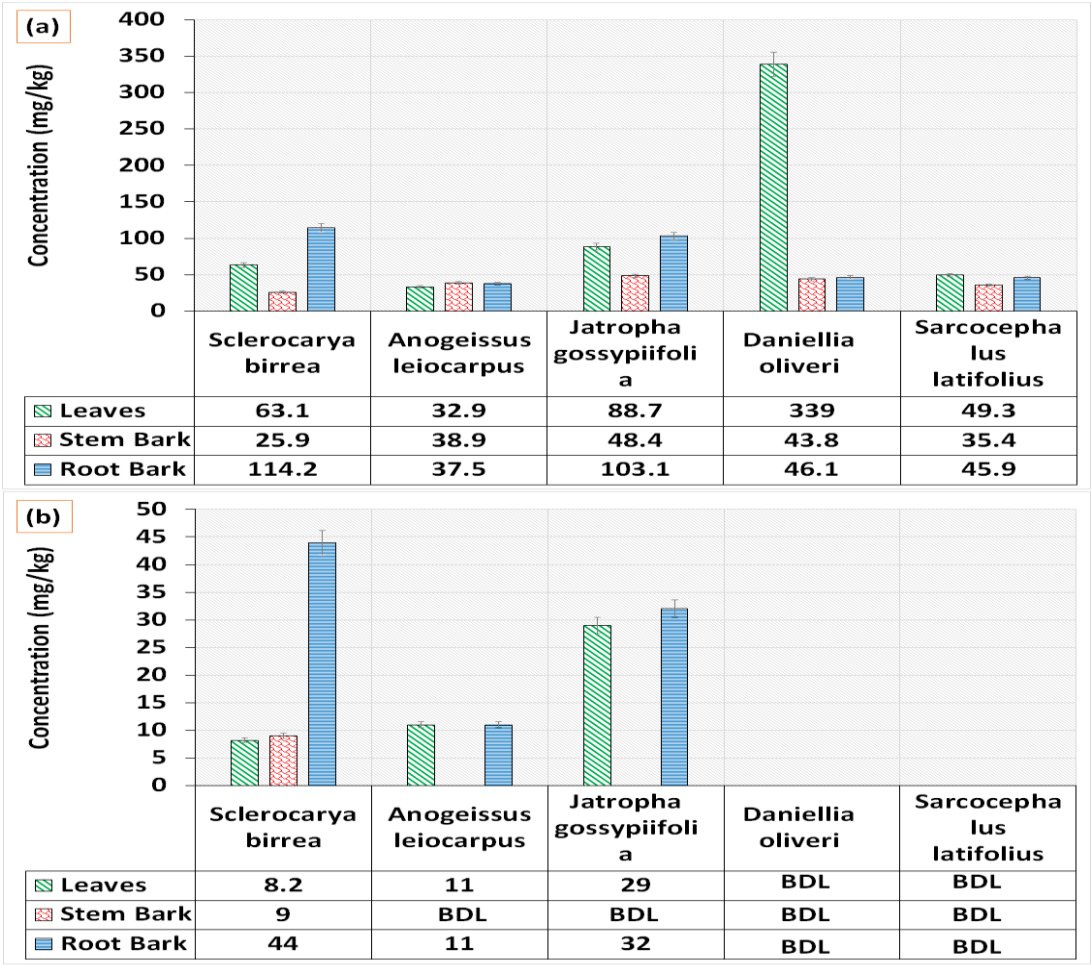
secretion which improves glucose tolerance under conditions of dietary stress These plants parts contain appreciable amount of Mn. In humans, the range between deficiency and toxicity of Mn is narrow. The recommended FAO/WHO values for adults range from 2 to 5 mg Mn/day [36]. Hence, the use of the anti-diabetic plants investigated in this study could serve as a suitable supplement in the management of diabetes mellitus.

In the management of diabetes mellitus using plant tissues with available Mn concentrations, the results provide a clue to that effect. These are the leaves of *Daniellia oliveri* (339.2±0.7 mg/kg) and the root bark of *Sclerocarya birrea* (114.2±0.5 mg/kg). *Jatropha gossypiifolia* leaves (88.7±0.4 mg/kg) and stem bark (48.4±0.2 mg/kg) are suitable alternative source of Mn.

The distribution of Zn in the leaves, stem bark and root bark of anti-diabetic medicinal plants investigated in this work are presented on Figure 4b. The result shows that Zn was not detected in all the samples of *Sarcocephalus latifolius* and *Daniellia oliveri* analyzed. It was also not detected in the stem bark samples of Anogeissus *leiocarpus*, and *Jathropha gossypiifolia*. The concentration of Zn in the stem bark was found only in *Sclerocarya birrea* (9.0±2.0 mg/kg). From the results, all pairwise concentration variation differences of Zn in the stem bark samples were statistically significant ($P<0.05$). The concentrations of Zn in the root barks ranged from *Anogeissus leiocarpus* (11±2.0 mg/kg) to *Sclerocarya birrea* (44.0±3.0 mg/kg) with a variability coefficient of 55%. From the results, all pairwise concentration variation differences of Zn in the root bark samples were statistically significant ($P<0.05$). On the average, the distribution was observed to follow the order, root bark>leaves>stem bark.

Some micro-elements have significant useful functions in the human body but the roles of some of them in fighting diabetes mellitus are not well understood. Zinc is one of such element which is an extremely important in activation and regulation of insulin levels in the blood [37] and improves the sensitivity of insulin in the management of diabetes mellitus [38]. In an experiment conducted and reported by Emdin *et al.*, [39], Zn was reported to play an active role in insulin's production in the B-cell in animal model. Played an important role in the microcrystalline character of the precipitated insulin granule. Other study shows that a reduction in Zn level was observed to affects the ability of the islet cell to produce and secrete insulin and further suggested that diabetes may be related to increased intracellular oxidants and free radicals associated with decreases in intracellular Zn and in Zn-dependent antioxidant enzymes [40, 41].

The root bark of *Sclerocarya birrea* (44.0±3.0 mg/kg) was indicated as the most potent source of Zn with *Jatropha gossypiifolia* (32.0±4.0 mg/kg) as suitable root bark alternatives. But for leaves samples, Zn may be reliably sourced in high concentrations from *Jatropha gossypiifolia* (29.0±3.0 mg/kg) and its possible substitute *Anogeissus leiocarpus* (11.0±2.0 mg/kg). Stem bark samples were indicated as relatively weak source of Zn, but where tissue availability is a challenge, the stem barks of *Sclerocarya birrea* (9.0±2.0 mg/kg) could suffice.



	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	63.1	32.9	88.7	339	49.3
Stem Bark	25.9	38.9	48.4	43.8	35.4
Root Bark	114.2	37.5	103.1	46.1	45.9

	Sclerocarya birrea	Anogeissus leiocarpus	Jatropha gossypiifolia	Daniellia oliveri	Sarcocephalus latifolius
Leaves	8.2	11	29	BDL	BDL
Stem Bark	9	BDL	BDL	BDL	BDL
Root Bark	44	11	32	BDL	BDL

Fig. 4: Analysis of Tissues Samples of Anti-diabetic Medicinal Plants Showing the Concentrations of (a) Manganese, and (b) Zinc. The results are presented as Mean±SD of three replicate analysis. Where "BDL" signified below detection limit.

Figure 5a, presents the distribution of Cr in the leave, stem bark and root bark samples of the anti-diabetic medicinal plants. In the stem bark samples, the concentration of Cr was detected only in *Sarcocephalus latifolius* (0.27±0.08 mg/kg). The concentration of Cr in the root bark sample varied from *Daniellia oliveri* (0.3±0.1 mg/kg) to *Jathropha gossypiifolia* (38.0±7.0 mg/kg) with a variability coefficient of 216%.The results revealed significant (P<0.05) differences between all pairwise concentrations in plants samples. Chromium was not detected in *Anogeissus leiocarpus*, and *Sclerocarya birrea*. The concentration of Cr in plants parts revealed that *Anogeissus leiocarpus* and *Sclerocarya birrea* were below detection limit

The result of this study suggests that the root bark sample is generally the most potent source of Cr, with the root bark of *Jathropha gossypiifolia* (38.0±20 mg/kg) being the most potent plant tissue. A suitable alternative is the root bark sample of *Sarcocephalus latifolius* (0.55±0. mg/kg).

Studies have shown that Cr potentiate insulin and potentiates the action of insulin by restoring glucose tolerance [42-44], while, poor Cr status is a factor contributing to the incidence of impaired glucose tolerance and type II diabetes [45].

The distribution of Cl in the leave, stem bark and root bark samples of the anti-diabetic medicinal plants were also investigated and presented in Figure 5b. From the results, it was observed that the concentration of Cl in leaves was higher in *Daniellia oliveri* (2035.0±35.0 mg/kg) and least in *Anogeissus leiocarpus* (77.0±10.0 mg/kg) with variability coefficient of 119%. The results further revealed significant (P<0.05) differences between all pairwise concentrations in the leave samples, except between *Anogeissus leiocarpus vs Sarcocephalus latifolius*. Stem bark concentration of Cl was highest in *Jathropha gossypiifolia* (4780.0±48.0 mg/kg) and least in *Anogeissus leiocarpus* (55.0±12.0 mg/kg) with variability coefficient of 88%. The results revealed significant (P<0.05) differences between all pairwise concentrations in the stem bark samples, except between *Anogeissus leiocarpus vs Sarcocephalus latifolius*. The concentration of Cr in the root bark samples was highest in *Sclerocarya birrea* (2291.0±34.0 mg/kg) and lowest in *Anogeissus leiocarpus* (109.0±12.0 mg/kg) with a variability coefficient of 92%. The results further revealed significant (P<0.05) differences between all pairwise concentrations in the plants samples, except between *Anogeissus leiocarpus vs Sarcocephalus latifolius.* On the average, the order of Cl concentrations distribution is stem bark> root bark>leaves.

Chloride acts as an anion of the extracellular fluid occurring in plasma, lymph, connective tissue, cartilage and bone [46].Chlorine helps to regulate acid alkali balance, stimulate production of hydrochloric acid, stimulate the liver to function as a filter for wastes and helps to distribute hormones [46]. Chloride works with Na and k, which carry an electrical charge in dissolved body fluids and is very important in regulating body pH. Chloride is also important for proper digestion of food and absorbs many elements that we need to survive as reported by Gopalakrishman *et al.*, [47].So occurrence of this element in this plants is important for these functions.

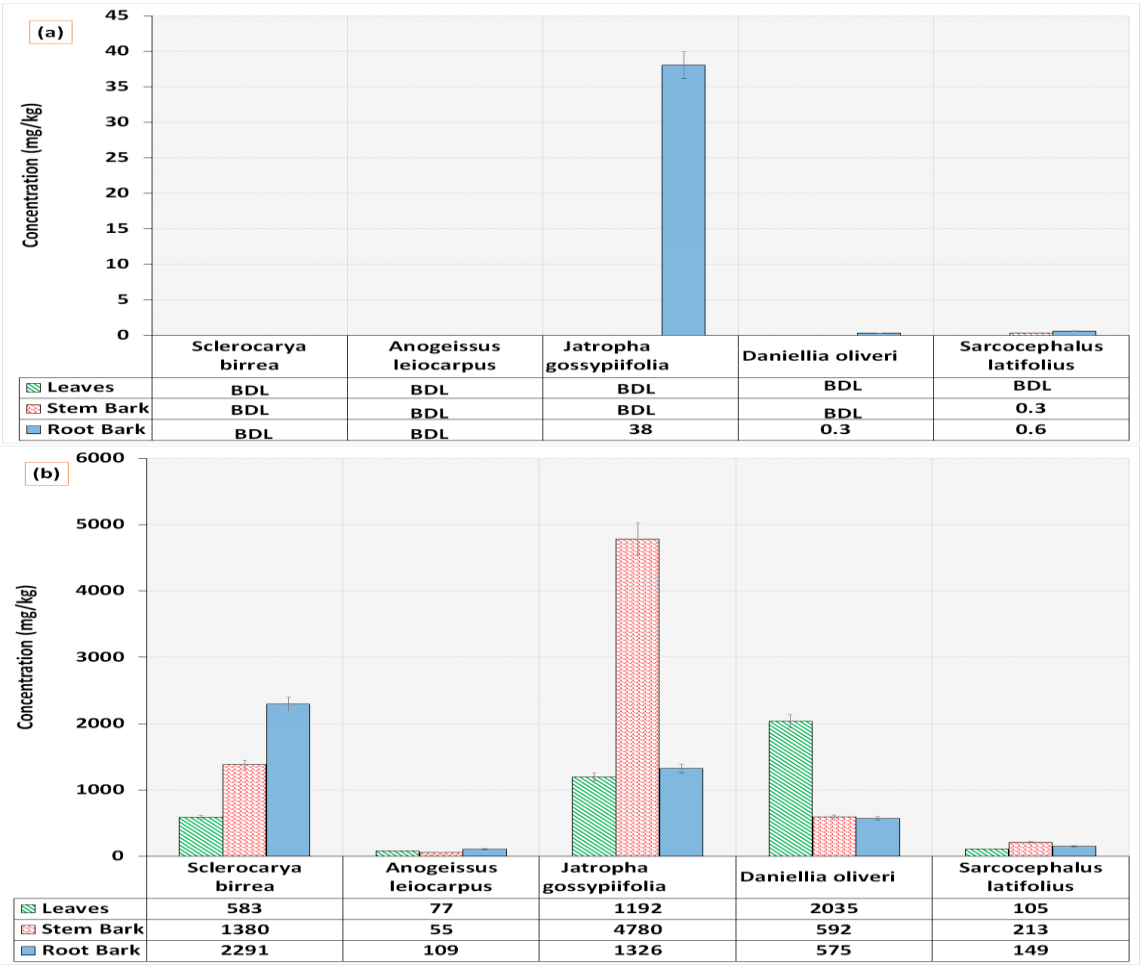


Fig. 5 Analysis of Tissues Samples of Anti-diabetic Medicinal Plants Showing the Concentrations of (a) Chromium, and (b) Chlorine. The results are presented as Mean±SD of three replicate analysis. Where "BDL" signified below detection limit.

Conclusion

In these study, INAA was used to determine quantitatively the concentrations of ten (10) elements, classified as Macro elements (Ca, K, Mg; Na), and Micro Element (Cl, Co, Cr, Fe, Mn; Zn).

From the results obtained, Ca, K, and Mg, were found in high concentrations at macro-nutrient level. Specifically, the results showed Ca in large concentrations in the stem bark of *Anogeissus leiocarpus* (79780±1277 mg/kg), while the highest concentration of K are contained in the root bark of *Jathropha gossypiifolia* (80250±321 mg/kg). Similarly, the root bark sample of *Jathropha gossypiifolia* was observed to contain the highest concentration of Na (558±2.0 mg/kg).

Under the category of micro-nutrient, Fe and Cl are toping the chart, with the stem bark containing the highest concentration of Cl (4780±48.0 mg/kg). The highest concentrations of Cr (38±20 mg/kg) and Fe (1506±50 mg/kg) were all found in the root bark samples of *Jathropha gossypiifolia*. The highest concentrations of Mn was recorded in leave sample of *Daniellia oliveri* (339.2±0.7 mg/kg) and Zn (44±3.0 mg/kg) were found in the root bark of S*clerocarya birrea*.

In general, the results indicate that all the 5 anti-diabetic medicinal plants are rich in more than one of the macro and micro elements, with about 90 % of the elements found in the tissues samples of *Jathropha gossypiifolia*. These element investigated are reported to play a vital roles in potentiating insulin, thereby aiding in the management of diabetes mellitus. These plants can served as reliable suppliers of mineral elements and medicines for the treatment and management of diabetes mellitus

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