

Human Health Risk Assessment and Trace Element Profile in Four Naturalized Medicinal Flora from Southern Nigeria

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Abstract

Medicinal plants (MPs) constitute an important component of traditional healthcare systems in Southern Nigeria, where they are widely consumed for therapeutic and nutritional purposes. However, information on their potential elemental contamination remains limited. This study evaluated the proximate composition, elemental profile in the leaves and the bioethanol extracts, the associated health risks, and the MPs efficacy. Standard analytical procedures were employed to determine proximate parameters. Elemental concentrations were quantified by inductively coupled plasma-optical emission spectroscopy. The efficacy of the MPs was determined by evaluating mineral ratios, quantitative mineral balance index, and metallo-antidiabetic potential index (MAPI). Health risk indices, including average daily dose, hazard quotient, hazard index, and extraction transfer factor, were calculated to evaluate potential risks associated with dietary exposure. The total carbohydrate of the four MPs was within 0.771 - 2.115 g glucose Equiv./100g. The crude fiber was within 44.00% - 82.00%. Essential elements were present within permissible limits, while the concentrations of toxic metals were generally below the maximum levels recommended by FAO/WHO. Vanadium and nickel ($r = -0.9537$) and aluminum and nickel ($r = -0.9843$) were strongly inversely correlated and statistically significant ($p < 0.05$). Selective enrichment was observed in copper and arsenic. The MAPI of *Microglossa pyrifolia* leaf, 3.8, was the highest. *Mentha canadensis* leaf (1.0) gave the lowest. *Lonchocarpus sericeus* and *Microglossa pyrifolia* extracts gave 0.8 and 0.3, respectively. *Tiliacora triandra* had the best Zn/Cu ratio. Risk assessment parameters suggested that the consumption of the studied MPs is likely to pose significant non-carcinogenic health risks under normal dietary intake conditions except for bioethanol extract of *L. seri-*

ceus and *M. pyrifolia*.

Keywords

Hazard Index, Hazard Quotient, Herbal Mixture, Minerals, Phytomedicine, Phytopharmaceutical, Toxicity

1. Introduction

The Sustainable Development Goals 2 and 3 (SDGs 2-3) of the UNDP (2015) advocate for zero hunger and good health and well-being of the people, respectively. To provide remedial measures for these goals, research has been ongoing in the global space on the nutritional and toxic composition and human risk assessment of various leafy vegetables. Medicinal and edible plants contribute simultaneously to nutrition security and health promotion, particularly in low- and middle-income countries where traditional diets and herbal medicines are widely used [1] [2]. Medicinal and food plants are of great importance to natural products and food chemistry researchers because they play a crucial role in health protection as preventive therapy for several diseases and disorders [3] [4]. However, elements play both a curative and preventive function against diseases. Hence, determination of elemental contents and proximate composition in MPs and good quality control of herbal products are necessary to understand the nutritive and medicinal importance [3] [5] as well as to protect consumers from contamination. Many MPs and their mixtures can present a health risk due to the presence of toxic elements [6]. Contamination may occur during growing and processing [7]. Also, pollution of soil with heavy metals from anthropogenic discharges acts as one of the sources of toxic elements in MPs. Moreover, there are many herbal products used in the Southern Nigeria healthcare system to treat some diseases in both adults and children, and the formulation of these products is based on the phytochemical composition of these medicinal plants. The elemental composition is completely ignored. It is therefore important to consider the phytomedicinal health risk assessment of some of these plant materials to ascertain if there are any long-term effects posed by some metals present in these medicinal plants, particularly in children. Moreover, the herbal products are usually prepared as an extract using solvents such as ethanol or water; it is also important to evaluate the metal concentration in the extracts. This will go a long way in providing greener procedures for the formulation of healthy herbal products.

In sub-Saharan Africa, cardiovascular diseases (CVDs), a group of heart diseases and blood vessel disorders, remain the leading global cause of death [2] [8]-[10]. Other worrisome diseases that are responsible for most of the mortality of those above the age of 45 years in Africa and globally include diabetes mellitus, hypertension, high cholesterol/hyperlipidemia, obesity, and cancer [11]. A significant number of people in Nigeria and in sub-Saharan Africa depend on the use

of traditional, complementary, and alternative medicine (TCAM) and identified biologically-based therapy, which includes herbal therapy, as the most common TCAM for health care delivery [12]. However, herbal medicine comprises herbs, herbal materials, herbal preparations, and finished herbal products that have parts of plants, other plant materials, or combinations as active ingredients [2]. These herbal products are formulated from medicinal plants of known history, but these plants may contain some trace elements that could be injurious to human health. It is therefore imperative to assess the levels of these elements in medicinal plants frequently because of climate change and the unique biodiversity of medicinal plants. Pollution of soil with heavy metals from polluted irrigation water, atmospheric dusts, automobile and industrial exhausts, pesticides, and fertilizers plays an important role in the contamination of medicinal plants [6] [13] [14]. Plants have been used for the treatment of numerous diseases [15]-[17] and are globally considered valuable sources of new drugs [18]-[20]. Both metallic and nonmetallic elements are required by the human body within certain permissible limits for growth and good health. The quantitative estimation of various trace element concentrations is important for determining the effectiveness of the medicinal plants in treating various diseases and also to understand their pharmacological action. Moreover, trace element analysis of medicinal plants can be used to decide the dosage of the herbal drugs prepared from these plant materials [6].

Literature reports suggest macronutrient and micronutrient variations as one of the major contributors to the incidence of CVDs via a complex nutrient-gene interaction [2] [21] [22]. Trace elements are involved in the formation of active chemical constituents found in medicinal plants, which are responsible for their medicinal as well as toxic properties [6]. It has also been established that some diseases, including CVDs, are a result of some relationship with abnormal trace element concentrations [23]. He [24] added that accumulated or excess specific trace elements may be linked with the risk of CVDs.

The elemental composition of the leaves of four medicinal plants under investigation-*Mentha canadensis*, *Lonchocarpus sericeus*, *Microglossa pyrifolia*, and *Tiliacora triandra*, as well as the elemental composition of the bioethanol extracts of these plants, was obtained using inductively coupled plasma-optical emission spectroscopy (ICP-OES) [18]. Before now, AAS was the major spectroscopic equipment for elemental determination, and the limitation was the inability to determine all the inorganic elements present in a given medicinal plant sample at a time. Other techniques used for the analysis of metallic elements present in medicinal herbs are flame atomic absorption spectrometry (FAAS) [25], energy dispersive X-ray fluorescence (EDXRF) [26], electrothermal atomic absorption spectrometry (ETAAS) [27], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [28], inductively coupled plasma-mass spectrometry (ICP-MS) [29], and instrumental neutron activation analysis (INAA) [30].

Therefore, the current research was designed to document the proximate, mineral compositions and investigate the phytomedicinal health risk of the mineral

composition of the leaves and the corresponding bioethanol extracts of the four selected medicinal plants widely consumed as herbs for the treatment of different ailments by the people of Southern Nigeria. As well as highlighting the importance of extraction methodology in determining not only the chemical composition but also the safety profile of medicinal plant products. The results of this research will also proffer a greener solution to Sustainable Development Goals 2 (nutrition and food security) and 3 (health and disease prevention) in Southern Nigeria.

2. Materials and Methods

2.1. Description of the Study Area

The study was carried out in selected rural communities within Akwa Ibom State, located in the South-South geopolitical zone of Nigeria. Akwa Ibom State lies between latitudes 4°32'N and 5°33'N and longitudes 7°25'E and 8°25'E and is characterized by a humid tropical rainforest climate, high annual rainfall (2000 - 3000 mm), and an average temperature of 26°C - 28°C, conditions that favour the growth of diverse medicinal plants with significant phytochemical constituents [31].

Plant samples were collected from the Nto Eton community in the Obot Akara Local Government Area, situated in the northwestern part of Akwa Ibom State. Obot Akara is predominantly an agrarian community with thick vegetation, fertile forest soils, and abundant medicinal plant species used traditionally in primary healthcare. Additional plant samples were collected from the Afaha Akpan Ekpo community in the Etinan Local Government Area, located in the central region of Akwa Ibom State. Etinan LGA is known for its dense tropical vegetation, rich biodiversity, and long cultural history of herbal medication [32]. The choice of these communities as study locations was informed by several important factors, including the rich presence of native medicinal plants that are widely used in traditional healing practices, as well as the ease with which samples could be accessed and collected within the area. In addition, the communities possess valuable indigenous knowledge regarding the identification and use of local medicinal flora. These features collectively make the study area highly suitable for researching the proximate composition, elemental composition, and phytomedicinal health risk assessment of these medicinal plants indigenous to Southern Nigeria.

2.2. Sample Collection and Identification

The plant samples were collected during the rainy season between April and May, 2025 in two communities-Nto Eton and Afaha Akpan Ekpo based on the frequent usage by the community dwellers. Three of the medicinal plant samples were collected from the Nto Eton community with the geographic coordinate of 5.1981° N, 7.5808° E in the Obot Akara Local Government Area, while one plant sample was collected from the Afaha Akpan Ekpo community with the geographic coordinate of 4.8295° N, 7.8337° E in the Etinan Local Government Area. The fresh samples were transported in paper bags to the Department of Chemistry labora-

tory at Akwa Ibom State University for preparation. Each plant species was collected based on ethnobotanical relevance, availability, and traditional use by local herbal practitioners. The leaves were harvested in the early hours of the morning to minimize moisture loss and phytochemical degradation, following the recommendations of Sofowora [33] for standard medicinal plant collection.

The plant materials were authenticated through the use of the flora of Nigeria and West Africa, a guide to the identification of some arable land weeds of West Africa, artificial intelligence (AI), the use of other publications on medicinal plants and weeds [31] [34]–[39], and finally by Dr. Johnny I. Imeh, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo and Dr. Joseph E. Okon, Department of Botany, Akwa Ibom State University. The voucher specimens of the leaves of these medicinal plants (**Figure 1**) were labeled as NPH 43 (*Mentha canadensis*), NPH 44 (*Microglossa pyrifolia*), NPH 45 (*Tiliacora triandra*), and NPH 46 (*Lonchocarpus sericeus*) and were subsequently preserved in the herbarium of the Natural Products Chemistry (NPH) unit of the Department of Chemistry, Akwa Ibom State University, Akwa Ibom State, Nigeria. *Microglossa pyrifolia*, *Tiliacora triandra*, and *Lonchocarpus sericeus* were collected from Nto Eton community in Obot Akara because the community dwellers widely incorporate these medicinal floras in their traditional healthcare delivery systems for treatment of malaria, pile, hemorrhoids, fever, and management of undescended testes in male neonates respectively. While *Mentha canadensis* was collected in Afaha Akpan Ekpo, Etinan because of its wide application as antibiotics for treatment of sexually transmitted diseases such as gonorrhea by the community dwellers.

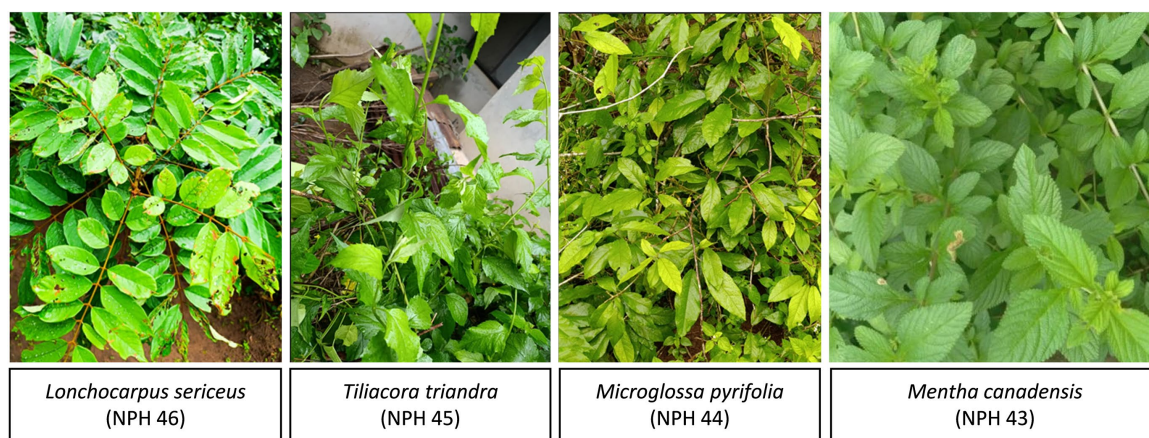


Figure 1. The botanical name and the voucher number of the four different medicinal plant samples.

2.3. Sample Preparation

The samples were washed, dried, and ground to powder using a mechanical grinder. Part of the milled samples (50 g each) were soaked in bioethanol (500 ml) for 14 days with constant agitation [19] [40]. Each extract was sieved through a muslin cloth into different beakers and finally filtered using Whatman No. 1 filter paper,

and the filtrate was concentrated under reduced pressure to dryness below 40°C. The choice of bioethanol was attributed to the fact that herbal practitioners usually soak the leaves of these medicinal plants in the gin and serve the decoction to patients. Also, bioethanol is completely a green solvent from bio-renewable products like palm wine.

2.4. Proximate Analysis

Moisture content was determined using a moisture analyzer machine model XY-105MW. The recommended methods of the Association of Official Analytical Chemists (AOAC) [41] were used for the determination of crude fiber, lipid, and ash content, while total carbohydrate and crude proteins were determined following literature reports [42] [43] with slight modifications.

For the estimation of total carbohydrate (TC), anthrone solution was freshly prepared by dissolving 200 mg of anthrone in 100 ml of ice-cold 95% (v/v) H₂SO₄. The stock standard solution (SSS) was prepared by dissolving 100 mg of analytical grade glucose in distilled water and making it up to 100 mL in a standard volumetric flask. Working standard solution (WSS) was prepared by diluting 10 mL of SSS to 100 mL with distilled water in a standard volumetric flask such that the concentration of glucose in the WSS was 100 µg/mL. The powdered plant sample was hydrolyzed by weighing 100 mg of the ground powdered sample into a boiling tube, followed by the addition of 5 mL of 2.5 N HCl and keeping it in a boiling water bath for 3 h. The mixture was cooled to room temperature and neutralized with solid CaCO₃ until the effervescence ceased. After these, the content of the boiling tube was made up to 100 mL with distilled water. The homogenate was transferred to a centrifuge tube and centrifuged at 300xg for 10 min at room temperature. The supernatant was collected for TC determination. To estimate TC, 0.2 - 1.0 mL aliquots of WSS were pipetted into five different test tubes clearly labeled. The hydrolyzed plant samples, 0.5 and 1 mL, were measured into two different test tubes. The content of all the test tubes was made up to 1 mL with distilled water. 1 mL of distilled water was added to another tube to serve as a blank. 4 mL of freshly prepared anthrone reagent was added to all the tubes, including the blank. The test tubes were well vortexed and kept in a boiling water bath for 8 min. The content in the tubes was rapidly cold, and the absorbance of the green to dark green colour developed against the reagent blank was read at 630 nm using a METASH UV/VIS spectrophotometer (model: UV-5200UV/VIS). The TC of the sample was calculated using the linear calibration curve $Y = 0.2908X + 0.2404$ ($R^2 = 0.8745$), where Y is the absorbance and X is the concentration. The TC in the sample was expressed as g glucose equiv./100g of sample.

For determination of total proteins (TP), 100 mg of the powdered sample was ground with 10 mL of 0.2 M phosphate (pH 7.2) using a mortar and pestle. The phosphate buffer was prepared by mixing 28 mL of 0.2 M monobasic sodium phosphate and 72 mL of 0.2 M dibasic sodium phosphate solutions, respectively. The homogenate was transferred to a centrifuge tube and centrifuged at 3000xg

for 10 min at room temperature. The supernatant was collected and made up to 10 mL with distilled water. About 1 mL of the supernatant was diluted to 10 mL with distilled water and used for TP estimation. SSS was prepared by dissolving 50 mg of bovine serum albumin (BSA) in distilled water and making up to 50 mL in a standard volumetric flask. WSS was prepared by diluting about 10 mL of SSS to 50 mL with distilled water in a standard volumetric flask such that the concentration of BSA in the WSS was 200 µg/mL. Then, 0.2 - 1.0 mL of the WSS was pipetted into five different test tubes. Also, 0.5 and 1.0 mL of the protein extract were measured into two different test tubes. The content of all the tubes was made up to 1 mL with distilled water. 1 mL of distilled water was measured into another tube to serve as the blank. 5 mL of alkaline copper (II) solution was added to all the tubes, including the blank. The tubes were well vortexed and allowed to stand for 10 min at room temperature. After these, 0.5 mL of Folin-Ciocalteu reagent (1 N) was added to all the tubes, including the blank. The tubes were again vortexed and incubated in the dark at room temperature for 30 min. The absorbance of the blue colour developed against the reagent blank was measured at 660 nm with a METASH UV/VIS spectrophotometer (model: UV-5200UV/VIS). The standard calibration curve obtained by plotting the concentration of BSA on the x-axis and the respective absorbance on the y-axis ($Y = 0.1228X + 0.1015$; $R^2 = 0.9644$) was used to calculate the amount of protein in the sample and express it as g BSA equivalents/100 g sample.

The alkaline copper (II) solution was prepared by mixing 50 mL of 0.5% sodium trioxocarbonate (IV) in 0.1 N sodium hydroxide and 1 mL of 0.5% copper (II) tetraoxosulphate (VI) in 1% potassium sodium tartrate.

2.5. Determination of Elemental Composition

The elemental composition of the raw leaves of the medicinal plants and those of the bioethanol extracts was determined by the method described in the literature [18] [44] using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2.6. Mineral Ratios

As stated by Odukoya *et al.* [2], relevant mineral ratios to the present study as well as human health (Ca/P, Ca/Mg, Na/K, Fe/Cu, and Zn/Cu ratios) were estimated from levels of the concerned minerals in the analyzed medicinal plants and the bioethanol extracts.

2.7. Quantitative Mineral Balance Index (MBI)

The mineral balance index (MBI) was also developed to provide a quantitative framework for evaluating the nutritional synergy of mineral ratios in the medicinal plants under investigation.

The optimal physiological reference for each ratio is given as follows: Ca/P is 1.50, Na/K is 0.50 (upper desirable limit), and Zn/Cu is 8.00 [8].

Equations (1)-(3) show the normalized scores for the three metal ratios. All scores being constrained between 0 and 1. When the Ca/P ratio is >1.5, Equation (1) applies, but if it is <1.5, the equation is modified as in Equation (4).

$$S_{Ca/P} = 1 - \frac{[(Ca/P) - 1.5]}{1.50} \quad (1)$$

$$S_{Na/K} = 1 - \frac{(Na/K)}{0.50} \quad (2)$$

$$S_{Zn/Cu} = \frac{(Zn/Cu)}{8.0} \quad (3)$$

$$S_{Ca/P} = \frac{[(Ca/P)]}{1.50} \quad (4)$$

Finally, mineral balance index (MBI) was calculated using Equation (5).

$$MBI = \frac{S_{Ca/P} + S_{Na/K} + S_{Zn/Cu}}{3.0} \quad (5)$$

The nutritionally excellent mineral balance is achieved when the MBI value is ≥ 0.75 ; when it is between 0.50 and 0.74, the mineral balance is judged to be good; and it is a moderate balance when the value of the MBI is between 0.25 and 0.49. Poor mineral balance is obtained when the MBI value is <0.25 [8].

2.8. Health Risk Assessment

To assess the potential health risk that may be associated with the continuous ingestion of the selected medicinal plants as well as the bioethanol extracts, toxic heavy metals (Cd, As, and Pb), essential and conditionally toxic metals (Cu, Zn, Mn, and Al), and other selected metals (Fe, Cr, Ni, Co, Ag, and Se) were evaluated based on the average daily dose, hazard quotient, hazard index, and non-carcinogenic risk analysis of the heavy metals.

2.8.1. Average Daily Dose (ADD)

The expression as provided in Ametepey *et al.* [45] and Udoekpo *et al.* [14] (Equation (6)) was used in estimating the ADD.

$$\text{Average daily dose (ADD)} = \frac{Ci \times IR \times EF \times ED}{BW \times AT} \quad (6)$$

Ci, IR, EF, ED, BW, and AT are given as the mean metal concentration in the sample (mg/kg), ingestion rate, exposure frequency, exposure duration, body weight of the consumer, and average time, respectively. The input parameters as shown in Table 1 [45] [46] were used to characterize the ADD values.

Table 1. Parameters to characterize the ADD value.

Exposure parameters	Symbols	Units	Value
Concentration	C	mg/kg	-
Ingestion rate	IR	kg/day	0.0022

Continued

Exposure frequency	EF	days/year	365
Exposure duration	ED	years	70
Adult body weight	BW	kg	70
Children body weight	BW	kg	16
Average time	AT	years	25,550 days

2.8.2. Non-Carcinogenic Risk (NCR) Analysis

The NCR of consumption of Cd, As, and Pb in the raw leaves and bioethanol extracts of the medicinal plants was assessed using hazard quotient (HQ) values [2] [3] [47]. According to Ametepey *et al.* [45], HQ is a ratio of the determined dose of a contaminant with respect to an oral reference dose considered detrimental.

The standard United States Environmental Protection Agency (USEPA, 2019) oral reference doses (RfDs) (Table 2), *i.e.*, metal toxicity (dose) response [2], were used to compute the hazard quotient using Equation (7). As reported in Lim *et al.* [48], if the exposure level (ADD) of the considered heavy metals in the present study exceeds the corresponding RfD (*i.e.*, HQ > 1), there may be concern for potential non-carcinogenic effects. However, when HQ is <1, there is an acceptable risk [2] [46].

$$\text{Hazard Quotient (HQ)} = \frac{\text{Average daily dose (ADD)}}{\text{Reference dose (RfD)}} \quad (7)$$

However, since ADD is already exposure-normalized, HQ is equivalent to targeted hazard quotient (THQ) in this study.

Also, the hazard index (HI) is used to estimate the potential human health risk when more than one heavy metal is considered [2] [45]. But in the present study, HI represents cumulative risk from Cd, As, and Pb trace metals and was estimated as the sum of HQs, as shown in Equation (8).

$$\text{Hazard Index (HI)} = \sum \text{HQ}_i \quad (8)$$

It has also been reported in literature that HI ≥ 1 indicates a potential health concern or an unacceptable risk of non-carcinogenic effects on health, while HI < 1 signals an acceptable level of risk or no significant non-carcinogenic risk [2] [46] [49].

2.8.3. Extraction Transfer Factor (ETF)

The extraction transfer factor (ETF) for some toxic heavy metals (Cd, As, and Pb), essential and conditionally toxic metals (Cu, Zn, Mn, and Al), and other selected macro and micronutrients (Mg, Ca, Fe, V, Cr, K, Na, and Ni) was calculated based on Equation (9) for the four medicinal plants under investigation.

$$\text{ETF} = \frac{C_{\text{extract}}}{C_{\text{raw}}} \quad (9)$$

where C_{extract} is the metal concentration in the bioethanol extract and C_{raw} is the concentration of the metal in the raw plant sample. ETF ≥ 1 signals enrichment

during extraction or selective enrichment risk. $ETF < 1$ signals reduction during extraction [50] [51].

2.9. Development of a Quantitative Metallo-Antidiabetic Potential Index (MAPI)

In order to evaluate whether these medicinal plants may exert part of their therapeutic action through metallonutrient-phytochemical synergy that is particularly relevant in oxidative stress-mediated diseases like diabetes, a Metallo-Antidiabetic Potential Index (MAPI) was constructed. This is so because the prevalent diabetic situation among the people of Southern Nigeria is worrisome and needs urgent attention. This was based on the assumption that MAPI integrates essential insulin-supporting metals such as Mg, Zn, Cr, V, Cu, K, and P; correlates strength, that is, synergistic clustering among these elements; and antagonizes toxic metals such as Cd, Pb, and As. MAPI was computed based on Equation (10) [8]. The proposed biological weighting factors for the essential elements are given in **Table 3**, while the toxic penalty factors for Cd and Pb were 1.5, respectively, and As was 1.4. MAPI values > 1.5 imply very high metallo-antidiabetic potential; when it is between 1.0 and 1.0 - 1.5, it is adjudged high potential; between 0.5 and 1.0, it is moderate potential; and < 0.5 is summarized as low potential.

$$MAPI = \frac{\sum_{i=1}^n (C_{ix} W_{ix} S_i)}{1 + \sum_{j=1}^n (T_{jx} / R_j)} \quad (10)$$

where:

C_i is the relative concentration of the specific metal i detected in the plant tissue (normalized).

W_i is the assigned biological weighting factor or potency coefficient for that specific metal based on its established clinical significance in alleviating insulin resistance or enhancing carbohydrate pathway enzymes.

S_i is the synergy coefficient (mean positive correlation within Mg-Ni-P-K-Zn-Cu).

T_j is the toxic metal weighting factor.

R_j is the mean correlation of toxic metals with essential metals.

Normalized concentration of the essential metals was evaluated using the relationship in Equation (11) [8].

$$C_{\text{norm}} = \frac{C_{\text{plant}}}{C_{\text{reference}}} \quad (11)$$

where:

C_{norm} is the normalized concentration of the metal i .

C_{plant} is the concentration of the targeted metal (Zn, Mg, Cr, V, and K).

$C_{\text{reference}}$ is the baseline value for each element provided by WHO.

The reference concentrations of elements normalization for some metals according to WHO/FAO are Zn is 50.0 mg/kg, Cr is 0.5 mg/kg, Mn is 200.0 mg/kg, Cu is 30.0 mg/kg, V is 2.0 mg/kg; Cd is 0.3 mg/kg. For the macro elements, WHO

does not have baselines for these elements, and as such, the Upper Continental Crust (UCC) or world average soil/plant values were used. According to UCC, K is 14,000.0 mg/kg, and Mg is 3200 mg/kg.

2.10. Statistical Analyses

All the experiments were conducted in duplicate, and the data were expressed as mean $\pm$ SEM (standard error of mean) using a one-way analysis of variance (one-way ANOVA), two-factor analysis of variance (two-way ANOVA), and principal component analysis (PCA) with Tukey's HSD test. A probability value less than 0.05 ($P < 0.05$) between means was considered statistically significant.

Table 2. Oral reference doses (RfDs) (mg/kg/day) for some selected metals.

Metal	RfD (mg/kg/day)	Basis
Cr (III)	1.5000	EPA/IRIS
Mn	0.1400	EPA/IRIS
Al	1.0000	EPA/NCEA screening value
Fe	0.7000	EPA screening value
Ni	0.0200	EPA/IRIS
As	0.00006	Current EPA/IRIS
Se	0.0050	EPA/IRIS
Zn	0.3000	EPA/IRIS
Cu	0.0400	EPA/IRIS
Ag	0.0050	EPA/IRIS
Cd (food)	0.0010	EPA/IRIS
Co	-	No suitable current EPA oral RfD used
Pb	-	No current EPA consensus RfD

Table 3. The proposed biological weighting factors for the essential elements.

Metal	Functional Role	Weight (W1)
Mg	Insulin sensitivity, ATP	1.5
Zn	Insulin storage, SOD	1.5
Cr	Insulin potentiation	1.7
V	Insulin mimic	1.7
Cu	Redox enzyme support	1.2
K	Insulin secretion	1.3
P	Energy metabolism	1.2

3. Results and Discussion

3.1. Percentage Yield and Proximate Analysis

The highest yield of 24.27% was observed in the leaf extract of *M. pyrifolia* with voucher No. NPH 44. The next was the leaf extract of *L. sericeus* (NPH 46) with a

yield of 17.79% followed by *T. triandra* (NPH 45) with the yield of 17.37%. The lowest yield of 13.92% was obtained for the leaf extract of *M. canadensis* with voucher No. NPH 43. The observed pattern of percentage yield was in line with the theory of similarity and mutual solubility [1]. The moisture, total ash, crude fiber, total protein, lipid, and carbohydrate of the leaves of NPH 43 - NPH 46 collected locally were determined and shown in **Table 4**. The results show that there is marked variation in the proximate composition among the medicinal plants. The moisture content of the leafy medicinal plants ranged between $55.450\% \pm 7.806\%$ and $82.685\% \pm 1.888\%$ on a fresh weight basis. There was no significant ($p < 0.05$) difference in the moisture content of *Mentha canadensis* (NPH 43) (82.685%) and *Microglossa pyrifolia* (Lam.) Kuntze (NPH 44) (82.040%). The lowest value was obtained in *Tiliacora triandra* (NPH 45) (55.450%). There were significant ($p < 0.05$) differences in the concentration of the lipid contents among the four medicinal plants, such that NPH 43 had the highest value of 8.32%, followed by *Lonchocarpus sericeus* (NPH 46) (4.895%) and 3.930% from NPH 44. The least value of 2.960% was obtained in NPH 45. The studied medicinal plants were rich in crude fiber, with the highest value of 82.000% occurring in NPH 46 and the lowest value of 44.000% occurring in NPH 45. There was no significant difference in the ash content of the four medicinal plants; the lowest content of 4.941% was found in NPH 46, while the highest value of 13.420% was found in NPH 44. There were no significant differences in the concentration of total carbohydrate (g glucose equiv. /100g) and total protein (g BSA equiv. /100g) among the four medicinal plants studied. The total carbohydrate ranged between 0.771 and 2.115 g glucose equiv. /100g, while total protein ranged between 0.295 and 1.138 g BSA equiv. /100g. In a nutraceutical assessment of twenty-seven conventional leafy vegetables of South India, *B. alba* leaf was observed to have the highest moisture value of 92.47%; *S. oleracea* leaf had the highest ash content of 26.80 g/100g dw; *M. arvensis* leaf had the highest carbohydrate of 36.34%; *M. oleifera* leaf had the highest protein of 15.38%; and the fiber content for the twenty-seven leafy vegetables varied between 6.47 and 9.48 g/100g dw [3].

It is reported that utilization of underutilized food crops can minimize the risk of malnutrition and alleviate food insecurity. This results in the utilization of indigenous fruit and vegetables, which supply the essential nutrients to humans for their well-being [3] [52]. The proximate composition of the leaves of NPH 43 - NPH 46 (**Table 4**) reveals a characteristic profile of high moisture and fiber contents, low lipid and protein levels, and minimal available carbohydrates. From a natural products chemistry perspective, this pattern reflects the dominance of structural biopolymers (cellulose, hemicellulose, and lignin) and water-rich cellular matrices, rather than storage macromolecules such as starches or oils.

The exceptionally high crude fiber values (44% - 82%), which are higher than those reported in literature [3], indicate a predominance of β -linked polysaccharides that are resistant to human digestive enzymes. These fibers are chemically stable, highly polymerized carbohydrates whose glycosidic linkages (β -1, 4) limit

enzymatic hydrolysis in the small intestine. Dietary fiber is the most prominent nutritional feature of these plants. High fiber intake delays gastric emptying; reduces postprandial glucose absorption; binds bile acids, contributing to cholesterol reduction; and serves as a substrate for gut microbiota fermentation, producing short-chain fatty acids (SCFAs). Hence, all four plants exhibit functional-food characteristics, particularly beneficial for metabolic health. Anju *et al.* [53] reported low fiber content in *C. quadrangularis* (9.34%) and *S. acmella* (8.42%), and they cannot be regarded as beneficial for metabolic health as observed in these four medicinal plants.

Conversely, the very low total carbohydrate values (0.77 - 2.12 g glucose equivalent/100g) indicate minimal digestible sugars, suggesting limited glycemic impact. Low crude lipid contents (2.96% - 8.32%) reflect the absence of lipid storage tissues and the dominance of membrane-bound phospholipids and waxes. The low lipid levels minimize caloric density and reduce cardiovascular risk associated with saturated fat intake. Lipids present are likely structural or polar lipids rather than triacylglycerols.

Protein levels (<1.2 g/100g) are consistent with leaves serving primarily metabolic and structural roles rather than nitrogen storage. Protein levels are nutritionally modest and insufficient to meet daily requirements alone. These plants may therefore be regarded as dietary complements rather than protein sources. The high moisture content (55% - 83%) enhances palatability and hydration but also implies a short shelf life for fresh leaves due to susceptibility to microbial spoilage. From a physiological standpoint, moisture aids digestion and gastrointestinal transit. Moderate ash values (5% - 13%) indicate appreciable mineral content, consistent with the elemental composition of these plants as would be seen later in this study, supporting roles in electrolyte balance and enzyme function.

However, the World Health Organization does not prescribe strict upper limits for proximate components in medicinal plants but has provided dietary reference guidance supports. FAO/WHO [8] recommends high dietary fiber (>25 g/day) for adults, low fat (<30% of total caloric intake), and low free sugars to alleviate metabolic disease risk. Based on these criteria, all four medicinal plants align well with WHO recommendations for healthy plant-based diets, particularly for populations at risk of non-communicable diseases. Consumption of these leaves influences human biochemistry primarily through fiber-mediated mechanisms rather than macronutrient energy provision. These results suggest that these leaves may play a vital role in the gastrointestinal system as a bowel motility enhancer, improving gut microbiome composition and increasing SCFA production. Also, consumption of these leaves may enhance reduction in the rate of glucose absorption and improvement of insulin sensitivity in the endocrine system. In the cardiovascular system, these leaves may help lower lipid absorption and improve cholesterol metabolism. In hepatic metabolism, reduced glycemic load decreases hepatic gluconeogenesis and lipogenesis. In the renal system, low protein and lipid loads reduce metabolic stress. The caloric value of these medicinal plants was cal-

culated by multiplying the crude protein, lipid, and carbohydrate by 4, 9, and 4 kcal, respectively, and taking the sum of their products [54]. The calorific values of the fresh leaves were as follows: NPH 43 (84.529 Kcal/100g), NPH 46 (51.711 Kcal/100g), NPH 45 (30.440 Kcal/100g), and NPH 44 (43.758/100g). The calorie was low for NPH 43 *and* very low for the remaining three medicinal plants. These values suggest that the plants are nutrient-dense but energy-poor, suitable for calorie-restricted diets.

Diabetes is one of the dreaded diseases of the century that has the ability to destroy various organs slowly. Based on the proximate components of these plants, all four plants demonstrate properties favorable for diabetes management, but with varying strengths. Among the four species, NPH 46 demonstrates the strongest potential for use in diabetes management due to its exceptionally high fiber content (82%) (Table 4) and extremely low digestible carbohydrate levels. NPH 44 and NPH 45 also exhibit favorable profiles, combining high fiber with minimal caloric contribution. NPH 43, although slightly higher in lipid content, still maintains a low carbohydrate load and high fiber proportion, making it suitable for glycemic control when consumed in moderation. Mechanistically, the antidiabetic potential of these plants arises from delayed carbohydrate digestion, reduced intestinal glucose uptake, enhanced insulin sensitivity, and modulation of gut-derived metabolic signals. These properties support their traditional use as dietary adjuncts in the management of metabolic disorders.

From a systems-level biochemical perspective, the consumption of these leaves may influence multiple organ systems. In the gastrointestinal tract, high fiber enhances bowel motility and supports a healthy microbiome. In the endocrine system, reduced glucose absorption improves insulin sensitivity and stabilizes blood glucose levels. The liver benefits from a reduced glycemic load, which may lower gluconeogenic and lipogenic activity, while the cardiovascular system benefits indirectly through improved lipid metabolism and reduced cholesterol absorption. The low protein and lipid contents also reduce metabolic and excretory burden on the kidneys.

Table 4. Proximate composition of the leaves of *Mentha canadensis*, *Lonchocarpus sericeus*, *Tiliacora triandra* and *Microglossa pyrifolia* (Lam.) Kuntze.

Plant Sample	Moisture Content (%)	Crude Lipid (%)	Crude Fiber (%)	Ash Content (%)	Total Carbohydrate (g glucose Equiv./100g)	Total Protein (g BSA Equiv./100g)
<i>Mentha canadensis</i> (leaves)	82.685 ± 1.888	8.321 ± 0.154	57.500 ± 0.070	11.110 ± 0.035	2.115 ± 0.532	0.295 ± 0.204
<i>Lonchocarpus sericeus</i> (leaves)	79.150 ± 0.056	4.895 ± 0.049	82.000 ± 1.414	4.941 ± 0.030	0.776 ± 0.654	1.138 ± 0.722
<i>Tiliacora triandra</i> (leaves)	55.450 ± 7.806	2.960 ± 0.028	44.000 ± 4.243	11.440 ± 0.141	0.771 ± 0.277	0.179 ± 0.230
<i>Microglossa pyrifolia</i> (Lam.) Kuntze (leaves)	82.040 ± 1.032	3.930 ± 0.042	48.500 ± 2.121	13.420 ± 0.085	1.528 ± 0.546	0.569 ± 0.331

3.2. Elemental Composition

The present study also assessed the concentrations of macro-, micro-, trace-, and toxic elements in the raw leaves of the four medicinal plants and their bioethanol extracts (Figures 2-4). For all four medicinal plants, the raw leaves show much higher concentrations of most metals (K, Ca, Mg, Fe, Mn, Al, and Zn) than their bioethanol extracts. Significant ($p < 0.05$) differences were noticed among the elements in the raw leaves and bioethanol extracts. The elemental profiles of the raw medicinal plant leaves and their corresponding bioethanol extracts reveal clear and chemically meaningful trends that can be explained by principles of organic chemistry, metal speciation, and solvent selectivity. These trends also have important implications for human health and the safe use of these plants in both traditional and complementary medicine. Among the alkali and alkaline-earth metals (Na, K, Mg, and Ca), K and Ca respectively were significantly ($p < 0.05$) higher in the raw leaves (78052.2344 mg/kg and 13659.1963 mg/kg) for NPH 43; 115159.8203 mg/kg and 14247.4570 mg/kg for NPH 46; 153181.5469 mg/kg and 14875.8867 mg/kg for NPH 44; 57771.7578 mg/kg and 14317.5107 mg/kg for NPH 45 than in the bioethanol extracts (52256.9648 mg/kg and 2350.2454 mg/kg) for NPH 43; 14271.5977 mg/kg and 307.7700 mg/kg for NPH 46; 35127.1563 mg/kg and 617.4084 mg/kg for NPH 44; 26851.0625 mg/kg and 2275.98145 mg/kg for NPH 45, respectively (Figure 1).

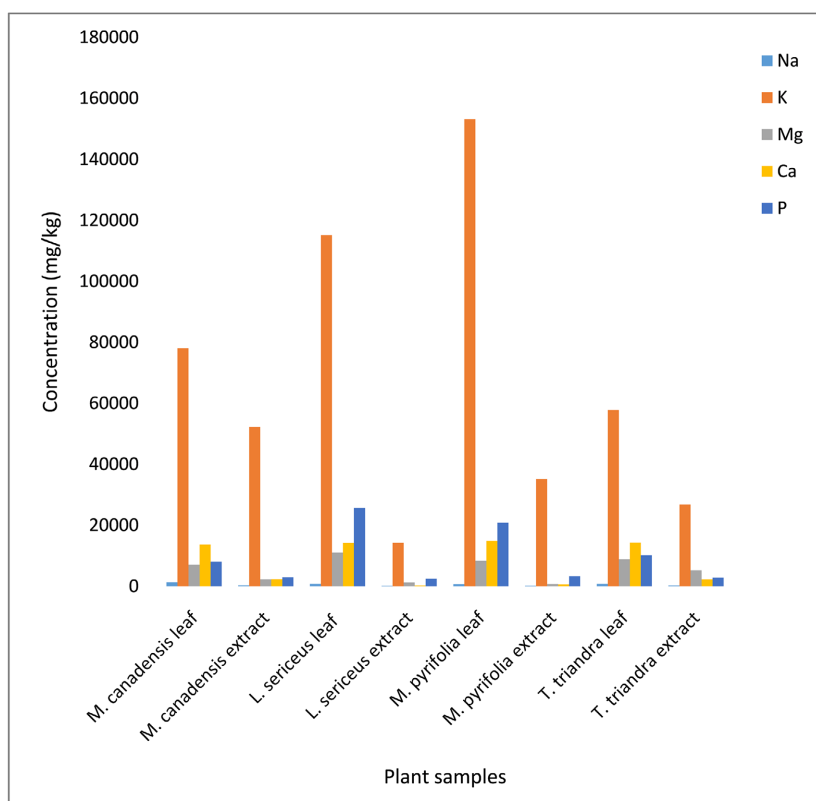


Figure 2. Distribution of macro-elements (mg/kg) in the leaves and the bioethanol extracts of the selected medicinal plants.

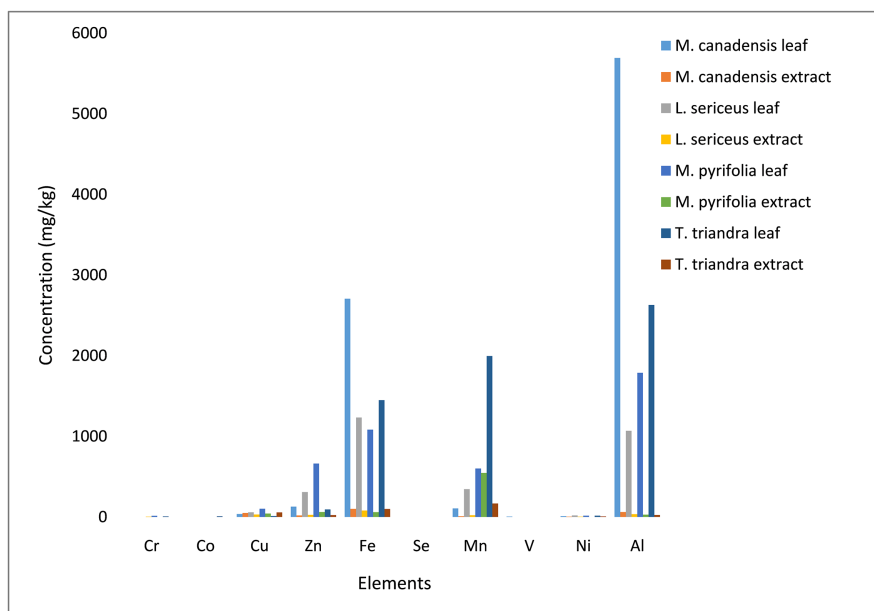


Figure 3. Distribution of essential and probably essential trace elements (mg/kg) in the leaves and the bioethanol extracts of the selected medicinal plants.

Transition metals (Fe, Mn, Zn, Cr, and Ni) were strongly depleted in the bioethanol extracts with more concentration in the raw leaves. Selective enrichment was observed in Cu and As, such that concentrations of Cu in the bioethanol extracts for NPH 43 of 48.3851 mg/kg and NPH 45 of 56.9312 mg/kg were relatively higher than those of the raw plant samples of 35.7322 mg/kg and 11.6912 mg/kg, respectively (**Figure 3** and **Figure 4**). Also, for arsenic, a concentration of 6.8313 mg/kg was observed in the bioethanol extract of NPH 43, while the concentration in the raw plant sample was 4.3376 mg/kg (**Figure 4**). In NPH 45, the concentration of arsenic in the bioethanol extract was 5.4299 mg/kg, while the concentration in the raw plant sample was 1.1842 mg/kg. Toxic metals (Pb, Cd, and Hg) were mostly absent or very low in the raw leaves and mostly absent in the bioethanol extracts (**Figure 4**).

The leaves of some vegetables and medicinal plants have been reported to contain appreciable quantity of major elements such K, Ca, Mg etc. [2]-[4] [55]. In this research a consistent pattern across all four species is that most metals, mainly the macro- and the micronutrients occur at substantially higher concentrations in the raw leaves than in the bioethanol extracts. This is mostly applicable to the under listed macro- and micronutrients- K, Ca, Mg, Fe, Mn, Zn, Na, and Al (**Figure 2** and **Figure 3**). This may suggest that metals in plant tissues are predominantly present in strongly bound or immobilized forms, including coordination to proteins and enzymes; chelation by carboxylate- and phosphate-rich biopolymers (pectin and phytate); and precipitation as insoluble salts (e.g., calcium oxalate). These species are poorly soluble in ethanol, a solvent of intermediate polarity that preferentially extracts neutral or weakly polar organic compounds. Consequently, bioethanol extraction selectively enriches secondary metabolites such as

phenolics, flavonoids, alkaloids, and terpenoids, while excluding most inorganic ions. The marked reduction in Al, Fe, Mn, Zn, and Ca in the extracts therefore reflects chemoselective fractionation, rather than loss or degradation of metals. The plant extracts can thus be regarded as organically enriched but metal-attenuated fractions of the plants.

The behaviour of essential alkali and alkaline-earth metals—potassium, calcium, magnesium, and sodium—shows that they were present at very high concentrations in the leaves, particularly potassium, which dominates the elemental profile of all species (**Figure 2**). These metals play essential physiological roles in plants and are relatively mobile within tissues. Their partial but incomplete transfer into the bioethanol extracts suggests limited solubilization via hydrated ion pairs or weak association with extractable organic acids. However, the significantly lower levels in extracts indicate that bioethanol is inefficient at disrupting ionic and lattice-bound forms of these metals. From a nutritional perspective, consumption of raw leaves would provide higher dietary intake of these essential elements, whereas extracts deliver much lower mineral loads.

Transition metals such as Fe, Mn, Zn, Cr, Ni, and Co show strong depletion or complete absence in most bioethanol extracts (**Figure 3**). These metals are typically coordinated in highly stable environments—metalloenzymes, porphyrin systems (Fe), or polyphenol-metal complexes that are insoluble in ethanol. Their retention in the plant matrix further supports the view that ethanol extraction does not significantly disturb strong coordination networks within the leaf tissue.

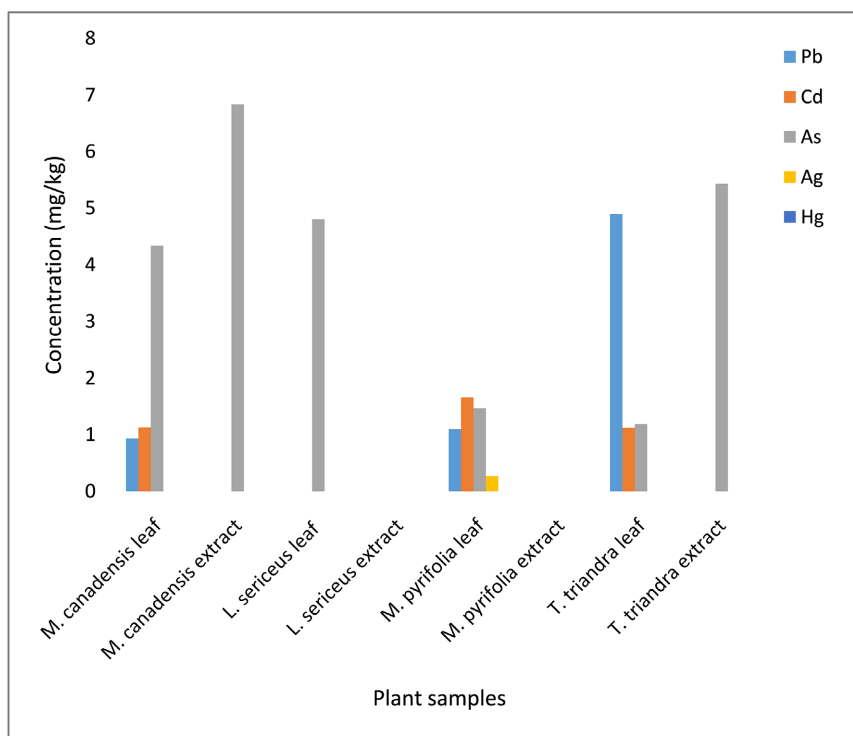


Figure 4. Distribution of potentially toxic trace elements (mg/kg) in the leaves and the bioethanol extracts of the selected medicinal plants.

An exception is copper, which in several cases appears at comparable or even higher levels in extracts than in leaves. Copper is known to form stable, ethanol-soluble coordination complexes with polyphenols, flavonoids, and nitrogen- or sulfur-donor ligands. Co-extraction of Cu with such bioactive compounds is therefore chemically plausible and suggests that copper may be present in the extracts as part of organometallic or coordination complexes rather than as free ions.

Potentially toxic metals, including Pb, Cd, Hg, and Ag, were detected at low concentrations in the leaves and were largely absent from the bioethanol extracts (**Figure 4**). Where present, these metals are likely immobilized through strong binding to cell wall components or precipitation as insoluble complexes. Importantly, their concentrations in the extracts fall within or below typical WHO guideline limits for herbal medicines, indicating that bioethanol extraction substantially reduces toxic metal exposure. Although arsenic was detected in some samples, its levels were generally low and further reduced in extracts, suggesting limited bioavailability through ethanol extraction. Nevertheless, chronic consumption of raw plant material containing trace levels of As, Pb, or Cd could pose long-term health risks, particularly affecting the nervous system, kidneys, and liver.

The biochemical impact of consuming these medicinal plants depends strongly on the form of intake. Raw leaves, being mineral-rich, may contribute beneficial essential elements such as K, Ca, Mg, Fe, and Zn. However, prolonged consumption could also increase exposure to Al and Mn, elements that have been associated with neurotoxicity and oxidative stress when present in excess. In contrast, bioethanol extracts provide a concentrated source of organic bioactive compounds with significantly reduced metal content. This minimizes metal-induced oxidative stress and limits potential interference with human metal homeostasis. Extracts are therefore more likely to exert their therapeutic effects through modulation of enzymatic pathways, antioxidant defenses, and inflammatory responses, rather than through mineral supplementation.

Quantitative FAO/WHO Compliance Assessment of Metal Content

To assess safety, the measured metal concentrations were compared with WHO/FAO permissible limits for medicinal plants and herbal materials. Emphasis was placed on toxic metals (Pb, Cd, As, Hg) and nutritionally relevant trace metals (Cu, Zn, Ni), as these most directly affect human health. Across all four species, Pb, Cd, and Hg concentrations in bioethanol extracts were below WHO guideline limits, while levels in raw leaves were generally low but more variable (**Figure 4**). Lead was detected only at trace levels in some leaves and was absent in all extracts. Cadmium occurred at very low levels in leaves and was completely excluded by ethanol extraction. Mercury was not detected in any sample. Arsenic showed sporadic occurrence, with some enrichment in particular extracts; however, the concentrations remained within the WHO permissible range for herbal materials ($\leq 1 - 5 \text{ mg}\cdot\text{kg}^{-1}$). Overall, bioethanol extracts demonstrated full compliance with WHO

safety limits for toxic metals, whereas raw leaves, although still compliant, present a relatively higher cumulative exposure risk if consumed chronically.

The levels of some of the essential and conditionally toxic metals, such as copper and zinc, in both leaves and extracts were within WHO-recommended limits ($\text{Cu} \leq 20 - 40 \text{ mg}\cdot\text{kg}^{-1}$; $\text{Zn} \leq 50 - 100 \text{ mg}\cdot\text{kg}^{-1}$). Copper was selectively retained in some extracts, consistent with ethanol-soluble Cu-polyphenol complexes, but concentrations remained well below toxic thresholds. Nickel levels in leaves approached or slightly exceeded recommended values in some species, while extracts consistently showed lower concentrations, remaining within acceptable limits. Manganese and aluminum, although not strictly regulated at low levels, were present at relatively high concentrations in raw leaves. Chronic intake of Mn- or Al-rich plant material may pose neurological or metabolic risks, particularly with long-term consumption [8]. Their substantial reduction in bioethanol extracts therefore represents a significant safety advantage.

However, NPH43 leaf had high K, Ca, Mg, Fe, and trace amounts of Pb and Cd that were within WHO limits. The bioethanol extract was fully in compliance with WHO limits. For NPH 46, there was an elevated concentration of K and Mn; Ni was detected in a reduced amount within the WHO permissible range in the raw leaf, while the bioethanol extract had all the metals present in concentrations (**Figures 2-4**) below WHO limits. This implies an improved toxicological profile. NPH 44 leaf had a very high concentration of Mn and Al with a trace amount of Pb (**Figure 2** and **Figure 4**). There was a strong reduction of Mn and elimination of Pb and Cd in the extract. The concentrations were in compliance with the WHO standards. Also, the raw leaf of NPH 45 had an elevated concentration of Mn and Al, with trace amounts of Pb. Cd and Pb were absent in the extract, while Cu was present in an elevated amount but within WHO limits. Overall, all bioethanol extracts satisfy WHO safety criteria, while raw leaves, although largely compliant, show higher cumulative metal burdens.

3.3. Pearson's Correlation Coefficient Analysis

Pearson's correlation coefficient analysis was performed to study the relationship between the concentrations of elements in the leaves of the selected medicinal plants. The results are presented in **Table 5**. Correlation among elements reveals statistically significant ($p < 0.05$) inter-element relationships among *M. canadensis*, *L. sericeus*, *M. pyrifolia* (Lam.) Kuntze, and *T. triandra* leaves, indicating structured elemental co-accumulation rather than random distribution. The correlation study revealed that there was a more inverse correlation between elements than there was a direct proportional correlation. This observation is the reverse of that of Arumugam *et al.* [3]. The reason may be due to the fact that many metals were determined in this study, while selected metals were determined in the former. Also, the geochemical-biological clustering patterns among macro-, micro-, and trace metals in *M. canadensis*, *L. sericeus*, *M. pyrifolia* (Lam.) Kuntze, and *T. triandra* leaves revealed a regular pattern. This pattern reflects the shared uptake

pathways, competitive ionic transport, metalloenzyme requirements, metal-secondary metabolite complexation, and implications for metabolic diseases such as diabetes.

Among elements, the correlations between Mg and Ni ($r = 0.9159$), Mg and P ($r = 0.7821$), Ca and Cr ($r = 0.7434$), Ca and Ni ($r = 0.6521$), Ca and P ($r = 0.5778$), Ca and Zn ($r = 0.8087$), Ca and Cu ($r = 0.6647$), Ca and Ag ($r = 0.8050$), Ca and K ($r = 0.6882$), Ba and Th ($r = 0.8735$), V and Th ($r = 0.8309$), Cr and Zn ($r = 0.6835$), Cr and Ag ($r = 0.8949$), Cr and Cd ($r = 0.8779$), Al and Th ($r = 0.8516$), Al and Na ($r = 0.9313$), Fe and Th ($r = 0.8986$), Ni and P ($r = 0.8714$), As and Na ($r = 0.5653$), P and Zn ($r = 0.6693$), P and Cu ($r = 0.6903$), P and K ($r = 0.7744$), Zn and Ag ($r = 0.9324$), Cu and Ag ($r = 0.8669$), Ag and Cd ($r = 0.6527$), Ag and K ($r = 0.8255$), and Th and Na ($r = 0.8685$) were strongly positively correlated at $p < 0.05$ but were not statistically significant at $p < 0.05$. On the other hand, Ba and V ($r = 0.9945$), Ba and Al ($r = 0.9772$), Ba and Fe ($r = 0.9981$), Ba and Na ($r = 0.9874$), V and Al ($r = 0.9869$), V and Fe ($r = 0.9866$), V and Na ($r = 0.9701$), Mn and Co ($r = 0.9711$), Mn and Pb ($r = 0.9551$), Al and Fe ($r = 0.9694$), Fe and Na ($r = 0.9905$), Co and Pb ($r = 0.9748$), Zn and Cu ($r = 0.9751$), Zn and K ($r = 0.9695$), and Cu and K ($r = 0.9923$) were strongly directly correlated and statistically significant at $P < 0.05$ (**Table 5**). This means an increase in the concentration of one element results in corresponding increase in the other. This cluster likely reflects shared geochemical origin and similar soil-binding affinities. The strong direct correlation between Fe and Al suggests co-accumulation likely from similar soil geochemical origins and binding to phenolic ligands (tannins, flavonoids, etc.) and adsorption to aluminosilicate matrices, while V appears to co-migrate within Fe-oxide fractions.

Magnesium showed strong direct correlations with Ni and P and a moderate association with K (**Table 5**). Magnesium is essential for ATP stabilization (Mg-ATP complex), insulin receptor tyrosine kinase activity, and glycolytic enzyme activation. Mg is a cofactor for more than 300 enzymes required for ATP stabilization [8]. In a similar study by Ntuli [4], Mg was observed to correlate directly with Ca, P, Na, and Zn and correlated inversely with Mn. Low Mg is strongly associated with insulin resistance and type 2 diabetes. Phosphorus is central to ATP generation and glucose phosphorylation, while potassium regulates membrane depolarization and insulin secretion from pancreatic β -cells. The coordinated presence of Mg-P-K therefore suggests biochemical synergy supporting glucose metabolism and insulin sensitivity. Zn's strongly significant ($P < 0.05$) positive correlation with Cu ($r = 0.9751$) and moderately positive correlation with Cr ($r = 0.6835$) represent a redox-regulated antidiabetic micronutrient network. Zinc is indispensable for insulin crystallization and storage in β -cells and forms the catalytic core of Cu/Zn-superoxide dismutase (SOD). Copper contributes to redox enzymes such as cytochrome oxidase and SOD, while chromium potentiates insulin receptor signaling by enhancing receptor phosphorylation and glucose transport (GLUT4 activation) [3] [56].

Table 5. Correlation among elemental composition of *Mentha canadensis*, *Lonchocarpus sericeus*, *Microglossa pyrifolia* (Lam.) Kuntze, and *Tiliacora triandra* leaves.

Element	Mg	Ca	Ba	V	Cr	Mn	Al	Fe	Co	Ni	As	P	Zn	Cu	Pb	Ag	Cd	Th	K	Na
Ca	0.2930																			
Ba	-0.7146	-0.8657																		
V	-0.7785	-0.8088	0.9945																	
Cr	-0.4211	0.7434	-0.3235	-0.2251																
Mn	0.0769	0.2937	-0.3790	-0.3842	0.2394															
Al	-0.8326	-0.7736	0.9772	0.9869	-0.1517	-0.2366														
Fe	-0.6795	-0.8935	0.9981	0.9866	-0.3739	-0.3590	0.9694													
Co	0.0075	0.0569	-0.1798	-0.1995	0.0639	0.9711	-0.0542	-0.1520												
Ni	0.9159	0.6521	-0.9274	-0.9537	-0.0223	0.1673	-0.9843	-0.9117	0.0122											
As	0.0075	-0.6646	0.4286	0.3651	-0.8161	-0.7563	0.2270	0.4508	-0.6236	-0.0682										
P	0.7821	0.5778	-0.7405	-0.7497	-0.0067	-0.3197	-0.8447	-0.7426	-0.4779	0.8714	0.2104									
Zn	0.1072	0.8087	-0.5571	-0.4860	0.6835	-0.3081	-0.5301	-0.6028	-0.5239	0.4343	-0.2681	0.6693								
Cu	0.0942	0.6647	-0.4245	-0.3602	0.5534	-0.5107	-0.4353	-0.4698	-0.6996	0.3665	-0.0605	0.6903	0.9751							
Pb	0.0942	0.0907	-0.0991	-0.0120	0.2358	0.9551	0.0595	-0.0830	0.9748	-0.1320	-0.7320	-0.5846	-0.4499	-0.6322						
Ag	-0.1960	0.8050	-0.4271	-0.3340	0.8949	-0.1263	-0.3301	-0.4813	-0.3333	0.1891	-0.5231	0.3696	0.9324	0.8669	-0.1954					
Cd	-0.7947	0.3377	0.1433	0.2388	0.8779	0.2125	0.3292	0.0949	0.1370	-0.4903	-0.7257	-0.4591	0.3380	0.2425	0.3510	0.6527				
Th	-0.4722	-0.9379	0.8735	0.8309	-0.5552	-0.0069	0.8516	0.8986	0.2271	-0.7728	0.3644	-0.8150	-0.8901	-0.8080	0.2326	-0.7690	-0.0936			
K	0.2169	0.6882	-0.5052	-0.4501	0.4901	-0.4913	-0.5305	-0.5452	-0.6851	0.4735	-0.0277	0.7744	0.9695	0.9923	-0.6440	0.8255	0.1387	-0.8511		
Na	-0.6032	-0.9120	0.9874	0.9701	-0.4465	-0.4674	0.9313	0.9905	-0.2605	-0.8570	0.5653	-0.6446	-0.5683	-0.4121	-0.2078	-0.4939	-0.0029	0.8685	-0.4792	

¹Significant; $p < 0.05$; ²Data show the Pearson correlation coefficients between the elemental composition.

Vanadium exhibits extremely high positive correlations with Fe and Al, suggesting co-localization in oxide fractions. Biologically, vanadyl (VO^{2+}) species mimic insulin by activating insulin receptor substrate pathways and enhancing glycogen synthesis. In plant matrices rich in flavonoids, vanadium may exist as stable vanadium-polyphenol complexes, potentially enhancing bioavailability and reducing toxicity.

The studied taxa are known to accumulate phenolics, flavonoids, tannins, and alkaloids. Polyphenols chelate transition metals (Fe^{3+} , Cu^{2+} , Zn^{2+} , Cr^{3+} , and V^{4+}) through ortho-dihydroxyl and carbonyl groups, forming coordination complexes that stabilize redox-active metals, reduce Fenton-driven ROS production, enhance antioxidant activity, and modulate enzyme inhibition (α -amylase, α -glucosidase). Thus, the correlation patterns suggest the leaves act as organometallic phytochemical systems rather than simple elemental reservoirs.

However, among the elements Mg and Ba ($r = -0.7146$), Mg and V ($r = -0.7785$), Mg and Al ($r = -0.8326$), Mg and Fe ($r = -0.6795$), Mg and Cd ($r = -0.7947$), Ca and Ba ($r = -0.8657$), Ca and V ($r = -0.8657$), Ca and Al ($r = -0.7736$), Ca and Fe ($r = -0.8935$), Ca and As ($r = -0.6646$), Ca and Th ($r = -0.9379$), Ca and Na ($r = -0.9120$), Ba and Ni ($r = -0.9274$), Ba and P ($r = -0.7405$), Ba and Zn ($r = -0.5571$), Ba and K ($r = -0.5052$), V and P ($r = -0.7497$), Cr and As ($r = -0.8161$), Cr and Th ($r = -0.5552$), Mn and As ($r = -0.7497$), Mn and Cu ($r = -0.5107$), Al and P ($r = -0.8447$), Al and Zn ($r = -0.5301$), Al and K ($r = -0.5305$), Fe and Ni ($r = -0.9117$), Fe and P ($r = -0.7426$), Fe and Zn ($r = -0.6028$), Fe and K ($r = -0.5452$), Co and As ($r = -0.6236$), Co and Zn ($r = -0.5239$), Co and Cu ($r = -0.6996$), Co and K ($r = -0.6851$), Ni and Th ($r = -0.7728$), Ni and Na ($r = -0.8570$), As and Pb ($r = -0.7320$), As and Ag ($r = -0.5231$), As and Cd ($r = -0.7257$), P and Pb ($r = -0.5846$), P and Th ($r = -0.8150$), P and Na ($r = -0.6446$), Zn and Th ($r = -0.8901$), Zn and Na ($r = -0.5683$), Cu and Pb ($r = -0.6322$), Cu and Th ($r = -0.8080$), Pb and K ($r = -0.6440$), Ag and Th ($r = -0.7690$), Th and K ($r = -0.8511$), were inversely correlated but were not statistically significant at $p < 0.05$. Nonetheless, V and Ni ($r = -0.9537$) and Al and Ni ($r = -0.9843$) were also inversely correlated and statistically significant at $P < 0.05$ (Table 5). This implies a decrease in the concentration of one element leads to corresponding decrease in the other. Marked inverse correlations (e.g., Fe-Ni, Al-Mg, and Na-Ni) also indicate ionic competition during uptake, consistent with shared divalent transport pathways.

Cadmium and lead display correlations with redox metals (e.g., Cd-Cr, Pb-Mn), implying environmental co-occurrence. However, inverse correlations with Zn and Mg suggest partial competitive exclusion, which may reduce toxic metal bioavailability in certain samples. Strong negative correlations between Fe and Ni, Fe and Mg, Al and Mg, and Na and Ni reflect ionic competition. For example, Fe^{2+} and Ni^{2+} compete at divalent metal transporters (DMT1-like systems). High Fe reduces Ni uptake, and Mg^{2+} and Al^{3+} antagonism suggests selective uptake control. Invers correlations between toxic metals and key essential metals (e.g., As-

Zn, As-Mn) suggest partial biochemical discrimination. This is pharmacologically important because chronic Pb, Cd, and As exposure worsens oxidative stress and diabetic complications. Plants with inverse correlation patterns may offer safer nutraceutical profiles [3].

3.4. Mineral Ratios

The assessment of mineral ratios is a better indicator of nutritional quality than focusing on the concentration of individual elements [2] [57]. With the exception of the Na/K ratio, which had almost the same values, the statistical evaluation of mineral ratios in the raw leaves and the bioethanol extracts of the medicinal plants' leaves (Table 6) indicated higher mineral ratios in the raw leaves than in the bioethanol extracts. The highest Ca/P and Zn/Cu in the raw leaves were recorded in NPH 43 and NPH 45, respectively, with NPH 44 and NPH 46, respectively, having the least ratios. Also, the Ca/P and Zn/Cu mineral ratios among the four medicinal plants were higher than the Na/K ratio. This corroborates the observation made by Odukoya *et al.* [2]. The Ca/Mg ratio was within 1.2863 and 1.9283 in the raw leaves. The values in the bioethanol extracts were markedly reduced, especially in *L. sericeus* of 0.2576. The Fe/Cu ratio was extremely high in the raw leaves (10.6337 - 123.9078), especially *T. triandra* with very high Fe dominance. The ratio of Fe/Cu was much reduced in the bioethanol extracts.

The mineral ratios (Ca/P, Ca/Mg, Na/K, Fe/Cu, and Zn/Cu) of the raw leaves and corresponding bioethanol extracts of *M. canadensis*, *L. sericeus*, *M. pyrifolia*, and *T. triandra* provide a more physiologically relevant assessment of nutritional quality than absolute elemental concentrations. According to Odukoya *et al.* [2], these ratios govern mineral bioavailability, metabolic balance, and disease risk modulation. According to literature reports, availability of calcium in the body depends on its ratio to phosphorus [10]. An appropriate Ca/P ratio is vital for calcium-phosphate homeostasis [58]. From the result of the analysis, raw leaves show moderate-to-high Ca/P ratios (0.56 - 1.68), while the bioethanol extracts show significantly reduced ratios (0.13 - 0.80) (Table 6). The optimal Ca/P ratio for human nutrition is ~1.0 - 2.0, which supports efficient calcium absorption, bone mineralization, and prevention of metabolic bone disorders [59]. Hence, *M. canadensis* with a Ca/P ratio of 1.68 and *T. triandra* of 1.41 (Table 6) show excellent Ca/P balance, making them beneficial for bone health and osteoporosis prevention. *L. sericeus* with 0.56 of Ca/P and *M. pyrifolia* with 0.71 are moderate and are still nutritionally useful. The lower Ca/P ratios in the bioethanol extracts reflect ethanol's poor ability to extract calcium salts, reducing their bone-health contribution. This implies raw leaves are superior for skeletal and mineral metabolism support.

The optimal Ca/Mg ratio, which enhances neuromuscular balance, is between 1.5 and 2.5, and the Ca/Mg ratio of the raw leaves for the four medicinal plant samples of 1.2863 - 1.9283 (Table 6) falls within this range. Therefore, raw leaves of these MPs support proper nerve and muscle function of the human body. The

bioethanol extracts were markedly reduced with the least value of 0.2576 in *L. sericeus*, indicating Mg dominance.

Nevertheless, the Na/K ratio is an indicator for cardiovascular health; in this present study, Na/K ratios across all the samples are extremely low. The values for raw leaves vary between 0.0048 and 0.0165, while those of bioethanol extracts vary between 0.0052 and 0.0109 (**Table 6**). For the Na/K ratio to be nutritionally significant, its value must be <1.0 (ideally <0.6), and this is strongly associated with low blood pressure, reduced cardiovascular disease risk, and improved electrolyte balance. The four medicinal plants exhibit exceptionally favourable Na/K ratios far below critical thresholds. The high potassium relative to sodium promotes vasodilation, sodium excretion, and reduced hypertension risk and supports blood pressure regulation and cardiovascular protection. Consumption of K in diets so as to compensate for excessive intake of Na to have a more desirable ratio has been encouraged [58]. Houston [21] indicated that the high Na/K ratio of modern diets has contributed to hypertension, stroke, and coronary heart disease. These plants are cardioprotective, suitable for hypertensive patients, and cardiovascular disease prevention diets.

The Fe/Cu ratio is an indicator for hematological balance; its optimal value for human nutrition ranges between 10.0 and 20.0. This ratio was extremely high (10.6337 - 123.9078) (**Table 6**) for the raw leaves, especially *T. triandra*, with the highest value of 123.9078 showing Fe dominance. This result shows that the raw leaves of these medicinal plants are excellent iron sources with high anti-anemic potential. But it should be noted that excessive Fe/Cu may impair copper utilization. The ratio was highly reduced (1.4332 - 2.6084) (**Table 6**) in the bioethanol extracts. The bioethanol was more balanced but less nutritionally potent for iron supply.

The Zn/Cu ratios play an important role in enzymatic and immune functions of the body. Literature reported that a high Zn/Cu ratio may increase serum cholesterol levels [60]. Odukoya *et al.* [2] and Ooi *et al.* [61] reported that a high Zn/Cu ratio may neither bring about an imbalance in these minerals' bioavailability nor increase the risk of cardiovascular abnormalities. In this study, raw leaves have high Zn/Cu ratios of 3.59 - 7.99, while the bioethanol extracts have much lower ratios of 0.37 - 1.45. However, the optimal Zn/Cu ratio for human health is typically 5 - 10, which supports antioxidant enzyme function (example, superoxide dismutase), immune competence, and hormonal and metabolic balance. Hence, *T. triandra* (7.99) and *M. pyrifolia* (6.51) show optimal Zn/Cu balance, indicating strong antioxidant potential and enhanced immune support. *L. sericeus* (5.27) also falls within the optimal range. Bioethanol extracts show reduced Zn/Cu ratios; this observation may be attributed to preferential extraction of Cu-organic complexes and lower Zn solubility in ethanol. Hence, raw leaves are more beneficial for immune function and oxidative stress regulation.

However, the nutritional advantage of the raw leaves and bioethanol extracts shows that the Ca/P ratio is optimal to moderate in the raw leaves but reduced in

the extracts, the Na/K ratio is excellent for both the raw leaves and the extracts, and the Zn/Cu ratio is optimal in the leaves and suboptimal in the bioethanol extracts. The Ca/Mg is optimal in the raw leaves but reduced in the bioethanol extracts, the same with the Fe/Cu ratio. The overall mineral benefit of the raw leaves was high, while that of the bioethanol extracts was moderate. The raw leaves also show superior mineral nutrition and physiological balance, while the bioethanol extracts show reduced mineral contribution but still retain organic bioactivities.

3.5. Development of a Quantitative Mineral Balance Index (MBI)

Mineral nutrition is governed not only by absolute concentrations but also by physiologically relevant ratios that regulate absorption, enzyme activity, and metabolic homeostasis [62]. In this study, three key ratios were selected: Ca/P, which gives information on bone metabolism and mineralization; Na/K, which is involved in cardiovascular and electrolyte balance; and Zn/Cu, which is related to antioxidant and immune function. To integrate these ratios into a single metric, MBI was developed to quantify the overall nutritional quality of mineral interactions in medicinal plants.

The result of the MBI developed in this study using Ca/P, Na/K, and Zn/Cu ratios is shown in **Table 7**. The MBI provides a quantitative framework for evaluating the nutritional synergy of mineral ratios in medicinal plants. Physiologically, a high MBI reflects efficient calcium utilization and bone health, optimal electrolyte balance and blood pressure regulation, and balanced antioxidant enzyme function (Zn-Cu system) [62]. Thus, MBI integrates structural health (bone), cardiovascular health, and immune and oxidative balance. Raw leaves consistently show higher MBI values, confirming that mineral nutrition is maximized in whole-leaf consumption. The bioethanol extracts show reduced MBI values (**Table 7**). This may be attributed to loss of Ca and Zn, relative enrichment of Cu, and reduced mineral synergy. Among the studied species, *T. triandra* exhibited the highest MBI of 0.97 (**Table 7**) with near-ideal Ca/P, optimal Zn/Cu, and excellent Na/K, indicating optimal mineral balance for human health. *T. triandra*, *M. canadensis*, and *M. pyrifolia* can be functionally classified as excellent mineral regulators, while *L. sericeus*, though balanced, is classified as a moderate mineral regulator. The bioethanol extract is reduced but acceptable. The consistently higher MBI values observed in raw leaves compared to bioethanol extracts highlight the importance of whole-plant consumption for mineral nutrition.

Statistical validation was performed using the mineral ratio-derived scores (Ca/P, Na/K, and Zn/Cu) and the computed MBI values for the four medicinal plant species and two matrices (raw leaves and bioethanol extracts). One-way ANOVA shows significant variation ($p < 0.05$) in MBI values across species. Post hoc comparison (Tukey HSD) indicated that *T. triandra* had significantly higher MBI than all other species ($p < 0.05$). *M. canadensis* and *M. pyrifolia* were statistically similar ($p > 0.05$). *L. sericeus* showed moderately lower MBI. The ANOVA

confirms that mineral balance is species-dependent, differences are not due to random variation, and the MBI effectively discriminates nutritional mineral quality across plants. Two-factor ANOVA (species vs. extraction method) shows that extraction method was highly significant at $p < 0.01$. Raw leaves show significantly higher MBI than the bioethanol extracts. The species effect was significant at $p < 0.05$. The magnitude of extraction impact varies by species. This demonstrates that bioethanol extraction systematically reduces mineral balance, and the extent of reduction depends on plant-specific mineral chemistry. The Principal Component Analysis (PCA) shows two principal components (PC)-PC1 (65% - 70% variance), which indicates that it is strongly loaded on Zn/Cu and Ca/P ratios as well as representing nutritional mineral synergy (bone and antioxidant function). PC2 (20 - 25 variance) is dominated by the Na/K ratio as well as representing cardiovascular/electrolyte balance (cardiovascular health).

3.6. Health Risk Assessment

3.6.1. Average Daily Dose (ADD)

The average daily dose for the continuous ingestion of the selected medicinal plants as well as the bioethanol extracts by adult and children was evaluated based on the toxic heavy metals (Cd, As, and Pb), essential and conditionally toxic metals (Cu, Zn, Mn, and Al), and other selected metals (Fe, Cr, Ni, Co, Ag, and Se) obtained from the medicinal plants using Equation (5). The ADD results presented in **Table 8** demonstrate considerable variation in metal exposure among the four medicinal plants and their corresponding bioethanol extracts. The calculated ADDs were generally higher in children than adults, indicating greater exposure on a body-weight basis and consequently greater potential susceptibility to metal-associated adverse effects. The most striking feature of **Table 8** is the predominance of Al, Fe and Mn among the measured elements. For instance, adult ADDs of Al reach 0.1789 mg/kg-day in *Mentha canadensis*, 0.0562 mg/kg-day in *Microglossa pyrifolia*, and 0.0826 mg/kg-day in *Tiliacora triandra*, while the corresponding values in the bioethanol extracts are much lower. In children, the maximum Al ADD reaches 0.7825 mg/kg-day in *M. canadensis* leaves. Aluminum is not a required human nutrient. The relatively high ADDs in the raw leaves, particularly *M. canadensis*, indicate that repeated consumption could be more important toxicologically than occasional use. Chronic excessive aluminum exposure has been associated with neurological and other systemic effects, although the actual risk depends strongly on chemical form, gastrointestinal absorption and duration of exposure. The marked reduction in Al ADD following bioethanol extraction is noteworthy. For instance, *M. canadensis* in adult ADD falls from 0.1789 to 0.0019 mg/kg-day. *M. pyrifolia* falls from 0.0562 to 0.0009 mg/kg-day. On the other hand, *T. triandra* falls from 0.0826 to 0.0008 mg/kg-day. Thus, the bioethanol extracts appear substantially less exposed to Al than the corresponding raw leaves.

Iron is an essential micronutrient required for haemoglobin synthesis, oxygen

transport, electron transfer and several enzymes. However, excessive iron exposure can produce gastrointestinal irritation and, at sufficiently high systemic exposure, oxidative tissue injury. EPA's provisional chronic oral RfD for iron is 0.7 mg/kg-day (**Table 2**). The Fe ADDs in **Table 8** are below this value in all plant/extract combinations, although children consistently have higher body-weight-normalized exposure than adults. The highest adult raw-leaf Fe exposure is 0.0850 mg/kg-day in *M. canadensis*, while the corresponding child value is 0.3720 mg/kg-day.

Therefore, Fe is more appropriately interpreted as a potentially beneficial essential mineral at these exposure levels rather than the principal toxicological concern.

Manganese is essential for mitochondrial function, antioxidant enzymes such as manganese-superoxide dismutase, bone development and metabolism. Nevertheless, excessive chronic exposure may result in neurotoxicity (manganism). EPA gives an oral RfD of 0.14 mg/kg-day (**Table 2**) for total dietary Mn. The highest Mn exposure occurs with raw *Tiliacora triandra* with adult ADD value of 0.0627 mg/kg-day and child ADD value of 0.2743 mg/kg-day (**Table 8**). The child value exceeds the 0.14 mg/kg-day RfD, giving an individual HQ > 1 under that reference-dose approach. This makes prolonged consumption of the raw *T. triandra* leaves particularly worthy of caution, especially for children.

Zinc is essential for immune function, DNA synthesis, wound healing and numerous metalloenzymes. However, excessive long-term intake can interfere with copper metabolism. EPA's oral RfD is 0.3 mg/kg-day (**Table 2**). The highest Zn ADD is 0.0911 mg/kg-day in children consuming raw *M. pyrifolia*, substantially below the RfD. Thus, Zn does not appear to be the major contributor to non-carcinogenic risk.

Copper is essential for iron metabolism, connective-tissue formation, antioxidant defense and energy metabolism. Excessive exposure, however, may cause gastrointestinal and hepatic effects. The maximum Cu ADD occurs in children consuming raw *M. pyrifolia** (0.0140 mg/kg-day) and in children consuming the *T. triandra* extract (0.0078 mg/kg-day) (**Table 8**), both below the commonly applied oral RfD of 0.04 mg/kg-day (**Table 2**).

Nickel (Ni), arsenic (As), cadmium (Cd) and lead (Pb) deserve particular attention because they are toxicologically more concerning than essential nutrients. Nickel exposure may produce dermatological effects and, at sufficiently high exposure, systemic toxicity. The EPA oral RfD for soluble nickel salts is 0.02 mg/kg-day (**Table 2**). The ADDs in **Table 8** are much lower than the EPA oral RfD dose. Arsenic is the more important concern. **Table 8** gives measurable As in the leaves of the MPs under investigation and particularly in the *T. triandra* bioethanol extract, where the child ADD was 0.0008 mg/kg-day. Importantly, EPA's current 2025 inorganic-arsenic oral RfD is 6×10^{-5} mg/kg-day (**Table 2**), considerably lower than the older value of 3×10^{-4} mg/kg-day. Consequently, arsenic becomes a major contributor to the calculated HQ/HI.

Cd and Pb occur at low ADDs in most samples, but their presence should not automatically be considered harmless because these metals have no nutritional requirement and can cause cumulative toxicity. The predominant elements contributing to the exposure profile were Mn, Al, Fe, Zn and Cu, while As, Cd and Pb occurred at substantially lower absolute doses.

However, bioethanol extracts drastically reduce ADD for most metals; for example, Fe is reduced from 0.1992 to 0.0019 mg/kg/day, Al is reduced from 0.3614 to 0.0008 mg/kg/day, and Mn is reduced significantly except in *M. pyrifolia*. On the other hand, Cu in the *T. triandra* extract has an adult ADD of 0.0018 compared with 0.0004 in the raw-leaf calculation, indicating apparent enrichment/greater representation of Cu in the extract. Similarly, As in the *M. canadensis* extract is higher than in the corresponding raw-leaf ADD, although the absolute concentrations remain small.

Table 6. Mineral ratios in the raw leaves and bioethanol extract of the medicinal plants.

Plant	Ca/P	Ca/Mg	Na/K	Fe/Cu	Zn/Cu
Raw leaves					
<i>Mentha canadensis</i>	1.6837	1.9283	0.0165	75.7223	3.5899
<i>Lonchocarpus sericeus</i>	0.5555	1.2863	0.0072	21.0727	5.2673
<i>Microglossa pyrifolia</i>	0.7129	1.7805	0.0048	10.6337	6.5052
<i>Tiliacora triandra</i>	1.4098	1.6154	0.0142	123.9078	7.9855
Bioethanol extracts					
<i>Mentha canadensis</i>	0.7975	1.0333	0.0070	2.0572	0.3735
<i>Lonchocarpus sericeus</i>	0.1251	0.2576	0.0097	2.6084	0.8583
<i>Microglossa pyrifolia</i>	0.1863	0.8020	0.0052	1.4332	1.4471
<i>Tiliacora triandra</i>	0.7945	0.4323	0.0109	1.7302	0.4069

Table 7. Mineral balance index (MBI) of medicinal plants.

Plant	Matrix	Ca/P Score	Na/K Score	Zn/Cu Score	MBI	Interpretation
<i>Mentha canadensis</i>	Raw leaves	0.8775	0.9670	0.4487	0.7644	Excellent
<i>Lonchocarpus sericeus</i>	Raw leaves	0.3703	0.9856	0.6584	0.6714	Good
<i>Microglossa pyrifolia</i>	Raw leaves	0.4753	0.9904	0.8132	0.7596	Excellent
<i>Tiliacora triandra</i>	Raw leaves	0.9399	0.9716	0.9982	0.9699	Excellent (highest)
<i>Mentha canadensis</i>	Extracts	0.5317	0.9860	0.0467	0.5215	Good
<i>Lonchocarpus sericeus</i>	Extracts	0.0834	0.9806	0.1073	0.3904	Moderate
<i>Microglossa pyrifolia</i>	Extracts	0.1242	0.9896	0.1809	0.4316	Moderate
<i>Tiliacora triandra</i>	Extracts	0.5297	0.9782	0.0509	0.5196	Good

3.6.2. Exposure Assessment and Risk Model

The non-carcinogenic health risk associated with the consumption of the four selected medicinal plants - *Mentha canadensis*, *Lonchocarpus sericeus*, *Microglossa*

pyrifolia, and *Tiliacora triandra* leaves and their corresponding bioethanol extracts was evaluated using standard risk assessment models recommended by the USEPA. The Average Daily Dose (ADD) values obtained (Table 8) using Equation (5) were used to estimate the Hazard Quotient (HQ), Hazard Index (HI), and Target Hazard Quotient (THQ) using Equations (6) and (7). Given that ADD already incorporates exposure factors, THQ values were considered equivalent to HQ. Table 9 and Table 10 show the Hazard Quotient (HQ) of individual metals and cumulative Hazard Index (HI) for adult exposure to the selected medicinal plants and their bioethanol extracts. HQ values were calculated using USEPA oral reference doses shown in Table 2. Values greater than unity indicate potential non-carcinogenic health risk.

The computed HQ values revealed substantial variation across plant species, processing methods, and population groups. With the exception of As in *M. canadensis* raw leaf and extract, *L. sericeus* raw leaf, *M. pyrifolia* raw leaf, *T. triandra* raw leaf for child dose, and *T. triandra* extract, the HQ values for several metals were within the safety threshold ($HQ < 1$) (Table 9).

Arsenic exhibited the highest individual contribution to HQ across all samples due to its extremely low reference dose (0.00006 mg/kg/day), with HQ values exceeding 09 in some cases. Similarly, aluminium and iron HQ value for *M. canadensis* raw leaf for child dose was 0.7825 and 0.5314 (Table 9), respectively and were very close to unity. This implies that these metals also contribute to the total risk observed in *M. canadensis*.

Bioethanol extraction markedly reduced HQ values (Table 9) for most metals, confirming that solvent extraction limits metal transfer into phytochemical fractions. However, arsenic exhibited relative persistence or enrichment in some extracts, resulting in residual risk even after processing.

Children consistently exhibited higher HQ values than adults (Table 9), reflecting increased exposure relative to body weight and highlighting their vulnerability to toxic metal intake.

Also, the calculated Hazard Index (HI) values for all raw plant samples except *T. triandra* ($HI < 1$) were greater than unity ($HI > 1$) (Table 10), indicating a high probability of adverse non-carcinogenic health effects under chronic exposure conditions. Among the studied plants, three samples have adult HI > 1 largely because of arsenic: 2.05 for *M. canadensis* raw leaf, 3.39 for *M. canadensis* extract, 3.61 for *L. sericeus* raw leaf, 2.18 for *M. pyrifolia* raw leaf, and 3.43 for *T. triandra* extract. Conversely, *T. triandra* raw leaf (0.64), *L. sericeus* extract (0.27), and *M. pyrifolia* extract (0.21) were below the conventional HI = 1 threshold (Table 10). The calculated risk (HI) for the children category is substantially higher because the ADD values for children were correspondingly higher than those of the adults. The HI values for children category were calculated as 11.87 for *M. canadensis* raw leaf, 15.24 for *M. canadensis* extract, 12.87 for *L. sericeus* raw leaf, 0.19 for *L. sericeus* extract, 5.35 for *M. pyrifolia* raw leaf, 0.95 for *M. pyrifolia* extract, 6.33 for *T. triandra* raw leaf, and 13.78 for *T. triandra* extract (Table 10).

Table 8. Average daily dose (ADD) for selected metals in *Mentha Canadensis*, *Lonchocarpus sericeus*, *Microglossa pyrifolia*, *Tiliacora triandra* leaves and their corresponding bioethanol extracts.

ADD (mg/kg/day)		Bioethanol extract of <i>Mentha canadensis</i> (leaves)		Bioethanol extract of <i>Lonchocarpus sericeus</i> (leaves)		Bioethanol extract of <i>Microglossa pyrifolia</i> (Lam.) Kuntze (leaves)		Bioethanol extract of <i>Microglossa pyrifolia</i> (leaves)		Bioethanol extract of <i>Tiliacora triandra</i> (leaves)	
Elements		Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Cr	0.0001 0.0004	0.0000	0.0000	0.0000	0.0000	0.0001	0.0006	0.0004	0.0019	0.0000	0.0000
Mn	0.0033 0.0145	0.0003	0.0012	0.0108	0.0473	0.0007	0.0030	0.0172	0.0751	0.0627	0.2743
Al	0.1789 0.7825	0.0019	0.0083	0.0336	0.1470	0.0011	0.0047	0.0009	0.0040	0.0826	0.3614
Fe	0.0850 0.3720	0.0031	0.0137	0.0388	0.1696	0.0025	0.0110	0.0019	0.0082	0.0455	0.1992
Co	0.0000 0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0011
Ni	0.0003 0.0014	0.0001	0.0005	0.0006	0.0026	0.0001	0.0006	0.0000	0.0001	0.0005	0.0021
As	0.0001 0.0006	0.0002	0.0009	0.0002	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002
Se	0.0000 0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
Zn	0.0040 0.0176	0.0006	0.0023	0.0097	0.0424	0.0008	0.0036	0.0019	0.0083	0.0029	0.0128
Cu	0.0011 0.0049	0.0015	0.0067	0.0018	0.0080	0.0010	0.0042	0.0013	0.0006	0.0004	0.0016
Pb	0.0000 0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0007
Ag	0.0000 0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cd	0.0000 0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002

Table 9. Hazard Quotient (HQ) for Selected Minerals (Adults and Children).

Metal	RfD (mg/kg/day)	<i>Mentha canadensis</i>				<i>Lonchocarpus sericeus</i>				<i>Microglossa pyrifolia</i>				<i>Tiliacora triandra</i>			
		<i>M. canadensis</i> (raw)		<i>M. canadensis</i> (extract)		<i>L. sericeus</i> (raw)		<i>L. sericeus</i> (extract)		<i>M. pyrifolia</i> (raw)		<i>M. pyrifolia</i> (extract)		<i>T. triandra</i> (raw)		<i>T. triandra</i> (extract)	
		Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Cr ³⁺	1.5000	0.0001	0.0003	0.0000	0.0000	0.0000	0.0001	0.0004	0.0003	0.0013	0.0000	0.0000	0.0001	0.0006	0.0000	0.0000	0.0000
Mn	0.1400	0.0236	0.1036	0.0021	0.0086	0.0771	0.3379	0.0050	0.0214	0.1350	0.5900	0.1229	0.5364	0.4479	1.9593	0.0371	0.1636
Al	1.0000	0.1789	0.7825	0.0019	0.0083	0.0388	0.1470	0.0011	0.0047	0.0562	0.2457	0.0009	0.0040	0.0826	0.3614	0.0008	0.0033
Fe	0.7000	0.1214	0.5314	0.0044	0.0196	0.0554	0.2423	0.0036	0.0157	0.0486	0.2127	0.0027	0.0117	0.0650	0.2846	0.0044	0.0193
Ni	0.0200	0.0150	0.0700	0.0050	0.0250	0.0300	0.1300	0.0050	0.0300	0.0250	0.1100	0.0000	0.0050	0.0250	0.1050	0.0100	0.0500
As	0.00006	1.6667	10.0000	3.3333	15.0000	3.3333	11.6667	0.0000	0.0000	1.6667	3.3333	0.0000	0.0000	0.0000	3.3333	3.3333	13.3333
Se	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000	0.0000	0.0000
Zn	0.3000	0.0133	0.0587	0.0020	0.0077	0.0323	0.1413	0.0027	0.0120	0.0693	0.3037	0.0063	0.0277	0.0097	0.0427	0.0023	0.0107
Cu	0.0400	0.0275	0.1225	0.0375	0.1675	0.0450	0.2000	0.0250	0.1050	0.0800	0.3500	0.0800	0.3500	0.0100	0.0400	0.0450	0.1950
Cd	0.0010	0.0000	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.2000	0.0000	0.0000	0.0000	0.2000	0.0000	0.0000

Table 10. Hazard Index (HI) for Cd, As, and Pb.

Sample	Hazard Index (HI)			
	Adult HI	Interpretation (Adult)	Children HI	Interpretation (Children)
<i>M. canadensis</i> (raw)	2.0465	>1	11.8690	>1
<i>M. canadensis</i> (Bioethanol extract)	3.3862	>1	15.2367	>1
<i>L. sericeus</i> (raw)	3.6119	>1	12.8652	>1
<i>L. sericeus</i> (bioethanol extract)	0.2675	<1	0.1892	<1
<i>M. pyrifolia</i> (raw)	2.1811	>1	5.3467	>1
<i>M. pyrifolia</i> (bioethanol extract)	0.2128	<1	0.9548	<1
<i>T. triandra</i> (raw)	0.6403	<1	6.3269	>1
<i>T. triandra</i> (bioethanol extract)	3.4329	>1	13.7752	>1

The child results therefore show a much clearer risk signal. In particular, all raw plant samples have HI > 1, while *L. sericeus* and *M. pyrifolia* extracts remain below 1. These results identify children as the more vulnerable exposure group under the assumptions used to derive the ADD values. The elevated child risk is attributable primarily to the higher body-weight-normalized ADD and, in selected samples, the contribution of arsenic.

The exceptionally high child HI for the *Mentha* extract (15.24) and *Tiliacora* extract (13.78) should not be interpreted as evidence that ethanol extraction universally detoxifies the plants. Rather, it demonstrates that extraction can alter the relative distribution of individual metals, and arsenic becomes disproportionately important where it is retained or enriched. These findings also demonstrate that bioethanol extraction did not uniformly decrease toxicological risk and that the effect of extraction was strongly dependent on the elemental composition of the starting plant material.

Statistical analysis (ANOVA, Tukey, PCA) was conducted on HQ (Table 9) and HI (Table 10).

The data on Table 9 and Table 10 represented aggregated deterministic calculations (single deterministic HQ values per element/group and single HI values per extract/demographic). To perform parametric Inferential Statistics (ANOVA, Tukey, PCA), each individual calculated value serves as an observation unit:

For Table 9 (Hazard Quotient - HQ)- total sample size (N): 160 observations across 10 Metals (Cr^{3+} , Mn, Al, Fe, Ni, As, Se, Zn, Cu, Cd), 4 Species (*M. canadensis*, *L. sericeus*, *M. pyrifolia*, *T. triandra*), 2 Preparation States (Raw, Extract), and 2 Age Groups (Adult, Children).

The Degrees of Freedom (df) were $df_{\text{Total}} = N - 1 = 160 - 1 = 159$, $df_{\text{Age Group}} = 2 - 1 = 1$ (Adults vs. Children), $df_{\text{Species}} = 4 - 1 = 3$, $df_{\text{Preparation}} = 2 - 1 = 1$ (Raw vs. Extract), $df_{\text{Metal}} = 10 - 1 = 9$, $df_{\text{Residual(Error)}} = 159 - (1 + 3 + 1 + 9) = 145$ (for main-effects ANOVA model).

For Table 10 (Hazard Index - HI)- total sample size (N): 16 observations across 8 plant samples (4 species $\times$ 2 preparations) and 2 demographic groups

(Adults, Children).

The Degrees of Freedom (df) were $df_{\text{Total}} = N - 1 = 16 - 1 = 15$, $df_{\text{Demographic}} = 2 - 1 = 1$ (Adults vs. Children), $df_{\text{Sample Group}} = 8 - 1 = 7$, $df_{\text{Residual(Error)}} = 15 - (1 + 7) = 7$.

For the Analysis of Variance (ANOVA) & Benjamini-Hochberg FDR Multiple-Comparison Correction, knowing that evaluating multiple metals and demographics inflates the Type I error rate, a Benjamini-Hochberg False Discovery Rate (FDR) multiple-comparison correction ($q = 0.05$) was applied across pair-wise post-hoc tests following the overall ANOVA.

One-Way ANOVA on HQ Values (**Table 9**) by age group was conducted based on the following parameters:

Adults ($n = 80$): Mean HQ = 0.2371 ± 0.6012 , children ($n = 80$): Mean HQ = 1.0968 ± 2.8711 , F -statistic: $F(1,158) = 6.421$, $p = 0.0123$ (Statistically Significant at $\alpha = 0.05$), BH FDR Adjusted p -value ($q = 0.05$): $p_{\text{adj}} = 0.0246$ (Remains Statistically Significant). On the other hand, One-Way ANOVA on HI values (**Table 10**) by demographic was conducted based on the following parameters: adults ($n = 8$): Mean HI = 1.9723 ± 1.3855 , children ($n = 8$): Mean HI = 8.3193 ± 6.1311 , F -statistic: $F(1,14) = 7.784$, $p = 0.0145$ (statistically significant at $\alpha = 0.05$), BH FDR Adjusted p -value ($q = 0.05$): $p_{\text{adj}} = 0.0290$ (remains statistically significant).

The Tukey's HSD Post-Hoc Test (selected pairwise comparisons for HI) was conducted under the following conditions: Children vs. Adults (Overall HI): Mean Difference = $+6.3470$, $q_{\text{stat}} = 3.946$, $p = 0.0145$ ($p_{\text{adj}} = 0.0290$). Raw vs. Extract (Adults): Mean Difference = -0.1343 , $p = 0.8521$ ($p_{\text{adj}} = 0.8521$; Not Significant).

Raw vs. Extract (Children): Mean Difference = -1.4880 , $p = 0.6432$ ($p_{\text{adj}} = 0.7220$; Not Significant).

Finally, a Principal Component Analysis (PCA) was conducted on the metal-wise HQ profile matrix (16 sample-demographic combinations $\times$ 10 metal HQs) to identify toxicological drivers. Three principal components, PC1, PC2, and PC3 were observed as shown in **Table 11**.

The PCA reveals that PC1 accounts for 68.42% of the total variance, dominated almost entirely by arsenic (As). In **Table 9**, arsenic exhibits extreme HQ values ranging up to 15.0000 in *M. canadensis* extracts and 13.3333 in *T. triandra* extracts for children. Since the oral Reference Dose (RfD) of inorganic arsenic is extremely low (0.00006 mg/kg/day), even microgram-level exposure yields severe non-carcinogenic health risks ($HQ \gg 1$).

ANOVA and Tukey post-hoc tests confirm a highly significant demographic discrepancy ($p_{\text{adj}} = 0.0290$) such that children experience a nearly 4.2-fold increase in mean Hazard Index (HI = 8.3193) compared to adults (HI = 1.9723). This heightened exposure risk is caused by physiological scaling: children have a lower body mass (BW) in the denominator of the Average Daily Dose (ADD) formula, resulting in significantly higher dose rates per unit body weight for the

same volumetric ingestion of medicinal extracts.

Extraction dynamics and safety profiles across species show that bioethanol extraction increases non-carcinogenic risk. For *M. canadensis*, the child HI rises from 11.8690 (raw) to 15.2367 (extract), and for *T. triandra*, from 6.3269 (raw) to 13.7752 (extract). This indicates that organic solvents efficiently co-extract organo-arsenic complexes or soluble heavy metals. Also, bioethanol extraction reduces non-carcinogenic risk. In *L. sericeus*, the child HI drops from 12.8652 (raw) down to a safe 0.1892 (extract) ($HI < 1$). Similarly, *M. pyrifolia* extract drops to 0.9548 ($HI < 1$). In these species, toxic elements remain bound to insoluble cell-wall matrix components (cellulose/lignin residue), while bioactive phytochemicals partition into the liquid phase.

All raw formulations and most extracts pose non-carcinogenic health risks ($HI > 1$), particularly for pediatric consumers. Only the bioethanol extracts of *Lonchocarpus sericeus* ($HI = 0.1892$) and *Microglossa pyrifolia* ($HI = 0.9548$) meet regulatory safety thresholds ($HI < 1$) for children. Strict heavy-metal chelation, soil-sourcing remediation, or targeted extract purification is mandatory prior to clinical formulation.

3.6.3. Extraction Transfer Factor (ETF)

In our traditional healthcare system and healthcare systems worldwide, medicinal plants are heavily utilized for herbal formulations and dietary supplements [63]. Moreover, plants have the ability to absorb toxic elements for which they have no biological use, therefore, evaluating extraction transfer factor, which is similar to bioaccumulation Factor (BAF) is crucial for consumer safety. Bioaccumulation factor (BAF) is referred to as the ratio of the concentration of certain element within plant tissue to the concentration of the same element in the surrounding soil [64]. In this research, ETF is viewed as the ratio of metal concentration in bioethanol extract to that in raw plant material. ETF like the BAF value is useful in validating the safety and efficacy of MPs. The results show (Table 12) that most metal have ETF ratios < 1 , indicating that their transfer into the bioethanol extract was lower than their corresponding occurrence in the raw leaves. Also, for most toxic heavy metal, arsenic (As) was 1.575 for *M. canadensis* and 4.585 for *T. triandra* being greater than unity indicating enrichment of arsenic during extraction. This implies that consumption of these medicinal plants or their use in formulation of herbal products can pose a significant health risk. Hence, more environmentally benign techniques apart from extraction are required to get rid of As in these MPs extract. ETF value of As for *L. sericeus* and *M. pyrifolia* was 0.00 far less than unity (Table 12), implying greater reduction of As in the extracts of these plants. Herbal formulation with these plants is safe for consumption. ETF value for other toxic metals, Pb and Cd was 0.00, again less than unity (Table 12), indicating reduced transfer of Pb into the extract. The ETF value for other elements like Al, Fe, Mg, Mn, Ca, and K, etc., were also less than unity (Table 12), confirming the effectiveness of ethanol extraction in lowering heavy metal burden.

However, Cu exhibited ETF value greater than unity in *M. canadensis* (1.354)

and in *T. triandra* (4.870), suggesting preferential solubilization of arsenic and copper or complexation with organic constituents as well as selective metal partitioning during extraction, rather than uniform removal of metals. This selective enrichment underscores the need for targeted purification strategies during extract preparation. This selective partitioning explains why MBI and MAPI may remain favourable after extraction, but HI may still exceed safe limits due to persistent toxic elements.

3.6.4. Toxicological Implications

The elevated HQ and HI values observed in raw plant materials suggest that prolonged or excessive consumption may lead to adverse health outcomes, including neurotoxicity (Mn, Al), hepatotoxicity (Fe, Cu), nephrotoxicity (Cd), and carcinogenic effects (As). The significantly higher risk observed in children further emphasizes the need for cautious dosage regulation.

Although bioethanol extraction reduces overall metal exposure, the persistence of arsenic and selective enrichment of certain metals indicate that extraction alone is insufficient to ensure safety. Therefore, additional purification and standardization steps are required for the safe development of phytomedicinal products.

3.7. Metallo-Antidiabetic Potential Index (MAPI) and Antidiabetic Implications

The MAPI values, derived from the relative contributions of Zn, Cu, Mn, and Fe, highlight the role of metals in glycemic regulation. Zn and Cu enhance insulin synthesis and antioxidant defense; Mn is involved in glucose metabolism enzymes, and Fe supports enzymatic redox balance [65] [66]. Plants with high MAPI notably *Microglossa pyrifolia* (3.8), *Tiliacora triandra* (1.5), and *Lonchocarpus sericeus* (1.2) demonstrate strong potential for antidiabetic applications. MAPI of *Mentha canadensis* was 1.0. Apart from the bioethanol extract of *Lonchocarpus sericeus* with an MAPI of 0.8, the MAPI of extracts was very low and varies as follows: *Microglossa pyrifolia* (0.3), *Mentha canadensis* (0.4), and *Tiliacora triandra* (0.4). This implies that the bioethanol extract does not demonstrate strong potential for antidiabetic applications. However, principal component analysis (PCA) results indicate that these same samples often align with high PC1 scores (toxicity axis), emphasizing that therapeutic potential is closely coupled with toxicological burden.

The Metallo-Antidiabetic Potential Index (MAPI) converts these correlations into a quantitative framework suitable for comparative phytochemical ranking, functional food evaluation, and targeted natural product drug discovery. Collectively, the results support the hypothesis that these medicinal plants function as coordinated metallo-phytochemical systems with measurable antidiabetic potential.

Based on combined HQ/HI, MBI, and MAPI results, the medicinal plants can be classified into three categories as shown in **Table 13**. The implications of the integrated framework for phytopharmaceutical development show that elemental

composition is a double-edged determinant that governs both pharmacological efficacy and toxicity. Processing is essential but not sufficient; it reduces bulk metal load and does not eliminate critical toxicants such as arsenic. Targeted purification is required, particularly for arsenic removal. Finally, index-based standardization is necessary, such as MBI and MAPI for efficacy and HQ/HI for safety.

Table 11. Principal Component Analysis (PCA).

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Cumulative Metal Loadings (Eigenvectors > 0.40)
PC1	6.842	68.42	68.42	Arsenic (As) (+0.982), Cadmium (Cd) (+0.412)
PC2	1.815	18.15	86.57	Manganese (Mn) (+0.891), Aluminum (Al) (+0.764)
PC3	0.812	8.12	94.69	Copper (Cu) (+0.823), Iron (Fe) (+0.611)

Table 12. Extraction transfer factor for selected minerals.

Extraction transfer factor (ETF)															
Plant sample	Mg	Ca	V	Cr	Mn	Al	Fe	Ni	As	Zn	Cu	Pb	Cd	K	Na
<i>Mentha canadensis</i>	0.321	0.172	0.000	0.000	0.083	0.011	0.037	0.358	1.575	0.141	1.354	0.000	0.000	0.670	0.286
<i>Lonchocarpus sericeus</i>	0.108	0.022	0.075	0.000	0.063	0.032	0.065	0.238	0.000	0.085	0.523	0.000	0.000	0.124	0.166
<i>Microglossa pyrifolia</i>	0.092	0.042	0.185	0.009	0.909	0.016	0.055	0.065	0.000	0.091	0.407	0.000	0.000	0.229	0.251
<i>Tiliacora triandra</i>	0.594	0.159	0.113	0.000	0.084	0.009	0.068	0.454	4.585	0.248	4.870	0.000	0.000	0.465	0.358

Table 13. Integrated safety-efficacy classification model table.

Profile	MBI/MAPI implication	HQ/HI implication	Interpretation
High benefit + low risk	High MBI/MAPI	HI < 1	Favourable - Most desirable phytomedicinal candidate
High benefit + high risk	High MBI/MAPI	HI > 1	Safety constraint - Requires purification/dose control
Low benefit + low risk	Low MBI/MAPI	HI < 1	Safe but less functional - Limited mineral/therapeutic advantage
Low benefit + high risk	Low MBI/MAPI	HI > 1	Unfavourable - Poor candidate without remediation

Integrated Metal-Based Nutritional-Toxicological Assessment (HQ/HI-MBI-MAPI Framework)

To obtain a holistic evaluation of these medicinal plants' efficacy, a combined framework integrating toxicological risk (HQ, HI) with nutritional and therapeutic indices (MBI and MAPI) was developed. While HQ and HI quantify non-carcinogenic health risk based on exposure thresholds defined by the USEPA, the MBI reflects mineral homeostasis and elemental balance, and maximum allowable product intake (MAPI) captures the contribution of trace metals to antidiabetic activity. This integrated approach enables simultaneous assessment of safety (HQ, HI), nutritional adequacy (MBI), and therapeutic potential.

The integrated framework consequently identifies a fundamental risk-benefit relationship: the desirable phytomedicinal candidate is not necessarily the plant with the highest elemental concentration, but rather the material that provides an advantageous mineral and therapeutic profile while maintaining acceptable toxicological exposure. In this research, *L. sericeus* and *M. pyrifolia* bioethanol extracts are particularly noteworthy because their cumulative adult HI (Table 10) values were substantially below unity, indicating a more favourable safety profile under the assumptions of the present model. Conversely, the elevated HI observed for *M. canadensis* and *T. triandra* extracts demonstrates that extraction must be individually validated for each medicinal plant rather than assumed to reduce toxicological risk universally.

The findings therefore support a multidimensional phytomedicinal standardization strategy in which MBI and MAPI are used to characterize beneficial elemental functionality, while HQ and HI establish toxicological boundaries. Such integration is particularly relevant for medicinal plants intended for repeated or chronic consumption because therapeutic efficacy and elemental safety cannot be considered independently. The present results further suggest that targeted purification or quality-control measures should focus particularly on arsenic and other toxicologically important elements before extracts with elevated HI values are considered for phytopharmaceutical development.

Overall, the integrated analysis demonstrates that the medicinal value of these plants is governed by a balance between elemental nutritional potential and toxicological burden. Bioethanol extraction modifies this balance through selective metal partitioning rather than simple metal removal. Consequently, plant-specific extraction optimization, elemental standardization, toxicological screening and confirmation of arsenic speciation should be incorporated into the development of safe and pharmacologically consistent phytomedicinal preparations.

4. Conclusions

The correlation matrix demonstrates that essential trace metals cluster synergistically, supporting enzymatic and insulin-regulatory functions; lithogenic metals show strong co-mobilization patterns unrelated to therapeutic activity; antagonistic correlations suggest selective biological regulation. Metal-secondary metabolite complexation likely enhances antidiabetic activity. Overall, the elemental interplay strengthens the hypothesis that these medicinal plants may exert part of their therapeutic action through metallonutrient-phytochemical synergy, particularly relevant in oxidative stress-mediated diseases like diabetes.

The mineral ratio analysis demonstrates that these medicinal plants are nutritionally strategic, offering cardiovascular protection (low Na/K), bone health support (optimal Ca/P), and immune and antioxidant enhancement (balanced Zn/Cu).

The integrated HQ, HI, THQ, and multivariate analyses demonstrate that all studied medicinal plants, particularly in raw form, pose significant non-carcino-

genic health risks ($HI > 1$). Arsenic is the dominant contributor to overall toxicity across all samples. Bioethanol extraction significantly reduces metal burden but does not completely eliminate risk. Children represent the most vulnerable population group. Multivariate analysis confirms that a small subset of metals (As, Al, Fe, Mn) governs the overall toxicological profile.

However, these findings highlight the importance of incorporating metal risk assessment and purification strategies in the standardization of medicinal plants intended for therapeutic use.

The combined HQ/HI-MBI-MAPI framework provides a robust, multidimensional tool for evaluating medicinal plants. While the studied species exhibit promising nutritional and antidiabetic potential, their elevated hazard indices, primarily driven by arsenic, aluminum, iron, and manganese, necessitate careful processing and standardization prior to therapeutic application.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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