**Cr and Mn total, accessible species, and protein-fraction contents in plants used for traditional anti-diabetes treatment**

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**ABSTRACT**

*Background:* Our survey has found eleven plants that have being consumed for traditional treatment of diabetes mellitus, particularly in Saudi Arabia and generally in many countries across the world. The literature reported about trace elements such as Cr and Mn positively affecting diabetes mellitus. The aim of this work is to determine the total, accessible element species, and protein-fraction contents of Cr and Mn in the edible parts of those plants.

*Methods:* The total contents of Mn were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), while the total contents of Cr was determined by ICP-sector field (sf)-mass spectrometry (MS) due to lower concentration. The protein-fraction contents were determined in accessible element species by size-exclusion chromatography (SEC)-ICP-MS.

*Results and discussion:* The separation was successfully carried out to quantify Cr and Mn bound up to 11 protein fractions. The examined plants recorded wide ranges of total contents (Cr 44.7–1880.0 µg/kg and Mn 3.7–59.0 mg/kg) and accessible element species contents (Cr 0.93–29.40 µg/kg and Mn 0.82–35.85 mg/kg). Also wide ranges of percentages of accessible element species contents to total contents of Cr (0.65–4.21%) and Mn (5.43–68.42%) were obtained. The hazardous indices of both trace elements Cr and Mn for all examined plants consumed by both children and adults were all < 1, indicating no probability of health risk to occur. Moreover, Cr as carcinogen element reported no probability of cancer risk to occur from the consumption of all examined plants. Irrespective of plant species, Cr was quantified in all SEC fractions (mainly protein fractions), with the exception of 1.9-3.7 kDa, while Mn was quantified in all SEC fractions with the exception of 100–120 and 1.3-3.7 kDa. Nevertheless, the majority of accessible Cr species contents bound to the 10–14 and 0.05–0.40 kDa fractions, while that of Mn bound to 0.05–0.40 kDa fraction. To gather, the benefits of specific plant species in terms of accessible Cr and Mn species contents, in addition to accessible Zn species contents reported in our previous study, *Haloxylon Salicornicum*, *Olea Europaea* *Momordica* and *Charantia* are recommended to be consumed for traditionally controlling T2DM.

**Keywords** medicinal plants; anti-diabetes; trace elements; SEC-ICP-MS; ICP-OES; ICP-MS

**1. Introduction**

Trace elements have been considered necessary for the maintenance of several essential physicochemical processes for all organisms [1]. According to their essentiality for the maintenance of health, trace elements have been classified into three categories; essential such as Fe, Mn, Cu, Cr, Zn, and Se, probably essential such as Ni, V, and Co and potentially toxic such as Al, As, Cd, Pb, and Hg. Both essential and probably essential trace elements are required in the diet. However, these two categories may have toxic effects at high levels [2–5]. In contrast, due to their multipurpose usage in various manmade activities, considerable amounts of trace elements, of which the majority are considered to be heavy metals as well, release into the environment and take place in different environmental counterparts. Accordingly, trace elements enter organisms, which put them at risk particularly at uncontrollable levels [6–10].

Different studies examined trace element contents in serum and plasma samples of type 2 diabetes mellitus (T2DM). Mg contents were found lower in diabetic serum than healthy object serum and vice versa for Cu, Zn, and Se [11]. Additionally, positive correlation between diabetes and Cd, Cr, Fe, Ni, Ag, and Zn contents in blood, while negative correlation with Br were recorded [12]. Furthermore, the relationship between some trace elements in blood and T2DM duration was investigated in population from Norway [13]. Positive relationship was recorded for B, Ca, and Ag, while negative relationship was recorded for In, Pb, and Mg. Also, some trace elements were quantified in plasma and the relationship with T2DM was assessed by Li et al. [14]. The study reported different relationship trends among trace element contents including Mn. A review study [15] reported different trends in relationship between Cr, Fe, and Zn and T2DM, while the review reported inconsistencies in relationship between Cu and Se and T2DM. The review also reported different trends of relationship between Cu and Se in diets and supplementation with increased risk of T2DM. The review also highlighted effective antioxidant characteristics and insulin-mimetic action of Cu and Se. In contrast, distortions by Cu and Se in the molecular pathways of glucose metabolism were reported [15]. In conclusion, close relationship between trace elements and T2DM has become more evident.

In particular, Mn is essential to nearly all organisms. The average human body can accumulate 12–20 mg of Mn. Glutamine synthetase is the most abundant Mn containing enzyme that plays a prominent role in brain chemistry [16]. Mn(II), Mn(III), and Mn(IV) have been reported as the most important physiochemical forms. Hence, the majority of Mn biochemistry relies on its redox properties [17]. It has become evident that Mn at lower oxidation states protects cells from oxidative damage particularly at higher levels; a phenomena that is frequently recorded in bacterial cells [18]. However, the redox capability of Mn is also discussed as probable cause for oxidative stress [19], probably resulting in a damage of beta-cells. Aside, Mn was recently also found to affect iron redox balance, by that increasing oxidative stress, too [20]. The absorption of inhaled Mn is tens of times faster than intestinal absorption. This is because of the small particle sizes (0.1–0.45 µm) emitted into the atmosphere [21–23]. However, Mn is known to be a neurotoxic agent for several decades. The injury of neuronal tissue and other toxic effects depend on particular Mn-species [24]. On the other hand, Cr deficiency was believed to impair glucose tolerance as it associates with carbohydrate metabolism [25]. In this regard, the Australasian Society for Parenteral and Enteral Nutrition (ASPEN) has recommended Cr in daily supplementation [26]. Cr supplementation in patients with T2DM may have a modest beneficial effect on glycemia and dyslipidemia, whereas such an effect is not observed in those without diabetes [27]. In contrast, the toxicity of Cr(III)tris(picolinate) as the main form of Cr supplement is of concern due to possible damage to DNA [18]. Therefore, the physicochemical processes of Cr and Mn, as for many other trace elements, in organisms depend on their chemical species.

Our research team previously examined the total Zn contents, water Zn extract contents and Zn-protein contents in a collection of medicinal plants traditionally used for controlling T2DM in Saudi Arabia [28]. The previous study assigned specific protein fractions having higher levels of Zn capturing than other fractions. Based on the literature that reported relationship of Cr and Mn with diabetes, it has been proposed to continue our previous study in order to evaluate Cr and Mn species in the same previously examined plant species. The current study also includes the evaluation of Cr and Mn total and accessible element species contents. Despite this study demonstrates trace element profiles in plants traditionally used in Saudi Arabia for T2DM, all or part of these plants are well known across the world for traditional treatment of T2DM as well. Therefore, this communication would have global impact on trace elements, in general, and Cr and Mn, in particular, of medicinal plants.

This aim is pursued here using the hyphenation of size-exclusion chromatography-inductively coupled plasma-mass spectrometry (SEC-ICP-MS) for on-line separation of protein fractions and elemental quantification. In addition, ICP-sector field (sf)-MS was used for quantification of total and accessible element species of Cr, while ICP-optical emission spectrometry (ICP-OES) was used for quantification of total and accessible element species of Mn. This is because the levels of Mn in plants match the quantification levels of ICP-OES, whereas the levels of Cr in plants match the quantification levels of ICP-sf-MS [29].

**2. Material and Methods**

*2.1. Sampling*

After personal communication with physicians and their diabetic patients using additional treatment of traditional medicines in the eastern region in Saudi Arabia, 15 plants species were considered in this study. Five samples were examined for each species and analysis of each sample was carried out in triplicate. Table 1 shows the examined species and the parts considered for establishing trace element profiles.

*2.2. Sample treatment and analysis*

The targeted parts of plant species were manually separated and partitioned in two portions. While a portion was treated for quantification of accessible Cr and Mn species contents followed by quantification of Cr and Mn in protein fractions, the other portion was treated for quantification of total Cr and Mn contents.

In principle, the extraction procedure for accessible Cr and Mn species analysis is oriented at maintaining speciation and being close to regular use of the plants as tea, i.e. hot water extract [28,30,31]. 200 mg of plant samples were mixed with 5 mL of Milli-Q water and adjusted at pH 4.0 using 10 mmol/L HCl. The mixture was then shacked at 80°C for 24 h and centrifuged at 3.500 rpm for 30 min. Thereafter, the supernatant was filtered by 42 Whatmann® filter paper and the extracts were used for quantifications.

A wet digestion procedure [28] was applied for total quantification in plant samples. 100 mg of plant samples were mixed with 1 ml of concentrated HNO3 (analytical reagent grade obtained from Sigma-Aldrich and sub-boiled distilled). A microwave system (CEM Discover SP-D) was used for digestion, which was carried out at three programmable steps as follows: (i) ramp 4 min with increasing temperature to 200°C; (ii) hold temperature for 2 min; (iii) cooling for 10 min. The mixture was diluted to 8 mL by water of Milli-Q water standard.

*2.3. Trace elements measurements*

The ICP-OES used for the measurements of Mn total contents was *Optima 7300* system from Perkin Elmer, Rodgau-Jügesheim, Germany. Digested samples were introduced into the system using a peristaltic pump connected to a Seaspray nebulizer with a cyclon spray chamber. Mn was measured at the following conditions: spectral line 257.611 nm; radio frequency power 1400 W; plasma gas flow 13 L Ar /min; nebulizer gas flow 0.6 L Ar/min.

The ICP-sf-MS system used for Cr-quantification was an Element 2 from Thermo-Electron, Bremen, Germany. The internal standards of 103Rh and 193Ir with concentration of 1 μg/L were administered to each sample. The instrument was operated in the high resolution mode. Samples were introduced using a peristaltic pump connected to a Seaspray nebulizer with a cyclon spray chamber. The measured element isotope was 52Cr. Other operating conditions were set as follows: radio frequency power 1300 W; plasma gas flow rate 15 L Ar /min; nebulizer gas flow rate 0.9 L Ar/min after daily optimization.

For quality control of measurements by both ICP-OES and ICP-sf-MS, five blank samples were tested followed by certified control standards (CPI international) for each element and two blanks.

*2.4. Species characterization by size exclusion chromatography-ICP-MS*

For species characterization, a hyphenation of SEC with ICP-MS was employed. The HPLC system used in this set-up was a 1100 Smartline inert Series from Knauer, Berlin, Germany. To maintain satisfactory resolution of both low and high molecular masses (LMM/HMM) of protein fractions, two separation columns were installed serially in the HPLC system. The first column was filled with Toyopearl TSK HW 55S from TosoHaas, Stuttgart, Germany. The dimension of the column was 600 × 10 mm ID. The column obtained separation in the mass range from 1 to 700 kDa. The second column was equipped with Toyopearl TSK HW 40S from TosoHaas, Stuttgart, Germany. The dimension was 250 × 8 mm ID. This second column obtained separation of analytes with masses of < 2 kDa. Following Willkommen et al. procedure [32], isocratic elution was carried out using 9:1-mixture of 50 mmol/L NH4Ac, pH 5.8 and 500 mmol/L NH4Ac, 10 mmol/L Tris, 5 vol% MeOH, pH 8.0. Other operating conditions were set as follows: flow rate 0.7 mL/min, injection volume 20 µL. The column effluent was introduced to the ICP-MS system (NexIon, Perkin Elmer, Rodgau-Jügesheim) using a Meinhard nebulizer and a cyclon spray-chamber. The ICP-parameter were as follows: radio frequency power 1200 W; plasma gas flow rate 15 L Ar /min; nebulizer gas flow rate 0.94 L Ar/min after daily optimization, dynamic reaction cell-gas: NH3 at 0.6 ml/min, rejection parameter q 0.58. The hyphenated system was mass-calibrated for SEC-separation, using the following standards with the following masses: ferritin 440 kDa, γ-globulin 190 kDa, arginase 107 kDa, transferrin 78 kDa, HSA 66 kDa, β-lactoglobulin 37 kDa, oxidized (612 Da) and reduced (307 Da) glutathione, citrate 192 Da, inorganic Mn (55 Da), and Fe (56 Da). The retention times were correlated to respective molecular masses. The calibration equations for LMM and HMM are described by Equation 1 and Equation 2; where RT stands for retention time and R stands for correlation coefficient.

LMM: log(MW) = -0.014RT + 3.0747, R² = 0.9592 Equation 1

HMM: log(MW) = -0.0009RT3 + 0.0977RT2 – 3.5058RT + 46.998, R² = 0.9942 Equation 2

Evaluation of chromatographic data was performed by exporting the chromatograms from ICP-MS software (Syngistix) to PeakfitTM software (Systat) for peak area calculation.

*2.5. Statistical analysis*

Univariate statistical analyses (minimum, maximum, mean, standard deviation (SD), and relative standard deviation (RSD)) as well as bi-variate statistical analysis (Peasron's correlation coefficients) were carried out using the statistical package SPSS 20.0 (SPSS, USA). PCC was conducted using two-tailed test of significance. The correlation was considered significant at the 0.01 and 0.05 levels.

**3. Results and discussion**

*3.1. Cr and Mn total contents*

Table 2 shows the total and accessible species contents, with descriptive statistical analysis, of Cr and Mn of the investigated plants. Total Cr species contents show the following descending order: *Haloxylon Salicornicum* > *Olea Europaea* > *Calligonumcomosum* > *Momordica Charantia* > *Vacciniummyrtillus* > *Citrulluscolocynthis* > *Trigonellafoenum-graecum* > *Nigella sativa* > *Opuntia Ficus-indica* > *Pennisetumglaucum* > *Sesamumindicum*. High levels of both SD and RSD for total contents of both trace elements indicate wide variation of accumulation levels of Cr and Mn among the examined plant species. In this context, Mn uptake by *Trigonellafoenum-graecum* was 4500-fold of that by *Sesamumindicum*, while Cr uptake by *Haloxylon* *Salicornicum* was 16-fold compared to by *Vacciniummyrtillus*. The accumulation of trace elements in plants depends on several factors such as soil physiochemical properties, climate conditions, plant affinity, element speciation, and element concentration [33].

A previous study reported comparable results of Cr contents (65–368 µg/kg) in 20 species of medicinal plants consumed in South Africa [34]. However, Cr total contents in the current examined medicinal plant species were found lower (330–4210 µg/kg) than those reported in other medicinal plant species consumed in Turkey [35]. This previous study screened 22 medicinal plants used traditionally for treatment of different diseases. In addition, another previous study reported higher Cr total contents (17600–110100 µg/g) in ten medicinal plants consumed in India for treatments of different diseases [36].

Mn total contents in the current study were within previously reported ranges (12.7–194.0 µg/g) in some medicinal plants growing and consumed in Moldova [37]. Mn total contents were also within a range (14.0–198.0 µg/g) reported in leaves of some plants traditionally used for hepatitis treatment in Dembia, Ethiopia [38]. Furthermore, a wide range of Mn total contents (3.00–290 µg/g) in medicinal plants used in Pakistan [39].

*3.2. Accessible Cr and Mn species contents*

Likewise Cr and Mn total contents, wide ranges were observed for both Cr (0.93–29.4 µg/kg) and Mn (0.82–35.85 mg/kg) accessible element species contents. Accessible Cr species contents show the following descending order: *Calligonumcomosum* > *Olea* *Europaea* ≈ *Haloxylon Salicornicum* > *Momordica Charantia* > *Vacciniummyrtillus* > *Citrulluscolocynthis* > *Nigella sativa* > *Pennisetumglaucum* > *Opuntia Ficus-indica* > *Trigonellafoenum-graecum* > *Sesamumindicum*. Accessible Mn species contents show the following descending order: *Opuntia Ficus-indica* > *Haloxylon Salicornicum* > *Olea Europaea* > *Momordica Charantia* > *Calligonumcomosum* > *Citrulluscolocynthis* > *Pennisetumglaucum* > *Nigella sativa* > *Vacciniummyrtillus* > *Trigonellafoenum-graecum* > *Sesamumindicum*. These classifications show similar orders for some plants and different orders for others. For instance, *Momordica Charantia* was in the fourth order of both Cr and Mn classifications. *Haloxylon Salicornicum* and *Olea Europaea* changed with each other the second and the third orders. Nevertheless, while *Calligonumcomosum* was in the first order of Cr content, it was in the fifth order in of Mn content.

To gather the benefits of accessible species contents of both Cr and Mn in specific plant species, *Haloxylon Salicornicum* and *Olea Europaea* could be recommended to be consumed for supporting T2DM treatment. In our previous study [28], *Momordica Charantia* showed the highest accessible Zn species content. This species is also rich in both Cr and Mn. Therefore, it is highly recommended to be consumed by T2DM patients.

From the viewpoint of bioaccessibility percentage, also wide range of percentages for both Cr (0.65–4.21%) and Mn (5.43–68.42%) were observed. Accessible Mn species percentage was much higher than that of Cr, which is attributed to the number and amount of Mn water-soluble forms being higher than that of Cr. Despite Nigella sativa was in the sixth order of total Cr content, it was in the first order of accessible Cr species percentage. For Mn, *Opuntia Ficus-indica* was in the first order of total Mn content, while it was in the first order of accessible Mn species percentage.

*3.3. Relationships between total contents and accessible species contents of Cr and Mn*

The matrix of the Peasron's correlation coefficients (R), with the relevant *p* values, between total contents and accessible species contents of Cr and Mn is shown in Table 3. To have a more comprehensive picture of relationships, Zn total and accessible species contents reported in our previous study are also included in the matrix [28]. The matrix shows significant positive correlation between Cr total contents and accessible Cr species contents; at the 0.01 level, R = 0.936. Also, significant positive correlation (R = 0.826 at 0.01 level) between Mn total contents and accessible species contents was observed. From these results, it could be concluded that the ratios of contents of Cr soluble-forms to total Cr contents are similar to that of Mn among the examined medicinal plants. In addition, significant positive correlation (at 0.05 level, R (0.625) is significant) was observed for Mn total contents and Cr total contents. This result indicates that the behavior of Cr and Mn accumulation in the examined medicinal plants is significantly similar. Nevertheless, Zn did not record significant correlation between total and accessible species contents. Notably, negative correlations, but not significant at 0.05 level, were observed between Cr total contents and Zn total contents as well as between Mn total contents and Zn total contents. These results reflect that Zn accumulation differ from Cr and Mn accumulation.

*3.4. Risk assessment*

Since wide ranges for both Cr and Mn were observed in medicinal plants that may put consumers at risk from consumption of plants reported high levels, it is desirable to assess the exposure level and tracing the path of Cr and Mn. In general, human is exposed to contaminants in various environmental counterparts through mainly three pathways; direct ingestion, inhalation, and dermal contact [9, 10, 33]. For risk assessment of food, only direct ingestion is the appropriate pathway [2–5, 40]. Accordingly, the chronic daily intake (CDI) for Cr and Mn was calculated for each category, i.e. children and adult, using Equation 3 [40]. Ci stands for the total element concentration (mg/kg) in plant. IngR stands for the ingestion rate (200 mg/day for children and 100 mg/day for adult). ExFr stands for the exposure frequency (350 days for both children and adults). ED stands for the exposure duration (6 years for children and 24 years for adult). BW stands for the body weight. 15 kg have been assigned for children and 70 kg for adults. AT stands for the averaging time, which is 365 × 6 day for children and 365 × 24 for adult. The results for Cr and Mn are shown in Table 4 and Table 5, respectively.

$CDI= \left(\frac{C\_{i}×IngR×ExFr×ED}{BW×AT}\right)×CF$ Equation 3

In addition, the hazard index (HI) was calculated (Equation 4) to assess non-carcinogenic risk from the intake. RfD stands for the reference dose by ingestion for the health risk of non-carcinogenic adverse effects. It was reported that HI < 1 suggests no probability of health risk to occur, whereas HI ≥ 1 suggests moderate or high risk for adverse human health effects (USEPA 2007). Accordingly, as shown in Table 4 and Table 5, for both elements Cr and Mn, the HI values for all examined plants consumed by both children and adults were all < 1 indicating no probability of health risk to occur.

$HI\_{for non carcinogenic risk }=\frac{CDI}{RfD}$ Equation 4

Furthermore, the lifetime average daily dose (LADD) through the exposure pathway was calculated (Equation 5) for Cr as it was reported a carcinogenic element. Consequently, the carcinogenic risk (CR) was calculated (Equation 6) to evaluate the possibility of having cancer. CSF stands for the cancer slope factor of an element. The safe level of CR is < 1 × 10-6 [41]. As a result, Table 4 shows that there are no probability of cancer risk to occur from the consumption of all examined plants.

$LADD\_{Inh}=\frac{C×ExFr}{PEF×AT}×\left(\frac{R\_{Child}×ED\_{Child}}{BW\_{Child}}+\frac{R\_{IngAdults}×ED\_{Adults}}{BW\_{Adults}}\right)$ Equation 5

$CR=LADD×CSF $ Equation 6

*3.5. Cr and Mn protein-fraction contents*

Fig. 1 demonstrates the Cr chromatogram – including peak deconvolution and evaluation as well as the Mn chromatogram from *Olea europea* as an example. Table 6 and Table 7 show Cr and Mn protein-fraction contents in the examined medicinal plants, respectively. The recovery of the summation contents in all fractions to accessible element species contents was calculated and the results are shown in Table 6 and Table 7 as well.

**Fig. 1.** Chromatograms of Cr (peak deconvolution and evaluation) and Mn from Olea europea.

As shown in Table 6, the recovery values (sum of fractions / total Cr) were close to 100.0% (range 86–106 %), indicating accuracy of the quantification of Cr protein-fractions. On the other hand, despite the low levels of accessible Cr species contents, Cr was successfully quantified in all protein fractions irrespective of plant species, with the exception of the 1.9–3.7 kDa fraction. Notably, all plant species recorded detectable Cr levels in the 10–14 kDa protein fraction, with the exception of *Olea Europaea*. In another context, as *Calligonum comosum* recorded the highest accessible Cr species content, 42% and 28% of Cr contents were observed in the two sequential protein fractions of 70–78 and 50–65 kDa, respectively. Likewise, as *Olea Europaea* was in the second order of accessible Cr species content, the inorganic fraction (0.05–0.40 kDa) recorded 41% of Cr content followed by the 70–87 kDa protein fraction that bound 35% of accessible Cr species content. In the third order of accessible Cr species content is *Haloxylon Salicornicum* (25.00 µg/kg), which it is almost similar to *Olea Europaea* (25.70 µg/kg). 49% of accessible Cr species content in *Haloxylon Salicornicum* bound to the 50–60 kDa protein fraction.

 For Mn protein fraction contents, the recovery values (sum of fractions / total Mn) in all examined plants were close to 100.0%, too, ranging from 87 % to 102 %., suggesting accuracy of the quantification of Mn protein fractions. Interestingly, all accessible Mn species contents bound to the inorganic protein fraction for *Vaccinium myrtillus*, *Calligonum comosum*, *Momordica Charantia*, *Opuntia Ficus-indica* and *Haloxylon Salicornicum*. Moreover, the percentages of 99%, 97%, 81%, and 73% of accessible Cr species contents bound to the inorganic protein fraction for *Pennisetum glaucum*, *Citrullus colocynthis*, *Vaccinium myrtillus* and Nigella sativa, respectively. Nevertheless, no Cr was detected in the inorganic fraction for *Sesamumindicum Trigonella* and *Foenum-graecum*.

**4. Conclusions**

The present study reports Cr and Mn total, accessible species, and protein-fraction contents in eleven medicinal plants traditionally used as anti-diabetic. From the results obtained, the following conclusions can be drawn.

* To gather, the benefits of specific plant species in terms of accessible Cr and Mn species contents, in addition to accessible Zn species contents reported in our previous study, *Haloxylon Salicornicum*, *Olea Europaea Momordica* and *Charantia* are recommended to be consumed for traditionally controlling T2DM.
* The correlation analysis reveals that the ratios of accessible Cr species contents to total Cr total contents are similar to that of Mn among the examined medicinal plants. The correlation also indicates similar behavior of Cr and Mn uptake by the examined plants.
* For protein profile, considerable amounts of accessible Cr species content bound to the 50–60 kDa and inorganic (0.05–0.40 kDa) protein fractions. For Mn, the dominant accessible species contents bound to the inorganic protein fraction.
* Despite the wide ranges of total Cr and Mn contents in the examined medicinal plants, no probability of general health risk was observed from the consumption of these plants. In addition, no probability of cancer to occur by Cr was recorded as it is reported a carcinogenic element.

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**Fig. 1**

**Table 1**

The examined plant species and the parts used.

|  |  |  |
| --- | --- | --- |
| Plant scientific name | Plant common name  | Part used  |
| *Trigonella Foenum-graecum* | Fenugreek | Seeds |
| Cinnamomum*zylanicum* | Cinnamon | Bark |
| Citrullus colocynthis | Colocynth  | Fruit |
| Momordica Charantia | Bitter melon | Fruit |
| Vaccinium myrtillus | Bilberry  | Fruit |
| Opuntia Ficus-indica | Barbary  | Fruit |
| Calligonum comosum | Carthage | Whole plant |
| *Retama raetam* | Retem | Whole plant |
| *Pennisetum glaucum* | Pearl millet | Seeds |
| *Guieras enegalensis* | Guiera | Leaves |
| *Haloxylon Salicornicum* | Ramth | Whole plant |
| *Nigella sativa* | black cumin | Seeds |
| *Morus alba* | White mulberry | Leaves |
| *Olea Europaea* | Olive | Leaves |
| *Sesamumindicum* | Sesame | Seeds |

**Table 2**

Total contents of Cr (µg/kg) and Mn (mg/kg) in digested samples and water extracts.

|  |  |  |  |
| --- | --- | --- | --- |
| Plant species  | Total content | Bioaccessible  | Excretion efficiency (%)  |
| Cr | Mn | Cr | Mn | Cr | Mn |
| *Sesamumindicum* | 44.70 | 15.10 | 0.93 | 0.82 | 2.08 | 5.43 |
| *Olea Europaea* | 1560.00 | 45.80 | 25.70 | 16.25 | 1.65 | 35.48 |
| *Vacciniummyrtillus* | 396.00 | 3.70 | 9.22 | 2.43 | 2.33 | 65.68 |
| *Nigella sativa* | 95.00 | 26.00 | 4.00 | 2.91 | 4.21 | 11.19 |
| *Trigonellafoenum-graecum* | 197.00 | 15.20 | 1.28 | 1.65 | 0.65 | 10.86 |
| *Pennisetumglaucum* | 58.80 | 11.60 | 2.26 | 4.00 | 3.84 | 34.48 |
| *Calligonumcomosum* | 1240.00 | 27.60 | 29.40 | 9.79 | 2.37 | 35.47 |
| *Citrulluscolocynthis* | 205.00 | 10.60 | 8.00 | 6.32 | 3.90 | 59.62 |
| *Momordica Charantia* | 800.00 | 25.40 | 18.80 | 12.00 | 2.35 | 47.24 |
| *Opuntia Ficus-indica* | 65.30 | 52.40 | 1.76 | 35.85 | 2.70 | 68.42 |
| *Haloxylon Salicornicum* | 1880.00 | 59.00 | 25.00 | 18.25 | 1.33 | 30.93 |
| Minimum  | 44.70 | 3.70 | 0.93 | 0.82 | 0.65 | 5.43 |
| Maximum | 1880.00 | 59.00 | 29.40 | 35.85 | 4.21 | 68.42 |
| Mean | 594.71 | 26.58 | 11.49 | 10.02 | 2.49 | 36.80 |
| Standard deviation | 671.68 | 18.32 | 11.08 | 10.44 | 1.12 | 21.95 |
| Relative standard deviation | 112.94 | 68.90 | 96.46 | 104.17 | 44.87 | 59.66 |

**Table 3**

Pearson's correlation matrix (coefficient and *p* values) of Cr, Mn and Zn contents in bulk and water extract (bioaccessible).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Tot-Cra | Bio-Crb | Tot-Mn | Bio-Mn | Tot-Zn | Bio-Zn |
| Tot-Cr | 1.000 |  |  |  |  |  |
| Bio-Cr | 0.936c(0.000) | 1.000 |  |  |  |  |
| Tot-Mn | 0.625d(0.040) | 0.472(0.143) | 1.000 |  |  |  |
| Bio-Mn | 0.304(0.364) | 0.242(0.473) | 0.826c(0..02) | 1.000 |  |  |
| Tot-Zn | -0.582(0.060) | -0.595(0.053) | -0.306(0.360) | -0.470(0.145) | 1.000 |  |
| Bio-Zn | -0.126(0.712) | 0.064(0.853) | -0.347(0.295) | -0.220(0.516) | 0.040(0.906) | 1.000 |

a total content; b bioaccessible content; c content is significant at the 0.01 level; d correlation is significant at the 0.05 level

**Table 4**

Chronic daily intake (CDI), hazardous quotient (HQ), hazardous index (HI), lifetime average daily dose (LADD) and cancer risk (CR) of Cr total contents in medicinal plants.

|  |  |  |
| --- | --- | --- |
| Plant species | Children  | Adult  |
| CDI | HQ | HI | LADD | CR | CDI | HQ | HI | LADD | CR |
| *Sesamumindicum* | 5.71E-07 | 1.14E-04 | 1.14E-04 | 5.20E-11 | 2.09E-09 | 5.71E-07 | 1.14E-04 | 1.14E-04 | 5.20E-11 | 2.09E-09 |
| *Olea Europaea* | 1.99E-05 | 3.99E-03 | 3.99E-03 | 1.81E-09 | 7.29E-08 | 1.99E-05 | 3.99E-03 | 3.99E-03 | 1.81E-09 | 7.29E-08 |
| *Vaccinium myrtillus* | 5.06E-06 | 1.01E-03 | 1.01E-03 | 4.61E-10 | 1.85E-08 | 5.06E-06 | 1.01E-03 | 1.01E-03 | 4.61E-10 | 1.85E-08 |
| *Nigella sativa* | 1.21E-06 | 2.43E-04 | 2.43E-04 | 1.10E-10 | 4.44E-09 | 1.21E-06 | 2.43E-04 | 2.43E-04 | 1.10E-10 | 4.44E-09 |
| *Trigonella Foenum-graecum* | 2.52E-06 | 5.04E-04 | 5.04E-04 | 2.29E-10 | 9.21E-09 | 2.52E-06 | 5.04E-04 | 5.04E-04 | 2.29E-10 | 9.21E-09 |
| *Pennisetum glaucum* | 7.52E-07 | 1.50E-04 | 1.50E-04 | 6.84E-11 | 2.75E-09 | 7.52E-07 | 1.50E-04 | 1.50E-04 | 6.84E-11 | 2.75E-09 |
| *Calligonum comosum* | 1.59E-05 | 3.17E-03 | 3.17E-03 | 1.44E-09 | 5.80E-08 | 1.59E-05 | 3.17E-03 | 3.17E-03 | 1.44E-09 | 5.80E-08 |
| *Citrullus colocynthis* | 2.62E-06 | 5.24E-04 | 5.24E-04 | 2.38E-10 | 9.58E-09 | 2.62E-06 | 5.24E-04 | 5.24E-04 | 2.38E-10 | 9.58E-09 |
| *Momordica Charantia* | 1.02E-05 | 2.05E-03 | 2.05E-03 | 9.30E-10 | 3.74E-08 | 1.02E-05 | 2.05E-03 | 2.05E-03 | 9.30E-10 | 3.74E-08 |
| *Opuntia Ficus-indica* | 8.35E-07 | 1.67E-04 | 1.67E-04 | 7.59E-11 | 3.05E-09 | 8.35E-07 | 1.67E-04 | 1.67E-04 | 7.59E-11 | 3.05E-09 |
| *Haloxylon Salicornicum* | 2.40E-05 | 4.81E-03 | 4.81E-03 | 2.19E-09 | 8.79E-08 | 2.40E-05 | 4.81E-03 | 4.81E-03 | 2.19E-09 | 8.79E-08 |

**Table 5**

Chronic daily intake (CDI), hazardous quotient (HQ), hazardous index (HI), lifetime average daily dose (LADD) and cancer risk (CR) of Mn total contents in medicinal plants.

|  |  |  |
| --- | --- | --- |
| Plant species | Children  | Adult  |
| CDIa | HQb | HIc | LADDd | CDI | HQ Inge | HI | LADD |
| *Sesamumindicum* | 1.93E-04 | 3.86E-02 | 3.86E-02 | 1.76E-08 | 2.07E-05 | 4.14E-03 | 4.14E-03 | 1.72E-08 |
| *Olea Europaea* | 5.86E-04 | 1.17E-01 | 1.17E-01 | 5.33E-08 | 6.27E-05 | 1.25E-02 | 1.25E-02 | 5.20E-08 |
| *Vaccinium myrtillus* | 4.73E-05 | 9.46E-03 | 9.46E-03 | 4.30E-09 | 5.07E-06 | 1.01E-03 | 1.01E-03 | 4.20E-09 |
| *Nigella sativa* | 3.32E-04 | 6.65E-02 | 6.65E-02 | 3.02E-08 | 3.56E-05 | 7.12E-03 | 7.12E-03 | 2.95E-08 |
| *Trigonella Foenum-graecum* | 1.94E-04 | 3.89E-02 | 3.89E-02 | 1.77E-08 | 2.08E-05 | 4.16E-03 | 4.16E-03 | 1.73E-08 |
| *Pennisetum glaucum* | 1.48E-04 | 2.97E-02 | 2.97E-02 | 1.35E-08 | 1.59E-05 | 3.18E-03 | 3.18E-03 | 1.32E-08 |
| *Calligonum comosum* | 3.53E-04 | 7.06E-02 | 7.06E-02 | 3.21E-08 | 3.78E-05 | 7.56E-03 | 7.56E-03 | 3.14E-08 |
| *Citrullus colocynthis* | 1.36E-04 | 2.71E-02 | 2.71E-02 | 1.23E-08 | 1.45E-05 | 2.90E-03 | 2.90E-03 | 1.20E-08 |
| *Momordica Charantia* | 3.25E-04 | 6.49E-02 | 6.49E-02 | 2.95E-08 | 3.48E-05 | 6.96E-03 | 6.96E-03 | 2.89E-08 |
| *Opuntia Ficus-indica* | 6.70E-04 | 1.34E-01 | 1.34E-01 | 6.09E-08 | 7.18E-05 | 1.44E-02 | 1.44E-02 | 5.95E-08 |
| *Haloxylon Salicornicum* | 7.54E-04 | 1.51E-01 | 1.51E-01 | 6.86E-08 | 8.08E-05 | 1.62E-02 | 1.62E-02 | 6.70E-08 |

a chronic daily intake; b hazardous quotient; c hazardous index; d lifetime average daily dose, e ingestion

**Table 6**

Cr protein-fraction contents in medicinal plants.

|  |  |  |
| --- | --- | --- |
| Plant species | Protein fraction (kDa) | Ra |
| 330-430  | 153-220 | 125-145 | 100-120 | 70-87 | 50-60 | 22-28 | 10-14 | 1.9-3.7 | 0.9-1.5 | 0.05-0.40  |
| *Sesamumindicum* | n.d. | n.d. | 0.34 | n.d. | n.d. | n.d. | n.d. | 0.21 | n.d. | 0.34 | 0.04 | 86 |
| *Olea Europaea* | n.d. | n.d. | n.d. | n.d. | 8.97 | n.d. | 5.38 | n.d. | n.d. | 0.90 | 10.46 | 86 |
| *Vaccinium myrtillus* | n.d. | n.d. | n.d. | n.d. | 6.18 | n.d. | 0.69 | 0.59 | n.d. | n.d. | 1.77 | 94 |
| *Nigella sativa* | n.d. | n.d. | n.d. | n.d. | 1.96 | n.d. | 0.12 | 0.69 | n.d. | n.d. | 1.22 | 98 |
| *Trigonella Foenum-graecum* | n.d. | n.d. | n.d. | 0.47 | n.d. | n.d. | 0.24 | 0.38 | n.d. | 0.19 | n.d. | 87 |
| *Pennisetum glaucum* | 0.31 | n.d. | n.d. | n.d. | 0.19 | n.d. | n.d. | 0.70 | n.d. | 0.55 | 0.50 | 94 |
| *Calligonum comosum* | 2.45 | n.d. | 1.63 | n.d. | 12.25 | 8.17 | n.d. | 4.90 | n.d. | n.d. | n.d. | 102 |
| *Citrullus colocynthis* | n.d. | 0.71 | n.d. | 1.91 | n.d. | n.d. | 2.47 | 1.01 | n.d. | n.d. | 1.91 | 106 |
| *Momordica Charantia* | 1.71 | n.d. | 1.52 | 1.14 | n.d. | 3.99 | 3.23 | 1.52 | n.d. | n.d. | 5.70 | 99 |
| *Opuntia Ficus-indica* | n.d. | n.d. | 0.38 | n.d. | n.d. | 0.50 | n.d. | 0.29 | n.d. | 0.59 | n.d. | 87 |
| *Haloxylon Salicornicum* | n.d. | 1.75 | n.d. | n.d. | 3.00 | 12.25 | 6.25 | 1.75 | n.d. | n.d. | n.d. | 100 |

a recovery (%) of the summation contents in all fractions to bioaccessible contents

**Table 7**

Mn protein-fraction contents in medicinal plants.

|  |  |  |
| --- | --- | --- |
| Plant species  | Protein fraction (kDa) | R |
| 330-430  | 153-220 | 125-145 | 100-120 | 70-87 | 50-60 | 22-28 | 10-14 | 1.9-3.7 | 0.9-1.5 | 0.05-0.40  |
| *Sesamumindicum* | 0.40 | 0.00 | 0.10 | n.d. | n.d. | 0.06 | 0.00 | 0.26 | n.d. | n.d. | n.d. | 86 |
| *Olea Europaea* | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 16.25 | 100 |
| *Vaccinium myrtillus* | n.d. | n.d. | n.d. | n.d. | 0.44 | n.d. | n.d. | n.d. | n.d. | n.d. | 1.98 | 98 |
| *Nigella sativa* | 0.38 | n.d. | n.d. | n.d. | 0.27 | n.d. | 0.12 | n.d. | n.d. | n.d. | 2.13 | 90 |
| *Trigonella Foenum-graecum* | n.d. | 0.22 | n.d. | n.d. | n.d. | 0.15 | 0.13 | 0.35 | n.d. | 0.80 | n.d. | 99 |
| *Pennisetum glaucum* | 0.04 | n.d. | n.d. | n.d. | n.d. | n.d. | 0.01 | n.d. | n.d. | n.d. | 3.94 | 101 |
| *Calligonum comosum* | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 9.79 | 100 |
| *Citrullus colocynthis* | 0.06 | n.d. | 0.04 | n.d. | 0.06 | n.d. | n.d. | n.d. | n.d. | n.d. | 6.15 | 102 |
| *Momordica Charantia* | n.d. | n.d. | n.d. | n.d. | 0.05 | n.d. | n.d. | n.d. | n.d. | n.d. | 11.95 | 98 |
| *Opuntia Ficus-indica* | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 35.85 | 100 |
| *Haloxylon Salicornicum* | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 18.25 | 97 |

a recovery of the summation contents in all fractions to bioaccessible contents