First Comprehensive Study on Total Determination of Nutritional Elements in the Fruit of the *Campomanesia Adamantium* (Cambess.): Brazilian Cerrado Plant

Nayara Vieira de Lima¹, Daniela Granja Arakaki¹, Paula Fabiana Saldanha Tschinkel¹, Anderson Fernandes da Silva¹, Rita de Cássia Avellaneda Guimarães¹, Priscila Aiko Hiane¹, Marcos Antonio Ferreira Júnior², Valter Aragão do Nascimento¹

Abstract

**Introduction:** Fruits and leaves of *Campomanesia adamantium* (Cambess.) O. Berg commonly called guavira are used in dietary or as a mode of treatment of variety of ailments in indigenous and urban populations in the city of the Campo Grande, State of Mato Grosso do Sul, Brazil. However, comprehensive studies on their mineral composition are scarce.

**Objective:** In the present study, evaluation of mineral contents (Na, K, Ca, Mg, P, Fe, Zn, Ni, Mn, Co, Cu, Mo, Cr, Si and Al) from peel, pulp and seeds of guavira was carried out.

**Method:** The peel, pulp and seeds of guavira were studied by ICP-OES with microwave digestion. The contents of the elements in the parts of the guavira, were compared to the Recommended Dietary Allowance (RDA), values Adequate Intake (AI) and tolerable upper intake levels (ULs).

**Results:** The results are considered in terms of the utility of the natural herbal medicaments as rich (Cu, P, Cr and Mo) or a source of minerals indispensable for proper functioning of the human organism. The concentration of elements in seeds, pulp and peel the guavira was compared with value of UL and does not cause toxicity. The concen-
Introduction
Medicinal plants are indispensable for human well being and provides all or a significant number of the remedies required in health care. Medicinal plants are used in treatment of complex diseases such as cancer [1], nutrition, flavoring, fragrances, cosmetics and other industrial purposes [2]. Several Brazilian Therapeutic plants and fruits are used as a mode of treatment of variety of ailments in indigenous and urban populations. Scientific researches have been made on traditional use of medicinal plants used in the Amazon [3-6], southern and northeast of Brazil [2-9]. However, the vegetation of Brazil has a lot of plants and tree species that are endemic. Most of these are little exploited. In general in the literature there are few reports on medicinal plants in the region Midwest Brazil, mainly involving the biome of pantanal and cerrado [3].

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a bush that grows in the fields and pastures of Brazil’s cerrado. This species belongs to the family Myrtaceae. Its fruits commonly called gabiroba, guabirolba or guavira. The fruits present oval shape, the pulp was succulent, with flavor well-appreciated. Its fruits are very much consumed by natives and sold to the public at the street-traders’ stalls. Its fruits are used by urban and rural population to make juice, ice cream and in alcoholic drinks [10].

In Campo Grande, State of Mato Grosso do Sul, in Midwest Brazil, according to popular information, Campomanesia adamantium and its fruits are rich in Aluminum, Zinc, Phosphorus and Magnesium. Indigenous, rural and urban populations believe that guavira is rich in Iron and combats anemia. In this context, each chemical element has its chemical properties, health effects, and are associated with important applications in the treatment of ailments. Indeed, fruits are important sources of many nutrients, including Potassium, fiber and vitamin C. However, there are no scientific data confirming the concentration of these elements in the seeds, pulp and peel of guavira.

In folk medicine the leaves of Campomanesia adamantium (Cambess.) O. Berg and its fruits are used to treat urinary tract disease, inflammatory and obesity problems [11]. Preparation of the infusion of the leaves is used to control diarrhea,
bladder problems, throat infections and vomiting [12]. Also is used in folk medicine to treat inflammation and rheumatism [13], anti-inflammatory and antinociceptive activities [14]. The compound cardamonin isolated from leaves of this plant showed antiproliferative activity in the cell line in a bioactivity-guided study [15]. In previous studies in vitro, the fruit extracts of C. adamantium were evaluated against the microorganism Mycobacterium tuberculosis [16]. Moreover, studies in animals showed that the fruit extracts of C. adamantium has anti-inflammatory, antihyperalgesic, and anti-depressive properties in rodents without causing toxicity [17]. In the traditional medicine in Brazil, the root and leaves of Campomanesia adamantium O. Berg are used for antidiabetic effects. The roots for C. adamantium is used to lowering lipid peroxidation and lipid serum level, improving risk factors for cardiometabolic diseases development [18]. The essential oils obtained from the leaves and flowers from Campomanesia adamantium (Myrtaceae) in the flowering stage were analyzed. The constituent major in the leaf oil was limonene and in the flower oil was ledol [19]. Limonene shown gastroprotective action in animals [20], while ledol was associated to antileukaemic effects [21]. Despite the existence of various species of plants and fruits that are used in the treatment of leukemia [22], knowing the concentration of elements is essential. According with others studies, the fruits have antioxidant properties; results showed the hepatoprotective effects of pulp or peel/seed hydroalcoholic extracts on injured liver-derived HepG2 cells by CCl4. Partially, the result are associated with the presence of antioxidant compounds as flavonoids [23].

As noted on the above-mentioned findings, different studies have shown that fruits, roots, leaves, peel and pulp of Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) has medicinal properties but no elemental composition has been done. Minerals play a very important role in the formation of the active chemical constituents present in medicinal plants. They are essential to human health and have a preventive role in combating diseases, even though they comprise only 4-6% of the human body. It is very important, to know the concentration of Macroelements (Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P)) and microelements (Iron (Fe), Manganese (Mn), Zinc (Zn), Cobalt (Co), Copper (Cu), Chromium (Cr), Lead (Pb), Nickel (Ni), Cadmium (Cd), Aluminum (Al), Selenium (Se), Sulfur (S), Arsenium (Ar), Molybdenum (Mo) in medicinal plants to estimate their role as sources of these components in the human diet. The lack of knowledge of the elemental constituents of these medicinal plants often poses human lives at risk, these elements can also be dangerous and toxic.

In Brazil, the dose rate of medicinal plants is not well defined [24]. These precautions are indispensable when larger amounts of fruits, leaves or others parts of the plant are consumed in long-term. Thus, know the concentrations of minerals in medicinal plants are very important and need to be screened for their toxicity. There are no studies that performed the comparisons on chemical composition of seeds, pulp and peel of the fruit of Campomanesia adamantium (Cambess.) O. Berg in Brazil with the international safety standards for the consumption of human beings.

The aim of present work was to compare the macroelements and micro-elements obtained in fruits of Campomanesia adamantium Cambess. O. Berg used as treatment of ailments and foods by indigenous and urban communities of the Campo Grande, State of Mato Grosso do Sul, Brazil, with the limit specification of RDA/AI and the total daily intake of these minerals. The macro and microelements content, after microwave digestion, was determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES).
Method

Research area
Fruits of Campomanesia adamantium (Cambess.) O. Berg were collected in December 2015 in the city of Campo Grande, State of Mato Grosso do Sul, Midwest region of Brazil. Fruits of the same ripe stage were collected from different plants. The Figure 1 shows the Location of Campo Grande on a map of Brazil. It is located -20° 26´34´´S latitude and -54° 36´ 47´´W longitude and it is situated at elevation 592 meters above sea level.

In the Figure 2, there is one photo of fruit of the Campomanesia adamantium (Cambess.) O. Berg (on the left of Figure 2), popularly known as guavira from Cerrado, Campo Grande-MS, Brazil, on the right, the different parts (a) fruit of guavira, (b) peel and pulp guavira, (c) seed guavira. The specimen was identified by Arnildo Pott and deposited (No 53328) in the herbarium of the Federal University of Mato Grosso do Sul (UFMS)/Brazil.

Elemental analysis by ICP-OES technique
The peel, pulp and seed were removed and kept separately. The dried samples were then ground with a manual grinder into powder and sieve to get very fine powder. It was then weighed and digested in HNO₃ + H₂O₂ mixture. Samples were prepared as follows: processed with mixture of 0.5 g sample plus 5 mL HNO₃ (65% Merck) and 3 mL H₂O₂ (35%, Merck Millipore) in the microwave digestion system Speedwave®, Berghof, Germany. After digestion, samples were diluted to 100 mL with ultrapure water. Since the final acid concentration of the samples was quite high (4% HNO₃).

In the present paper, the concentration of the elements (K, Ca, Na, P, Mg, Fe, Si, Mo, Mn, Z, Cr, Cu, Al and Cu) was determined with the use of ICP-OES technique (Thermo Scientific – iCAP 6000 Series). The concentrations of the different elements in these samples were determined using the corresponding standard calibration curves obtained by using standard solutions of the elements of interest (Merck). Duplicate analyses were performed on each sample.

Comparative criteria
The contents obtained of the pulp, seeds and peel were compared with recommended values:

- Recommended Dietary Allowance (RDA): refer to the recommended average daily level of nutrients to meet the needs of nearly all healthy people in a particular age and gender group.
Adequate Intake (AI): established when RDA is insufficient and is set at a level assumed to ensure nutritional adequacy. It is a recommended average daily nutrient intake level, based on experimentally derived intake levels or approximations of observed mean nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

Tolerable Upper Intake Level (UL): maximum daily intake of nutrients that do not pose a risk of adverse effects on health. According to the recommended values, when the percentage of detected elements is greater than 15%, it is considered as a food source. However, when the concentration of elements detected is greater than 30%, it is considered as rich.

In the absence of established RDA or data on Adequate Intake (AI), some of values of concentration of macro and micro-nutrients obtained in this manuscript are compared with permissible limit set by FAO/WHO (Food and Agriculture Organization of the United Nations / World Health Organization) or with evidence of medicinal plants’ results published.

Results and Discussion

The concentration of mineral in the seeds, pulp and peels guavira fruit obtained in this work and compared with the limit specification of RDA/AI and the total daily intake of these minerals are in the Table 1. The results obtained within the framework of this study were compared with the estimates based on the regulatory limits of the WHO/FAO and published studies involving medicinal plants when available in the literature. In the present work, the concentration of macro-elements in the peel, pulp and seeds decreases in the order: K > Ca > Na > P > Mg, K > P > Na > Ca > Mg and P > K > Ca > Mg > Na. As well as the concentration of micro elements in the peel, pulp and seeds also decreases in the order: Fe > Si > Mo > Mn > Zn > Cr > Cu > Co, Si > Fe > Al > Mo > Zn > Mn > Cr > Cu and Fe > Al > Si > Zn > Mo > Cu > Mn > Cr > Ni > Co. Among the various elements K, Ca, Na, P are found present at the major level, Cr, Cu and Co are at minor level. Our studies demonstrated that the guavira seeds are rich in Copper, Iron, Phosphorus, chromium and molybdenum. However, this plant is not good source of other elements as Nickel, Zinc, Potassium, Magnesium, Manganese, Silicon, Sodium and Calcium.

In Table 1, a higher concentration of Sodium is found in the peel 23.34 mg/100g, while for pulp.
and seeds are 24.56 mg/100g and 5.82 mg/100g. Considering the Adequate Intake of Sodium of 1,500 mg/day, this fruit is not a good source of Sodium. Moreover, the tolerable upper intake level (UL) for consuming of Sodium in adults is 2,300 mg/day. Therefore, the results in Table 1 are below the values Adequate Intake and tolerable upper intake level. However, the results presented in Table 1 for Sodium concentration is higher than that obtained in other plants, such as the Terminalia chebula, with concentration of Sodium obtained of 0.06 mg/100g [25].

The concentrations of Potassium (K) in Table 1 were 254.8 mg/100g, 202.3 mg/100g and 175.15 mg/100g for guavira seeds, peel and pulp. These values are low when compared with others medicinal plants as Anethum graveolens (3,693.961 mg/100g) and Allium cepa (4,321.13 mg/100g) [26]. The fruit of guavira is not a source of Potassium, when considering the Adequate Intake of Potassium of 4,700 mg/day. The tolerable upper intake levels (UL) are not established for Potassium. However, several trials have evaluated the effects of Potassium supplementation [27].

On the other hand, minimum concentrations of Calcium were observed in the pulp (19.90 mg/100g) and maximum in seeds (46.08 mg/100g), which is less than 1,334.3 mg/100g in Brassica campestris, Pakistani medicinal plant [28]. The reported tolerable upper intake level of Calcium is 2,500 mg/day. It is clear that the concentration of Calcium in seeds, pulp and peel the guavira is lower than UL and does not cause toxicity. Calcium supplementation has been recommended for athletes who may require supplementation to improve bone density. In this case recommended daily dose of 1,500 mg [29]. However, higher doses cause a risk of serious gastrointestinal adverse event [30]. Until recently, the recommended adequate intake of Calcium for adults is 1,000 mg per day [31]. Based upon this information, the guavira is not considered a good source of Calcium.

In this study, in Table 1, a higher concentration of Magnesium is found in the seeds 19.81 mg/100g, while for pulp and peels are 10.37 mg/100g and 15.30 mg/100g. This result is inside of the interval obtained by others papers published, for example, in some Pakistani medicinal plants, Magnesium content ranged between 0.373 mg/100g in Punica granatum [32] to 224.188 mg/100g in Convolvulus arvensis [28]. The Recommended Dietary Allowance (RDA) of Magnesium (Mg) for adults is established the mean intake of 355 mg/day. So, the guavira fruit is not a good source of Magnesium. There is not current data to establish a safe upper level for the Magnesium intake. The UL for Magnesium is determined by supplementation only (350 mg/day) and does not regard the ingestion from food or water. Thus after this comparing, the contents of Magnesium obtained of the fruit of guavira do not cause toxicity.

The RDA for daily Phosphorus in adults is established the mean intake of 700 milligrams per day (see Table 1). From this comparison, the present study indicates that seeds of the guavira are rich in Phosphorus (406.52 mg/100g). Guavira have higher contents of Phosphorus than certain plants. In the case of Indian plant known as Sesbania bispinosa (Jacq.), the lowest concentration of Phosphorus was found in seeds 0.532 mg/100g followed by the concentrations in leaves 0.292 mg/100g, in roots 0.28 mg/100g [32]. The tolerable upper intake level (UL) for Phosphorus is 4,000 mg/day for generally healthy adults. In this case specifically, the contents of Phosphorus obtained of the fruit not cause toxicity.

In Table 1, Iron contents were 1.453, 1.089 and 5.022 mg/100g for the guavira peel, pulp and seed. Recommended Dietary Allowance (RDA) of Iron for adults is established the mean intake of 13 mg/day. After comparison, the present study indicates that seeds of the guavira are rich in Iron. The regulatory limits of the WHO/FAO (2005) have not been established yet for the Iron in herbal medicines. The
limited set by FAO/WHO (1984) in edible plants was 2 mg/100g. The Iron concentration found in Pakistani medicinal plants ranged with values between 18.163 and 679.688 mg/100g [28]. However, the concentrations of Iron presented in the Table 1 are minor that the values tolerable upper intake level (45 mg/day), so that, concentration of Iron obtained in guavira don’t cause toxicity.

In our work, in relation to guavira seeds were obtained the amount of Zinc 1.063 mg/100g, in the peel and pulp analyzed ranged between 0.0118 and 0.221mg/100g. There are no limits of Zinc concentration in medicinal plants by the World Health Organization (WHO-2005). Recommended Dietary Allowance (RDA) of Zinc for adults is established the mean intake of 9.5 mg/day. So, the guavira fruits are not rich in Zinc. However, the recommendation of Zinc is beneficial in the treatment of several disorders, such as several pro-inflammatory conditions and cancer [33]. In the Indian, the plant *Withania somnifera* known commonly as Indian ginseng has below concentration of Zinc in seeds 0.146 mg/100g [34]. The concentrations of Zinc presented in the Table 1 are minor that the values tolerable upper intake level (40 mg/day), so that, concentration of Zinc obtained in guavira don’t cause toxicity.

Nickel concentrations detected in the seeds of guavira were 0.017 mg/100g (Table 1). Recommended Dietary Allowance (RDA) is not established for Nickel. According to Food and Agriculture Organization (FAO) of the United Nations (1984) the permissible limit in edible plants is 0.163 mg/100g. Until 2005, there are no limit permissible for Nickel by WHO in medicinal plants. Scientific findings have shown that Ni is toxic as evidenced by lipid peroxidative damage to placental membrane, in this case the metabolic change may be responsible for decreased placental viability, altered permeability, and potential subsequent embryotoxicity [36]. The concentration of Nickel presented in the Table 1 are below of the values tolerable upper intake level (1 mg/day), for that reason, concentration of Nickel obtained in seeds of guavira don’t cause toxicity.

In Table 1, Manganese (Mn) contents were 0.269, 0.099 and 0.237 mg/100g for the guavira peel, pulp and seed. According to FAO/WHO the permissible limit set in edible plants was 0.2 mg/100g [35]. After this comparison, the concentrations of Manganese in fruits of guavira are in perfect harmony with those limits of FAO/WHO. However, for Manganese in medicinal plants limits not yet been established by WHO (2005). Considering the Adequate Intake of Manganese of 2.5 mg/day, this fruit is not good source of Manganese. The tolerable upper intake level (UL) for consuming of Manganese in adults is 11 mg/day. So, the results in Table 1 for Manganese are below the values Adequate Intake and tolerable upper intake level.

The contents of Cobalt varied from 0.01 mg/100 g in the peel, to 0.005 mg/100 g in the pulp and 0.013 mg/100 g in the seeds. Results on *Mentha piperita* shown 0.026 mg/100 g of Cobalt in a Spanish study [36]. A safe Recommended Dietary Allowance (RDA) or Adequate Intake for Cobalt (Co) hasn’t been set yet. The only role for Cobalt is a constituent of vitamin B12 [37]. Neither has Cobalt been assessed in relation to the WHO Guidelines for Drinking-water. Until now, Cobalt has not been evaluated by competent organs in order to establish a tolerable upper intake level. However, some reports have suggested that acute intakes following ingestion of > 30 mg Co/day may cause skin rashes and gastrointestinal upset in humans [38].

Copper (Cu) contents were 0.005 mg/100g, 0.0031 mg/100g, and 0.0326 mg/100g for the guavira peel, pulp and seed respectively. In our study, Copper content in peel and pulp are lesser than seeds. The Recommended Dietary Allowance (RDA) of Copper for adults is established the mean intake of 0.9 mg/day. The present results indicate that seeds of guavira are rich in cooper. In a recent study in Serbia, the concentration of Copper in *Foeniculum vulgare* was mentioned as
The permissible limit of Copper set by FAO/WHO (1984) in edible plants is 0.3 mg/100g. The WHO limit for Copper in medicinal herbs has not been established yet. However, some countries had set limits for Copper in medicinal plants at 20 and 0.150 mg/g, respectively [40]. The tolerable upper intake level (UL) for consuming of Copper in adults is 10 mg/day. So, the results in Table 1 for Copper are below the values Adequate Intake and tolerable upper intake level.

Molybdenum (Mo) contents in the guavira peel, pulp and seed were 0.626, 0.434 and 0.469 mg/100g respectively (see Table 1). The Recommended Dietary Allowance (RDA) of Molybdenum for adults is established the mean intake of 0.045 mg/day. The present results indicate that seeds, pulp and peel of guavira are rich in Molybdenum. In 1973, the WHO experts suggested that 2 µg/kg of body weight would be appropriate to maintain normal parameters in health [41]. In some countries the concentration of molybdenum in diet 0.23 mg/kg, this corresponds to a daily intake of 100 µg of molybdenum per day for adults. Studies on consumption fruits or leaves containing Molybdenum are scarce in the literature reports, and the values of dietary intake considering desirable (elements essential or beneficial to plants or animals) and undesirable (with intoxication risk) are yet incomplete. This is important information required in assessing risks to human health due to their overburden. So, knowledge of the current levels of dietary intake of guavira by Indigenous and rural populations are of primary importance [42]. The tolerable upper intake level (UL) for consuming of Molybdenum in adults is 2 mg/day. So, the results in Table 1 for Molybdenum are below the values Adequate Intake and tolerable upper intake level.

In Table 1, Chromium (Cr) contents were 0.101, 0.074, and 0.084 mg/100g for the guavira peel, pulp and seed. Considering the Adequate Intake of Chromium of 0.030 mg/day, all parts of this fruit is rich in Chromium. On others hand in the Pakistan, the range of Chromium varied between 0.12 mg/100g in Convolvulus arvensis and 2.949 mg/100g in Cannabis sativa [28]. The permissible limit set by FAO/WHO (1984) in edible plants was 0.002 mg/100g. The permissible limit of Chromium for plants is 0.130 mg/100g recommended by WHO. After cooperation, our results indicate that concentration of Chromium in fruit peel was well above the permissible limit set by WHO. Moreover, the tolerable upper intake level (UL) for consuming of Chromium in adult is not established yet. In individuals with type 2 diabetes were observed the beneficial effects of supplemental chromium [43].

According to data in Table 1, the range of Silicon (Si) varied with values between 1.346 mg/100g (peel), 1.182 mg/100g (pulp) and 1.104 mg/100g in guavira seeds. There are no guidelines to establish a permissible level of Silicon in herbs medicinal. A safe Recommended Dietary Allowance (RDA) or Adequate Intake for Silicon (Si) hasn’t been set yet. The daily intake from the British diet has been estimated to 20-50 mg corresponds to 0.3-0.8 mg/kg body weight/day in a 60 kg person [44]. Since Silicon is not considered an essential element, most plants will grow normally without it. Studies on deprivation of Silicon in humans have not been conducted. However, its have a beneficial role in bone health [45].

In Table 1 Aluminum (Al) contents were 0.567, and 2.037 mg/100g for the guavira pulp and seed. There are not Recommendation (RDA) and Adequate Intake for Aluminum (Al). According to FDA's 1993 Total Diet Study dietary exposure model and the 1987-1988 U.S. Department of Agriculture (USDA) Nationwide Food Consumption Survey, the authors estimated daily Aluminum intakes 0.12 mg Al/kg/day for adult males and females. [46]. However, excessive intake of Aluminum results in pathological aberrations such amnesia in young people. Aluminum is present in the brain of patients with
Alzheimer disease [47]. Aluminum has found numerous applications in modern pharmacology [48]. Clinical trials suggest that administration of medications containing Aluminum and citrate or beverages cause toxicity [49].

Conclusion
For the first time, the concentration of macro and micro-elements in seeds, pulp and peel in the *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae) were measured and compared with recommended values. Parts of the fruit as seeds only are rich in Phosphorus, Iron and Copper. However, peel, pulp and seeds area rich in Molybdenum and Chromium. All contents are below the values Adequate Intake (AI) and tolerable upper intake level (UL).

The analysis of Cr concentration showed higher in peel and the lowest value was found in pulp. It was found that highest amount of Mo was present in peel and pulp had the lowest value. The contents of Chromium (Cr) were reportedly found higher than the permissible levels as recommended by the WHO. However, others values of concentration of macro and micro-nutrients obtained in this work are according to permissible limit set by FAO/WHO or with studies published of medicinal plants.

The concentration of elements K, Ca, Na, P are higher in peel, pulp and seeds of fruit. The level of Zinc and Copper present in some parts of fruit is very low compared to other detected elements.

The mineral composition results of the guavira showed that their fruits are not source of mineral elements as Zn, Ca, K, Na, Ni, Mn, Co, Mg, Al and Si.

The gaps in knowledge about the level of contents in the *Campomanesia adamantium* (Cambess.) O. Berg was completed in this work. The data obtained would serve as a tool for deciding the dosage of prepared from this plant with medicinal and nutritional purposes.

The knowledge of the chemical composition and medicinal properties of various plants has economic interest crop production and global health.

References


