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Investigation of *Campomanesia* Components: A Fruit of Brazilian Cerrado

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Additional information is available at the end of the chapter

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Abstract

A survey of chemical composition of the fruit of *Campomanesia adamantium* used by rural and urban inhabitants of the cities of the Campo Grande, Mato Grosso do Sul State, Brazil, was carried out by inductively coupled plasma-optical emission spectroscopy (ICP-OES) aiming at the detection of minerals. Fifteen minerals were detected in the peel, pulp, and seeds of plant. The concentrations of elements K, Ca, Na, and P are found to be present at the major level in peel, pulp, and seeds of fruit. The zinc concentration is very low compared to other detected elements. The levels of some chemical elements in the fruit do not exceed the limits established by international legislation. Animal studies should be performed. The knowledge of the chemical elements in plants has economic interest, and involves global health problem.

Keywords: medicinal plants, guavira, inductively coupled plasma mass spectrometry

1. Introduction

Medicinal and aromatic plants are important source of natural wealth. It is estimated that there are about 350,000 species of existing plants. Therapeutic plants have been valued as a mode of

treatment of a variety of diseases and have played a very important role in the health. So, they serve as raw materials for manufacturing several traditional and modern medicines [1, 2].

The records of medicinal plants' use for treating diseases and ailments date back to centuries ago. However, currently, more than half of the world's population still uses plants for the development of new medicines. In countries such as China, Africa, India, and Brazil, the traditional medicine is still the support of health care, and most of the drugs and cures come from plants. The World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care [3]. As in other countries, in Brazil increasingly, medicinal plants are used by rural and urban inhabitants, especially for treating minor ailments.

The vegetation of Brazil is richly endowed with a wide variety of plants, some of which are yet to be fully exploited. Some of the plants are cultivated and used as food or drugs while a good number of others grow wild in the Brazilian Amazon Forest. In Brazil, an effort for documenting the traditional uses of medicinal plants in several Brazilian forests reported 117 medicinal plants used in the Brazilian Amazon [4]. With the exception of the Amazon, few studies on medicinal plants have been performed in other Brazilian areas such as the Pantanal and Cerrado.

Located in South America, the Brazilian Cerrado is located mainly in the Midwest region of Brazil. The Cerrado is the second largest biome in Brazil, behind only the Amazon Rainforest. The Cerrado is rich in traditional medicine. The Midwest region of Brazil, in Mato Grosso do Sul State, in the city of Campo Grande/MS, has several medicinal plants, the *Campomanesia adamantium* (Cambess.) O. Berg and other plants species are part of the Cerrado vegetation of this city. The *C. adamantium* (Cambess.) O. Berg is also known popularly as gabirola, guabirola, or guavira. It is a fruit produced by gabirola, a wild shrub that grows in the fields and pastures of Brazil's Cerrado that develops in hot tropical climate with low rainfall. This species belongs to the family Myrtaceae. The fruits are of round-shaped, with a soft pulp, and very smooth well-appreciated taste.

There are many varieties of guavira fruit in Brazil. The differences between guavira of a same variety are often greater than the differences between two different varieties. Some varieties of guavira have different characteristics among them, and they are not as great as those among the different varieties of oranges or mandarins. Sometimes, it is impossible to distinguish one variety from the other, since the difference among the fruit of the same plant is as great as that between the fruit of different varieties. The most common species are *C. xanthocarpa* (Mart.) O. Berg, *C. corymbosa* (Cambess.) O. Berg, *C. Cambessedeaniana* O. Berg, *C. adamantium* (Cambess.) O. Berg, and *C. pubescens* (Mart. ex DC.) O. Berg.

The reproduction of guavira relies largely on their interaction with animal pollinators and fruit and seed dispersers. These plants are found as deciduous shrub, with a height from 0.5 to 1.5 m; flowering is usually from September to October, the flowers are lily and plentiful. Fruiting occurs from November to January and the fruits generally range from 2 to 2.5 cm in diameter (**Figure 1**); however, the morphological variation from one species to another is evident.

The fruit has 90% sweet juicy pulp and is widely appreciated by the population of the Brazilian Cerrado region. They are mature when the fruit has turned from green to yellow (see **Figure 1**). These plants have a delicate epicarp, demanding care during transport when ripe. A quick processing or freezing is recommended. Refrigerated storage recommendations are at 25°C. In nature, the guavira has low caloric value, mainly due to the high moisture content and therefore a lower concentration of sugars, lipids, and proteins in their structure. The gabioba fruits have nutritional properties due to its high content of vitamin C, minerals, and phenolic compounds, which allows considering it as functional food [5–7]. In **Figure 1**, images represent different parts of the fruit of *C. adamantium* (Cambess.) O. Berg, popularly known as guavira, from Cerrado, Campo Grande-MS, Brazil: (a) fruits of guavira, (b) peel and pulp guavira, (c) magnified image of the fruit, and (d) seed guavira.

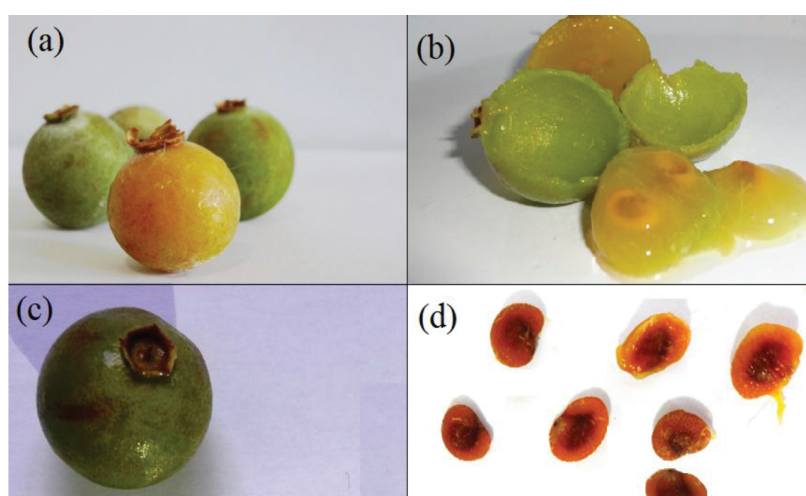


Figure 1. *Campomanesia adamantium* (Cambess.) O. Berg fruits, popularly known as guavira, from Cerrado, Campo Grande-MS, Brazil: (a) fruit of guavira, (b) peel and pulp guavira, (c) magnified image of the fruit, and (d) guavira seeds.

Several studies have shown the importance of the plant *C. xanthocarpa* Berg. (Myrtaceae) as potential effect in reducing blood cholesterol levels. In Brazil, one clinical study with hypercholesterolemic people investigated the effects of plant *C. xanthocarpa* Berg. (Myrtaceae) on inflammatory processes, oxidative stress, endothelial dysfunction, and lipid biomarkers in hypercholesterolemic individuals. According to results, the treatment reduced the blood total cholesterol (TC) and low-density lipoprotein (LDL-C) levels, reduced the oxidative stress in hypercholesterolemic individuals, and improved the levels of nitric oxide (NOx) [8]. In this work, the authors state that their results are in accordance with a previous preclinical study conducted in mice, which showed that this plant was effective in preventing gastric ulcerations and did not produce toxic symptoms [9].

Other studies on these species properties showed the effects of the aqueous extracts of these plants in rats fed on a high calorie diet. Comparing the results from the experimental group with the results of the control group, the chronic treatment with the *C. xanthocarpa* aqueous extract induced a significant reduction in weight gain in the rat. Equally, biochemical analysis

showed that this treatment reduced glycemia. However, no effects on lipidic levels were observed [10]. On the other hand, one study with some species of plants used for weight loss purpose in Brazil and around the world showed that scientific data found are not sufficient to guarantee the efficacy and safety of these plants for treating obesity [11]. Consistent with the results aforementioned studies *Campomanesia* species are used in folk medicine as anti-inflammatory, antirheumatic, antidiarrheal, and hypocholesterolemic [12].

During several years, this plant is used in Brazilian folk medicine for ulcer treatment. Although there is no study in humans, the oral administration of the extract in animals proved to be effective in preventing gastric ulceration in rats and did not produce toxic symptoms [13]. The determination of groups as presence of flavonoids, saponins, and tannins has been related to antiulcer activity in other published work [14].

Studies *in vivo* evaluated the antinociceptive and anti-inflammatory activity of ethanolic extract from *C. velutina* leaves and its fractions, using male Swiss albino mice. The results of the present study demonstrated that *C. velutina* has anti-inflammatory and antinociceptive. However, the complete mechanisms of these actions remain to be elucidated [15].

Indigenous and rural populations used the leaves of *C. adamantium* (Cambess.) O. Berg and its fruits to treat inflammatory, obesity, diarrheal, and aseptic urinary tract problems. The infusion of the leaves is used for bladder problems, high blood pressure, throat infections, vomiting, indigestion, and cramps. The leaves are also used for the treatment of rheumatic diseases and as cholesterol reducers. *C. adamantium* (Myrtaceae) is an antioxidant fruit, and results showed the hepatoprotective effects of pulp or peel/seed hydroalcoholic extracts on injured liver-derived HepG2 cells by CCl₄. The results in part are associated with the presence of antioxidant compounds, especially flavonoids [16]. Researches and studies prove that the oleic and linoleic fatty acids obtained from the oil of the seeds from *C. xanthocarpa* Berg. showed amounts of bioactive compounds, and the results of fatty acid contents indicated a high degree of unsaturation. Oleic acid is included in the normal human diet as a part of animal fats and vegetable oils. It plays a key role in the synthesis of hormones [17]. Linoleic acid is found in many vegetable oils, including flaxseed oil, sunflower seed oil, and corn oil. In the traditional medicine in Brazil, the roots and leaves of *C. xanthocarpa* Berg. are used for antidiabetic effects [18].

The ingestion of such plants for medicinal purpose can have imperative side effects. Scientific surveys of these plants are necessary because many of them may have detrimental effects, such as acute or chronic toxicity, or their use may inhibit the adoption of the proper and effective treatment. Hence, with regard to the toxicological consideration of medicinal plants, the major hazard that may be associated with the use of plants is the presence of potentially toxic mineral elements such as the accumulative elements copper, lead, cadmium, mercury, arsenic, fluorine, selenium, molybdenum, and vanadium. Currently, an effort has been made by Brazilian researchers to review the elemental contents and efficacy of traditional herbal medications.

Some people believe that *C. adamantium* (Cambess.) O. Berg is rich in zinc, aluminum, potassium, calcium, phosphorus, and magnesium. In this context, each chemical element has its chemical properties, health effects, and would be associated with important applications in

the treatment of ailments. In the case of zinc because of its effect in the fight against infections. On the other hand the adverse effect of zinc deficiency increases the susceptibility of children to infectious diarrhea and contributes to malnutrition increases the susceptibility of children to infectious diarrhea, and contributes to zinc deficiency and malnutrition [19]. According to information popular, this species is rich in aluminum. So, *C. adamantium* is popularly used to treat ulcer disease in Mato Grosso do Sul State, in Midwest Brazil. It is logical that among the various categories of antiulcer drugs in the market, many contain aluminum. According to the elders who live in some farms, the fruit of guavira has potassium and helps maintain muscle force. In fact, several investigations have shown that the health benefits of potassium include relief from stroke, blood pressure, heart and kidney disorders, anxiety and stress, as well as enhanced muscle strength. However, there are no scientific data confirming the concentration of this element in the guavira fruit.

Knowledge of element concentrations in highly consumed plant samples is of interest. Especially of trace elements toxic as well as nontoxic in plants are very important medicinally. The diets of the world's population lack one or more essential mineral elements. This can be remedied through dietary diversification, mineral supplementation, food fortification, or increasing the concentrations and/or bioavailability of mineral elements in produce. Some medicinal plants are rich in minerals important to human. Until now, we know that each mineral has a role in human metabolism. For example, sodium is essential to humans. An adult person requires about 2.5–3.0 g per day [3]. Any extra sodium may contribute to high blood pressure. High blood pressure is a leading cause of cardiovascular disease. It accounts for two-thirds of all strokes and half of heart disease [20]. Sodium helps cells to transmit nerve signals and regulates water levels in tissues and blood. On the other hand, potassium has opposite effects on heart health, while high potassium intake can help relax blood vessels and excrete the sodium and decrease blood pressure. Our bodies need far more potassium than sodium each day [21].

Studies demonstrated that elements such as potassium, calcium, sodium, magnesium, manganese, and copper could reduce cardiovascular disease in human beings [22]. Low amount of phosphorus and calcium determined in the sample may still contribute to bone formation. Calcium plays a role in final common pathway mediating stimulus-contraction coupling in cardiac and smooth muscle [23]. Also, low potassium may still reduce the risk of stroke while low sodium content may add value in osmotic regulation of the body fluids and transmission of nerve impulse [24].

Calcium is the most abundant element in the human body. According to a study published [25], the amount of calcium that the body loses through urination increases with the amount of salt that is ingested. Other element as magnesium is abundant in intracellular fluid. Nevertheless, the mechanism involved in its regulation is still unknown. The potential uses of magnesium include the treatment of eclampsia, myocardial infarction, and arrhythmias [26]. Vegetables, nuts, seeds, and legumes are the best sources for magnesium. In contrast to the calcium and magnesium, quantities of manganese in mammalian tissue are scant. But at the same time, this mineral is essential for bone mineralization and metabolism [27]. Studies using plasma of

conscious horses increased superoxide capacity in a manner related to the dose of manganese [28].

Based on the above information, the present chapter includes a preliminary study of the detection of chemical composition of medicinal plant (*C. adamantium* (Cambess.) O. Berg) used by the rural and urban communities of Campo Grande city, Mato Grosso do Sul State, Brazil. This study is necessary because to date there have been no definitive studies on the chemical composition of *C. adamantium* (Cambess) O. Berg fruits in Mato Grosso Sul State, Brazil.

Nowadays, *C. adamantium* (Cambess.) O. Berg (Myrtaceae) is used in folk medicine to treat inflammation and rheumatism. This could be used as an alternative medicine for other disease control. The literature search reveals that few studies have been done on this plant by Brazilian researchers [29, 6]. For this reason, the aim of this work is to characterize the chemical constituents of this plant species native to the Brazil. Using one inductively coupled plasma-optical emission spectroscopy (ICP-OES), we performed measurements of the chemical concentration in the peel, pulp, and seeds of the fruit.

2. Experimental background

2.1. Research area

The *C. adamantium* (Cambess.) O. Berg (Myrtaceae) fruits were collected in Campo Grande city, Mato Grosso do Sul State, Brazil, in October 2015. Fruits of *C. adamantium* in various stages of ripening were collected from various plants. **Figure 2** shows the geographic coordinates of

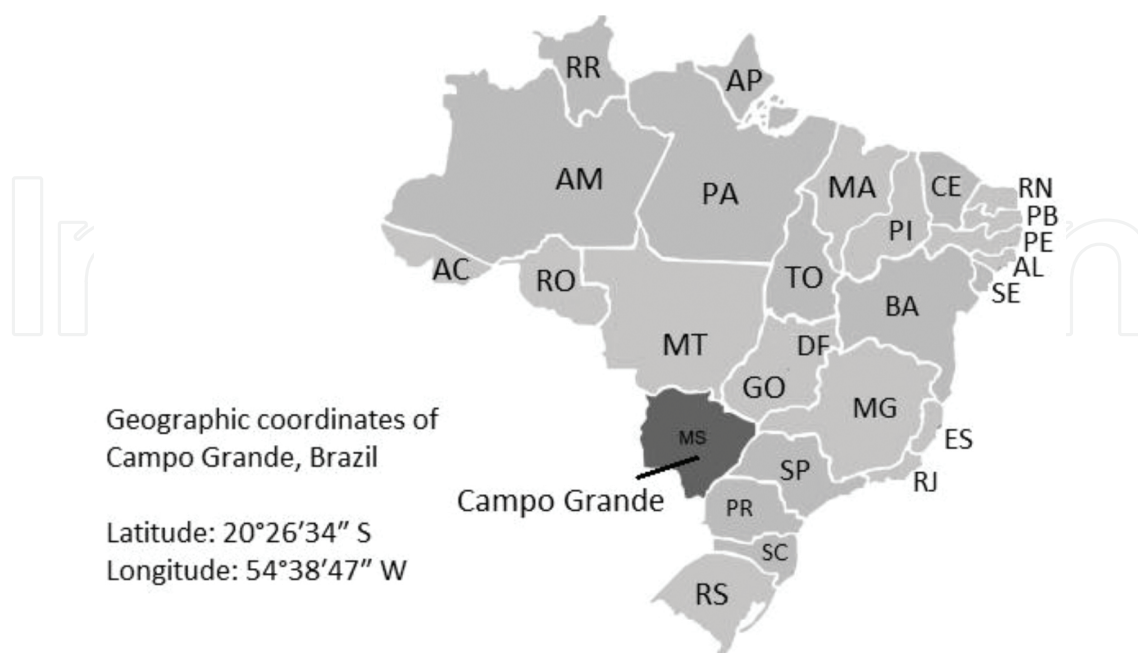


Figure 2. Geographic coordinates of Campo Grande, Mato Grosso do Sul State, Brazil.

Campo Grande, Mato Grosso do Sul State, Brazil, which is used in mapping and navigation, including GPS satellite navigation system (the global positioning system).

2.2. Elemental analysis by ICP-OES technique

All the samples of peel, pulp, and seeds of the fruit were weighed and digested in HNO_3 + H_2O_2 mixture. Samples were prepared as follows: a mixture of 0.5 g sample plus 5.0 mL HNO_3 (65% Merck) and 3.0 mL H_2O_2 (35%, Merck Millipore) was processed in the microwave digestion system Speedwave®, Berghof, Germany. After digestion, samples were diluted to 100 mL using ultrapure water. The final acid concentration of the samples was quite high (4% HNO_3).

In the present paper, the concentration of the elements (K, Ca, Na, P, Mg, Fe, Si, Mo, Mn, Z, Cr, 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). Triplicate analyses were performed on each sample.

The specimen has been identified by Dr. Arnildo Pott and deposited (N° 53328) in herbarium of Federal University of Mato Grosso do Sul (UFMS)/Brazil.

3. Results and discussions

The concentrations of different mineral elements of seed, pulp, and peel of guavira fruit analyzed are listed in **Table 1**. In the present work, the concentration of elements in the peel decreases in the following order: $\text{K} > \text{Ca} > \text{Na} > \text{P} > \text{Mg} > \text{Fe} > \text{Si} > \text{Mo} > \text{Mn} > \text{Zn} > \text{Cr} > \text{Cu} > \text{Co}$. The pulp of the fruit decreases in the following order: $\text{K} > \text{P} > \text{Na} > \text{Ca} > \text{Mg} > \text{Si} > \text{Fe} > \text{Al} > \text{Mo} > \text{Zn} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Co}$. The results attributed to seeds of fruit: $\text{P} > \text{K} > \text{Ca} > \text{Mg} > \text{Na} > \text{Fe} > \text{Al} > \text{Si} > \text{Zn} > \text{Mo} > \text{Cu} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Co}$. Among the various elements, K, Ca, Na, and P are found to be present at the major level, and 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 a good source of other elements such as nickel, zinc, potassium, magnesium, manganese, silicon, sodium, and calcium. The chemical characteristic of each chemical element obtained in this study will be described below.

Copper contents were 0.005, 0.0031, and 0.0326 mg/g for the guavira peel, pulp, and seed, respectively. In our study, the copper content of the seeds is the highest while that of the peel is the least. The present results indicate that seeds of guavira are a rich source of copper. In a recent study in Serbia, the concentration of copper in *Foeniculum vulgare* was mentioned as 0.001542 mg/g [30]. The permissible limit of copper set by Food and Agriculture Organization/World Health Organization (FAO/WHO) (1984) in edible plants is 0.003 mg/g. 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 [31].

Iron contents were 0.01453, 0.01089, and 0.05022 mg/g for the guavira peel, pulp, and seed, respectively. The seeds of guavira are a rich source of iron. The regulatory limits of the WHO/FAO (2005) have not been established yet for the iron in herbal medicines. The limit set by FAO/WHO (1984) in edible plants was 0.02 mg/g. The iron concentration found in Pakistani medicinal plants ranged with values between 0.18163 and 6.79688 mg/g [32]. Values of iron found in Egyptian species and medicinal plants ranged from 0.02696 to 1.046.25 mg/g [33]. Iron is necessary for several functions in the human body. However, iron toxicity has an adverse effect on various metabolic functions and cardiovascular system [34].

Elements	Peel (mg/g)	Pulp (mg/g)	Seeds (mg/g)
Cu	0.005	0.0031	0.0326
Zn	0.00118	0.00221	0.01063
Ca	0.2598	0.199	0.4608
K	2.0236	1.7515	2.5482
Na	0.2334	0.21566	0.0582
P	0.2332	0.5755	4.0652
Cd	ND	ND	ND
Fe	0.01453	0.01089	0.05022
Ni	ND	ND	0.00017
Mn	0.00269	0.00099	0.00237
Co	0.0001	0.00005	0.00013
Mg	0.15304	0.10371	0.1981
Al	ND	0.00597	0.02037
Cr	0.00101	0.00074	0.00084
Mo	0.00627	0.00434	0.00469
Si	0.01346	0.01182	0.01104
ND, non-detected.			

Table 1. Levels of inorganic elements in guavira fruit.

The present study indicates that the seeds of guavira are a rich source of phosphorus (4.0652 mg/g). In an Indian plant known as *Sesbania bispinosa* (Jacq.), the lowest concentration of phosphorus found in seeds was 0.00532 mg/g followed by the concentrations in leaves 0.00292 mg/g and in roots 0.0028 [35].

Chromium contents were 0.00101, 0.00074, and 0.00084 mg/g for the guavira peel, pulp, and seed, respectively. On the other hand in the Pakistan, the range of chromium varied between 0.0012 mg/g in *Convolvulus arvensis* and 0.02949 mg/g in *Cannabis sativa* [32]. The permissible limit set by FAO/WHO (1984) in edible plants was 0.00002 mg/g. The permissible limit of

chromium for plants is 0.00130 mg/g recommended by WHO. After comparison of researches of data above, the concentration of chromium in fruit peel was recorded above the permissible limit set by WHO. The beneficial effects of supplemental chromium in individuals with type 2 diabetes were observed at levels higher than the upper limit of the estimated safe and adequate daily dietary intake [36].

Molybdenum contents in the guavira peel, pulp, and seed were 0.00626, 0.00434 and 0.00469 mg/g, respectively. In 1973, the WHO experts suggested that 2 µg/kg of body weight would be appropriate to maintain normal parameters in health [37]. Representative diets of various countries showed an average concentration of molybdenum in diet 0.23 mg/kg; this corresponds to a daily intake of 100 µg of molybdenum per day for adults. The values of dietary intake of Mo are scarce in the literature reports in Brazil and other countries. 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 is of primary importance [38].

The nickel concentration was detected by only seeds (0.00017 mg/g). According to the Food and Agriculture Organization of the United Nations (1984), the permissible limit in edible plants is 0.00163 mg/g. Until 2005, there is no permissible limit 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 [39].

In India, the plant *Withania somnifera* known commonly as Indian ginseng has below concentration of zinc 0.0206 mg/g. In our work, in relation to guavira seeds, we obtained the amount of zinc 0.01063 mg/g. There are no limits of zinc concentration in medicinal plants by the World Health Organization (WHO, 2005). However, the zinc concentration in guavira is less than other Pakistani plants [32]. The guavira fruits are not rich in zinc, as some people claim. However, the recommendation of zinc is beneficial in the treatment of several disorders, such as several pro-inflammatory conditions and cancer [40].

The range of potassium varied between 2.023 mg/g in peel, 1.75 mg/g in pulp, and 2.54 mg/g in the seeds. These values are low when compared with other medicinal plants: *Rheum australe* (0.00622 mg/g) and *Anethum graveolens* (36.93961 mg/g). Potassium has been found in higher concentrations in *Allium cepa* (86.422 mg/g) [41]. On the other hand, minimum concentrations of Ca were observed in the pulp (0.199 mg/g) and maximum in seeds (0.4608 mg/g), which is less than 13.34 mg/g in *Brassica campestris*, Pakistani medicinal plant [32]. According to a study with several herbal medicaments, the reported tolerable upper intake level of calcium in herbs is 2500 mg/day. It has been suggested that for those athletes who may require calcium supplementation to improve bone density, building up to an intake of 1500 mg daily in doses of at least 500 mg at a time is recommended [42]; higher doses could result in adverse gastrointestinal symptoms in some people [43]. So, the guavira fruit is not a good source of potassium and calcium.

In this study, the concentration of magnesium obtained was 0.1981 mg/g. In some Pakistani medicinal plants, magnesium content ranged between 0.00333 mg/g in *Punica granatum* [44] to 2.24188 mg/g in *Convolvulus arvensis* [32]. There are no current data to establish a safe upper level for the magnesium intake.

The range of Mn varied between 0.00269 mg/g in peel and 0.00237 mg/g in seeds of guavira. According to FAO/WHO, the permissible limit set in edible plants was 0.002 mg/g [45]. Studies on medicinal plants in Nigeria obtained a concentration of manganese 0.000399 mg/g in *Fleurya aestuans* (Urticaceae). 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 have not yet been established by WHO (2005).

Silicon contents in the guavira peel, pulp, and seed were 0.01346, 0.01182 and 0.01104 mg/g, respectively. There are no guidelines to establish a permissible level of silicon in medicinal herbs. It is not certain that silicon is essential to all plants. No silicon deprivation studies have been conducted in humans. However, silicon appears to have a beneficial role in bone formation and in bone health [46].

The sodium concentrations for fruit studied ranged from 0.0582 mg/g (seed) to 0.2334 mg/g (peel). In the plant *F. aestuans* Linn. (Urticaceae) of Nigeria, the concentration of sodium obtained was 0.01225 mg/g. The minimum daily intake of sodium is 2.3 g.

In analyzed fruits, the sodium contents varied between 0.2598 mg/g (peel), 0.199 mg/g (pulp), and 0.4608 mg/g (seeds). In the reported plants [44], Na contents ranged from 0.0006 mg/g (*Therminalia chebula*) to 90.375 mg/g (*Linum usitatissimum*) [44]. The recommended daily allowance of sodium is 0.12–0.37 g/d for infants, 1.5–1.7 g/d for children, and 1.2–1.5 g/d for adults [47].

4. Conclusions

The results of the analysis showed that the guavira fruits are rich in mineral contents, especially potassium, calcium, sodium, and phosphorus.

The concentrations of elements K, Ca, Na, and P are found at the major level in peel, pulp, and seeds of fruit. The zinc concentration is very low compared to other detected elements.

The mineral composition results of the medicinal plants showed that these plants contain rich source of mineral elements; this result became so important when the usefulness of minerals such as Ca, Mg, P, K, and Na in body is considered. The knowledge of the current levels of dietary intake of guavira by indigenous and rural populations is of primary importance. The elemental analysis of the guavira showed significant variation among different elements. The analysis of Cr concentration showed the highest in peel and the lowest value was found in pulp. It was found that the highest amount of Mo was present in peel and pulp had the lowest value. The concentrations of chromium (Cr) and molybdenum (Mo) were reportedly found higher than the permissible levels.

Some minerals of guavira showed elemental contents above the permissible levels as recommended by the WHO.

Animal studies should be performed. The knowledge of the chemical elements in plants has economic interest and involves global health problem.

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