Metal Analysis in *Citrus Sinensis* Fruit Peel and *Psidium Guajava* Leaf

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ABSTRACT

The determination of metal traces is very important because they are involved in biological cycles and indicate high toxicity. The objective of the present study is to measure the levels of heavy metals and mineral ions in medicinally important plant species, *Citrus sinensis and Psidium guajava*. This study investigates the accumulation of Copper (Cu), Zinc (Zn), Cadmium (Cd), Aluminum (Al), Mercury (Hg), Arsenic (As), Selenium (Se) and inorganic minerals like Calcium (Ca) and Magnesium (Mg) in *C. sinensis* (sweet orange) fruit peel and *P. guajava* (guava) leaf, to measure the levels of heavy metal contamination. Dried powdered samples of the plants were digested using wet digestion method and elemental determination was done by atomic absorption spectrophotometer. Results are expressed as mean ± standard deviation and analysed by student's 't' test. Values are considered significant at $P < 0.05$. The results were compared with suitable safety standards and the levels of Cu, Zn, Cd, Mg and Ca in *C. sinensis* fruit peel and *P. guajava* leaves were within the acceptable limits for human consumption. The order of concentration of elements in both the samples showed the following trend: Mg > Ca > Al > Zn > Cu > Cd > Hg = As = Se. The content of Hg, As and Se in *C. sinensis* fruit peel and *P. guajava* leaves was significantly low and below detection limit. The content of toxic metals in tested plant samples was found to be low when compared with the limits prescribed by various authorities (World Health Organization, WHO; International Centre for Materials Research, ICMR; American Public Health Association, APHA). The content of Hg, As and Se in *C. sinensis* fruit peel and *P. guajava* leaves was not detectable and met the appropriate safety standards. In conclusion, the tested plant parts taken in the present study were found to be safe.

Key words: Atomic absorption spectrophotometer, *Citrus sinensis,* heavy metals, *Psidium guajava*

INTRODUCTION

Heavy metals are elements having atomic weight between 63.545 and 200.5 g and a specific gravity greater than four.[1] The elements play essential roles in biological processes, but at higher concentrations they may be toxic to the biota and they disturb the biochemical processes and cause hazards. These elements include metals (Cd, Chromium - Cr, Cobalt - Co, Cu, Zn, Palladium - Pd, Nickel - Ni, and Silver - Ag) and metalloids (Se, As, Antimony - Sb). Most of the trace elements are transition metals with variable oxidation states and coordination numbers. These metals form complexes with organics in the environment thereby increasing their mobility in the biota and manifest toxic effects. [2]

Heavy metals have been reported to have positive and negative roles in human life. Some like Cd, lead (Pb)

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and Hg are major contaminants of food supply and may be considered the most important problem to our environment while others like iron (Fe), Zn and Cu are essential for biochemical reactions in the body.[3] Intracellular free magnesium is involved in the energy reactions of phosphorylation and is necessary for the activation of hundreds of enzymatic reactions concerning adenosine-5'-triphosphate (ATP).[4] Ca is an essential nutrient required for critical biological functions such as nerve conduction, muscle contraction, cell adhesiveness, mitosis, blood coagulation and structural support of the skeleton.[5] Generally, most heavy metals are not biodegradable, have long biological half-lives and have the potential for accumulation in different body organs leading to unwanted side effects. The content of essential elements in plants is conditional, being affected by the characteristics of the soil and the ability of plants to selectively accumulate some metals.[3] Several plants are used widespread for their many therapeutic and pharmaceutical virtues, especially antioxidant, anti-tumoral, and anti-infectious activities. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses.[6] Food chain contamination by heavy metals has become a burning issue in recent years because of their potential accumulation in bio systems through contaminated water, soil and air.[7] Therefore, based on persistent nature and cumulative behaviour as well as the probability of potential toxicity effects of heavy metals as a result of consumption of medicinal plant species, there is need to test and analyze the medicinal plants to ensure that levels of trace elements meet the agreed international requirements. The objective of this study is to measure the levels of heavy metals and mineral ions in *C. sinensis* and *P. guajava*, the favourite fruits consumed widely in the tropics, and therefore, to evaluate the potential of these plants' for metal accumulation.

MATERIALS AND METHODS

Reagent and solutions

All solutions used in the study were prepared from analytical reagent grade chemicals. Nitric acid, hydrochloric acid and hydrogen peroxide were obtained from E-Merck (India) Ltd., Mumbai, India. High-performance liquid chromatography (HPLC) grade water was used in all experiments. The standard solutions of analytes for calibration procedure were produced by diluting a stock solution of 1000 parts per million (ppm) of all the investigated elements.

Sample collection

Fresh fruit peel of *C. sinensis* and fresh leaves of *P. guajava* were collected from Bahadurgarh, Haryana, India. The plant parts were taxonomically identified and authenticated by Dr. J.P. Yadav, Department of Biosciences, M.D. University, Rohtak, India. The voucher specimen of tested plant parts were deposited in Herbarium of Pharmaceutical Sciences, M.D. University (for *C. sinensis,* voucher number is DPS 0014, for *P. guajava,* voucher number is DPS 0015). The two samples were analyzed for determination of ash value, crude fibre content and presence of Cu, Zn, Cd, Mg, Ca, Al, Hg, Ag and Se.

Sample preparation

The collected *C. sinensis* fruit peels and *P. guajava* leaves were thoroughly washed and rinsed with distilled water. The samples were then dried in oven at 40°C. The dried samples were then ground into fine powder and stored in fresh plastic polythene bags ready for further use.

Determination of ash value

The sample (5 grams) was kept in a muffle furnace and ashed at a temperature not exceeding 525°C for 6 hours. The ash was then cooled in a dessicator and weighed. The ash content was recorded as gram per 100 gram fresh weight (g/100 g-f w).[8]

Determination of crude fibre content

The dried powdered drug samples of *C. sinensis* fruit peel and *P. guajava* leaves (2 grams each) were boiled separately with 10% v/v nitric acid and strained. Residues obtained were washed and boiled with 2.5% v/v sodium hydroxide solution for 30 seconds, then washed with hot water and transferred to dried crucible to obtain percent crude fibre content.[9]

Determination of metal content

Sample digestion

The powdered samples of *C. sinensis* fruit peel and *P. guajava* leaves were digested by wet digestion method. Two grams of dried plant sample was taken in a 100 ml beaker and 10 ml of nitric acid was added. The sample was placed on a hot plate and heated at 95°C for 15 minutes. The digest was cooled and 5 ml of concentarted nitric acid was added and heated for additional 30 minutes at 95°C. The later step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 2 ml of water and 3 ml of 30% hydrogen peroxide was added. With the beaker covered, the sample was heated gently to start the peroxide reaction. If effervescence becomes excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1 ml increments, followed by gentle heating until the effervescence was subsides. 5 ml of concentrated hydrochloric acid and 10 ml of water was added and the sample was heated for additional 15 minutes without boiling. The sample was cooled through a whatman no. 42 ashless filter paper and diluted to 60 ml with deionised water.

Table 1: Instrumental conditions for metal analysis by atomic absorption spectrophotometer mentioning details about parameters like wavelength (nm), slit setting (nm), light source, flame type and operating current

Parameter	Cu	Zn	Cd	Mg	Ca	Al	Hg	As	Se
Wavelength (nm)	324.7	213.9	228.8	285.2	422.7	309.3	253.6	193.7	196
Burner slit (mm)	1		0.5	0.5	0.5	0.5	0.5	1	1
Light source	hcl	hcl	hcl	hcl	hcl	hcl	hcl	hcl	hcl
Flame type	a	a	a	a	a	b	a	a	a
Operating current (mA)	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10

hcl: hollow cathode lamp; a: air/ C_2H_2; b: N_2O/ C^2H_2

Table 2: Concentration of metal/mineral elements present in *C. sinensis* fruit peel and *P. guajava* leaf sample in µg/g dry weight* and their corresponding permissible limits

Metal/ mineral	Maximum permissible limit (µg/g) 10,11,12	Metal content in µg/g dry wt*	
		Citrus sinensis fruit peel	*Psidium guajava* leaf
Cu	10	5.90±0.075	6.39±0.48
Zn	15 – 50	9.50±0.407	11.36±0.92
Cd	0.05	0.005±0.001	0.003±0.001
Mg	2000	1944±518.7	1917±622.3
Ca	--	340.35±111.5	315.85±79
Al	15	39.18±6.26	24.18±4.71
Hg	1	bdl	bdl
As	10	bdl	bdl
Se	0.01	bdl	bdl

bdl: below detection limit; *: average concentration ± SD (standard deviation), n=3 (µg/g = mg/kg)

Preparation of calibration curve using standard compounds

Standard solutions were prepared for each element depending upon the linear working range, corresponding five dilutions were made and their absorbances were measured. Standard dilutions, for each metal, was prepared from their respective stock solutions (1000 ppm) which is either available readymade or prepared from their respective salts. Calibration curves were plotted using standard operating procedure.

Sample analysis

Metal content in the digested samples were analyzed for Cu, Zn, Cd, Mg, Ca, Al, Hg, As and Se in triplicate.

The instrumental conditions during the analysis of ten metals are listed in Table 1, mentioning details about parameters like wavelength (nm), slit setting (nm), light source, flame type and operating current, which are defined for respective metals.

RESULTS

The values of different metals present in the plant samples (in µg/g) along with their maximum permissible limits are listed in Table 2. The elements like Cu, Zn, Cd, Mg, Al, Hg, Ar, Se and inorganic minerals like Ca and Mg have been determined by atomic absorption spectrophotometer. The order of concentration of elements showed the following trend: Mg > Ca > Al > Zn > Cu > Cd > Hg = As = Se.

In the present research, magnesium deposition in *C. sinensis* fruit peel was found to be higher than that found in *P. guajava* leaves. Calcium acts as a secondary messenger in various signal transduction cascades. There are no permissible limits of calcium.[12] The content of Ca was found to be higher in *C. sinensis* fruit peel when compared to *P. guajava* leaves. Also, *C. sinensis* fruit peel showed higher accumulation capacity for aluminium as compared to *P. guajava* leaves. The concentration of Zn in tested samples was found to be low or not significantly high. The content of Zn, a biologically active metal, was lower in *C. sinensis* than *P. guajava*. The Cd concentration in both the tested samples was much lower than the prescribed value. The content of Cd was found to be less in *P. guajava* than *C. sinensis*. The levels of Se, Hg and As in *C. sinensis* fruit peel and *P. guajav*a leaves were found to be negligible and not detectable.

The ash content of *C. sinensis* (sweet orange) fruit peel and *P. guajava* (guava) leaf studied was found to be 1.91 g and 1.87 g respectively. Sweet oranges peel and guava leaves had average crude fibre content of 43.33g/100 g-f w and 55g/100 g-f w respectively. Results are expressed as mean ± standard deviation and analysed by student's 't' test. Values are considered significant at $P < 0.05$.

DISCUSSION

Trace elements play an important role in human biology, because they are either inadequately synthesized or not synthesized in the body. Some of these trace elements, for example, manganese (Mn), copper, zinc, are essential micronutrients and have a variety of biochemical functions in all living organisms.[13] Plants and humans require adequate amounts of micronutrients like Fe and Zn, but accumulation of an excess or uptake of non-essential metals like Cd or Pb can be extremely harmful. Living organisms cannot synthesize mineral elements like other nutrients. Consequently, for the body to meet the needs of mineral elements, they are usually acquired through food intake. In the human body, the minerals function as structural components of body organs, tissues and constituents of body fluids and tissues as electrolytes, and catalysts in enzyme and hormone systems. The functional roles of elements are interrelated and balanced against each other, and most often cannot be considered as single elements with independent and self-sufficient roles in the organized bodily processes. A number of trace elements (e.g., Cu, Zn, Fe and Se), in addition to certain vitamins (e.g., vitamins A, D, F, D_{12} and folacin) and other nutrients are strongly related to adequate immune response. These nutrients act together and/or separately, to form an active immune response.[14]

Intracellular Mg deficiency is correlated with the impaired function of many enzymes utilizing high-energy phosphate bonds, as in the case of glucose metabolism.[4] Ca is an essential nutrient required for critical biological functions such as nerve conduction, muscle contraction, cell adhesiveness, mitosis, blood coagulation and structural support of the skeleton. In recent years, an adequate intake of calcium has been demonstrated to reduce the risk for chronic diseases such as osteoporosis, hypertension and possibly colon cancer, as well as a number of other disorders.[5] Aluminum (Al) occurs naturally and makes up about 8% of the surface of the earth. It is always found combined with other elements such as oxygen, silicon and fluorine. When aluminum enters into the environment, it binds to particles in the air. It can dissolve in lakes, streams and rivers depending on the quality of water. It can also be taken up into some plants from soil, however, it is not known to bioconcentrate upside in the food chain.[15]

Among heavy metals, Zn is an important element for both plants and animals. It plays an important role in several metabolic processes; it activates enzymes and is involved in protein synthesis and in carbohydrate, nucleic acid and lipid metabolism. It form complexes with deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and affects the stability of these compounds. Zn concentration in plants is influenced by the age and vegetative state of the plant. Usually, the highest Zn content is found in young plants.[16] Zinc is a cofactor of over two hundred enzymes involved in metabolic pathways but its high levels in human body can be toxic due to its interference with copper metabolism.[17]

Cu is an essential trace element important for the function of many enzymes, notably cytochrome oxidase and superoxide dismutase. Copper ions can adopt oxidized Cu (II) or reduced Cu (I) states, allowing the metal to play a pivotal role in cell physiology as a catalytic cofactor in the redox chemistry of mitochondrial respiration, iron absorption, free radical scavenging, and elastin cross-linking.[18] Copper plays a role in oxidative defense system, on the other hand, chronic copper toxicity can result in severe poisoning.[17] Cd is highly toxic to animals and plants. In plants, exposure to Cd causes reduction in photosynthesis, water and nutrient uptake.[19] Se has attracted attention because of its apparent ability, usually when administered as inorganic salts, to ameliorate the toxic effects of heavy metals such as Hg and Cd.[20]

Also, the inorganic minerals like Na (sodium), k (potassium), Ca, Mg and heavy metals like Fe, Mn, Pb, Hg, Cr, Cd, Ni, Co, Be (beryllium), Cu etc., when present above the permissible limit are harmful.[21] Of the numerous trace elements, that are present in contaminated soil, Cd, Pb, Hg, As, Se, Zn, Cu and Ni have been identified as elements of primary concern because of their potential hazard to man.[22] The main purpose of ash determination is to assess the quality of the food materials. The ash contents, in these medicinal plant parts are not high; this implies low quantities of inorganic compounds in the fruits. Thus, to get higher quantities of such compounds in the body, repeated intake of the fruits is recommended. Fibre helps to maintain the health of the gastrointestinal tract, but in excess it may bind trace elements, leading to deficiencies of Fe and Zn in the body.[23]

In the present study, it was found that the tested plant parts showed higher content for some elements (although not dangerous), while for others, the content was lower. The results were compared with suitable safety standards and the levels of Mg, Ca, Zn, Cu and Cd in *C. sinensis* fruit peel and *P. guajava* leaves were within the acceptable limits for human consumption. The content of toxic metals in tested plants was found to be significantly low. The content of Hg, As and Se in *C. sinensis* fruit peel and *P. guajava* leaves was below the detection limit and met the appropriate safety standards. In conclusion, the tested plant parts taken in the present study were found to be safe.

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Announcement

INVITATION

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