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COMPARATIVE STUDIES ON PHYTOCHEMICAL SCREENING AND METAL ANALYSIS OF ALCOHOLIC EXTRACTS OF *MUSA ACUMINATE, ACTINIDIA DELICIOSA* AND *MANGIFER INDICA* BY USING ICP-OES AND FLAME PHOTOMETER.

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Abstract:

The acceptance of remedies in any system of medicine depends upon their efficacy and non-toxicity. There were many reports of rejecting Indian herbal products in various countries because of higher levels of heavy metal content than permitted.

Though few are regarded as essential,some heavy metals, especially cadmium, mercury and lead, are potentially hazardous due to their intrinsic or selective toxicity, particularly in environmental contexts.

In view of the importance of herbal drug standardization, it was contemplated to carry out the heavy metal determination in Musa Acuminate ,Actinidia Deliciosa and Mangifer Indica fruits which were procured from local market..The metals like copper, iron, magnesium nickel, sodium and zinc in the alcoholic extracts of them by ICPOES. These extracts were also subjected to general phytochemical screening.

Its an alarming bell for humankind if the heavy metal content is more than permitted in common edible commodities. Further study may require understanding the factors influencing the heavy metal content in commonly used fruits .

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INTRODUCTION:

A metal is a word which is derived from Greek called as métallon[1][2] .Metal is a material (an element, compound, or alloy) that is typically hard, opaque, shiny, and has good electrical and thermal conductivity. About 91 of the 118 elements in the periodic table are metals. Metals are mainly divided into five types based upon its nature **[3] [4]**[5] . Some metals adopt both structures depending on the temperature.[6] .

S.no	Type of metals	Nature	Example
1	Base metal	Metal that oxidizes or corrodes relatively easily, and reacts variably with dilute hydrochloric acid (HCl) to form hydrogen	iron, nickel, lead and zinc
2	Ferrous metal	The term "ferrous" is derived from the Latin word meaning "containing iron".	Iron
3	Noble metal	Noble metals are metals that are resistant to corrosion or oxidation	gold, platinum, silver, rhodium, Iridium and palladium
4	Precious metal	A precious metal is a rare metallic chemical element of high economic value.Chemically, the precious metals are less reactive than most elements	Gold, silver, palladium ,ruthenium, rhodium, palladium, osmium, iridium, and platinum.
5	Heavy metal	A heavy metal is any relatively dense metal or metalloid and toxic to the human body.	Arsenic ,lead etc

According to IOM recommended guidelines the adequate intake of minerals are given below along with their advantageous and disadvantages [7-22]

Recommended adequate intake by the IOM for Minerals mg/day										
Age	Ca	Cr mcg/day	Cu	Fe	Pb Mcg /day	Mg	Ni	K	Na	Zn
0-6 months	200	0.2	0.20	0.27		30		400	120	2
7-12 months	260	5.5	0.22	11		75		700	370	3
1-3years	700	11	0.7	7		80		3000 -4500	1000	3-8
4-8 years	1000	15	1	10		130		4500 -4700	1200	8-11
9-18 years	1300	25	1.3	8		240	100-300	4700	1500	13
19-50 years	1000	35	1.5	11		410	400-600		1500	
51-70(male)	1000	35	1.7	8		400	500-700		1500	
51-70(female)	1200	30	1.7	8		420			1200 -1300	
71+ years	1200		1.7							

S.No	Name of the metal	deficiency	Toxicity
1	Ca	Insomnia,anxiety, nervousness, depression, fatigue, muscle / joint pains, muscle spasms / cramps, stomach acid, osteoporosis, seizures, birth defects, miscarriage, high blood pressure, irregular heartbeat,	Arteriosclerosis, cardiovascular disease, arrhythmia, ischemic heart disease and stroke, hypertension, low stomach acid, depression, fatigue, glaucoma, higher risk for several cancers, muscle / joint pains, osteoporosis, osteoarthritis, calcification, dry skin,constipation
2	Cr	Reduced glucose tolerance / impaired glucose metabolism, weakened immune system, increased susceptibility for infections (e.g. bladder, left tonsil), trabecular bone loss,	Spinal / joint degeneration, depressed immune system, lymphatic swelling
3	Cu	Anemia, increased susceptibility for infections,weakened immune system, hormonal disorders, increased risk for (colon) cancer, miscarriage, trabecular bone loss, inflammatory joint disease	Wilson's disease, anemia, nausea, vomiting, abdominal pain, moodiness, violent behavior, ADD / ADHD, depression, confusion, weight gain, hemangiomas, arthritis, joint / spinal degeneration,
4	Fe	Fatigue, anemia, depression, dizziness, asthma,gastrointestinal disorders, pale skin, miscarriage, amenorrhea (failure to menstruate), dysmenorrhea, (painful periods), migraineheadaches, Ménière's disease,	Hemochromatosis, migraineheadaches,arthritis,high blood pressure, heart disease, liver disease, dizziness, gastrointestinal disorders, nausea,higher risk for several cancers, fibroid tumors,benign prostatic hypertrophy (BPH),
5	Pb	---	lead toxicity is the nervous system, both in adults and children. damage to the brain and kidneys. reduce fertility, delayed puberty
6	Mg	Irregular heartbeat, cardiovascular disease, anxiety, insomnia, nervousness, fatigue, muscle / joint pains,osteoporosis, seizures, high stomach acid, asthma,high blood pressure,	Cardiovascular disease, arrhythmia, cardiac arrest, coma, muscle spasms, joint / spinal degeneration, bone loss, low stomach acid, low body temperature, low blood pressure, higher risk for several cancers,.
7	Ni	Hyperglycemia (high blood sugar), low blood pressure, depression, liver disease, anemia, low stomach acid, sinus congestion, fatigue, low adrenals	Angina, skin rash, hypoglycemia, decreased estrogen, shortness of breath, asthma, nausea, lowered pulse,vomiting, diarrhea, headache, stomach irritation,increased protein in urine, increased red blood cells,heart failure
8	K	Irregular and/or rapid heartbeat, palpitations,high blood pressure (hypertension), shortnessof breath, asthma, heart disease, chest pains,stroke, paralysis, muscle spasms / weakness,bladder weakness, edema (water retention),kidney disease,	Irregular or slow heart beat, low blood pressure,kidney disease, cystitis - bladder infections or burning, higher risk of several types of cancer,infrequent menstrual cycles, muscle spasms orcramps, ovarian cysts (right), joint / back pains,weakened immune system, impotence, anxiety,insomnia, irritability, reactive hypoglycemia, coma.
9	Na	Fatigue, depression, mental apathy, low bloodpressure, headaches, dehydration, confusion,dizziness, arthritis, kidney stones,	Edema, hypertension, stroke, dizziness, gout,headaches, kidney damage, kidney stones, stomach problems, nausea, vomiting, coma

		seizures,In some cases: greater risk for LDL-related heart disease, high blood pressure, or edema.	
10	Zn	Decreased growth, loss of taste and smell, sterility, low sperm count, decreased wound healing, skin rash, hair loss, heart disease, liver disease, kidney disease, muscle weakness, enlarged prostate (BPH), several types of cancer, calcium spurs, paralysis, high blood pressure, arthritis,	Nausea, vomiting, dehydration, stomach ulcers, gastrointestinal problems, prostatitis, higher risk of several types of cancer, loss of libido, impotence, joint / back pain, muscle weakness / cramps, anemia, dysmenorrhea (menstrual pain), ovarian cysts (left), numbness, tingling, tremors, seizures, insomnia, irritability, weakened immune system, hair loss.

World health organization currently encourages, recommends and promotes traditional/herbal preparation in National Health Programmers because such drugs are easily available at low cost, are comparatively safe and the people have faith in such remedies. Some traditional medicine/herbal preparation with ancient formulas have been found to contain some metals, such as lead, cadmium, mercury, arsenic, lithium etc. Even though the herbal preparations are safe, but some of the herbal preparations cause serious poisoning and toxic effect, due to the preparations containing dangerous toxic drugs or metals . Both medical professional and the general public should be alerted to the potential toxicity of herbal preparation. There should be frequent monitoring of herbal preparations, containing toxic drugs or metals.

The objective of the study was to determine the concentration of metals in plants that are used in medicine by the local community. Analysis of the metal in selected plant samples was performed by ICPOES and Flame photometry of metals at different wavelengths respectively

METHOD AND METHODOLOGY:

Collection and authentication of plant material:

Collection and authentication of plant material:

The plant material *musa acuminata,actinidia deliciosa* and *mangifera indica* was collected in the month of may 2018 from local market in madinaguda, Hyderabad.

Preparation of ethanolic extract:

Method: The Ethanolic extract of the plant was prepared using reflex condensation process. The fresh fruits about 200g was weighed and placed in a 500 ml round bottom flask with 200ml of ethanol and its refluxed for 5 hrs at 40^{o}c . Then suspension was filtered through a fine muslin cloth. The solvent was evaporated by heating until ¾ is reduced. The remaining solvent is evaporated under room temperature. A semisolid residue was obtained.

Phytochemical screening:

S.NO	PHYTOCHEMICAL	NAME OF THE TEST
1.	GLYCOSIDES	LEGALS TEST
2	SAPONINS	FROTH TEST
3	ALKALOIDS	DRAGENDORFFS TEST
4	TANNINS	CHROMIC TEST
5	FLAVOINIDS	SHINODA TEST
6	MUCILAGE	RUTHENIUM RED TEST
7	CARBOHYDRATES	MOLISCH TEST
8	PROTEINS	XANTHOPROTEIC TEST
9	PHYTOSTEROLS	SALKOWSKI TEST

PROCEDURE FOR ESTIMATION OF METALS:

Preparation of extract sample (acid digestion)

1g of the extract was weighed and transfer into 50 ml of beaker. Then add 5ml of conc. HNO_3 and placed it on hot plate until the organic fumes were completely stopped. Then add 25 ml of water for acid digestion on hot plate. Digestion to be taken until 50% of the sample was too evaporated and remaining sample was filtered and makeup to 50 ml, and gone for furthered dilutions.

Preparation of standard:

The standard reagent 1000ppm was purchased from The National Institute of Standards and Technology (NIST) from Pune. From the standard reagent bottles (1000ppm) there were furthered dilutions into 0.5, 0.75, 1 ppm was prepared.

Caluculation:

The amount metals(ppm)=conc of the test X dilution factor / weight of the test taken

RESULTS AND DISCUSSIONS:

8.1. PERCENTAGE YIELD OF THE EXTRACT:

S.No	Name of The Plant	Percentage Yield (%)
1	*ACTINDIA DELICIOSA*	2.6%
2	MANGIFERA INDICA	2.9%
3	MUSA ACUMINATA	3.6%

8.2. PHYTOCHEMICAL SCREENING:

Table:4

Name of the plant	Alk	Carb	Gly	Tan	Phytos	Flav	sapo	Pro	muci
ACTINDIA DELICIOSA	+	+		+	+		+	+	+
MANGIFERA INDICA	+	+	+	+	+		+	+	+
MUSA ACUMINATA		+				+	+	+	

The above table indicates the presence (+) or absence (-) of phytochemicals in ethanolic extract(Alk:Alkaloids , Carb:Carbohydrates , Gly:Glycosides, Tan:Tannins, Phtos:Phytosterol,Flav:Flavanoids , Sapo:Saponins , Pro:Proteins , Muci:Mucilages)

WEIGHT TAKEN FOR METAL ANALYSIS:

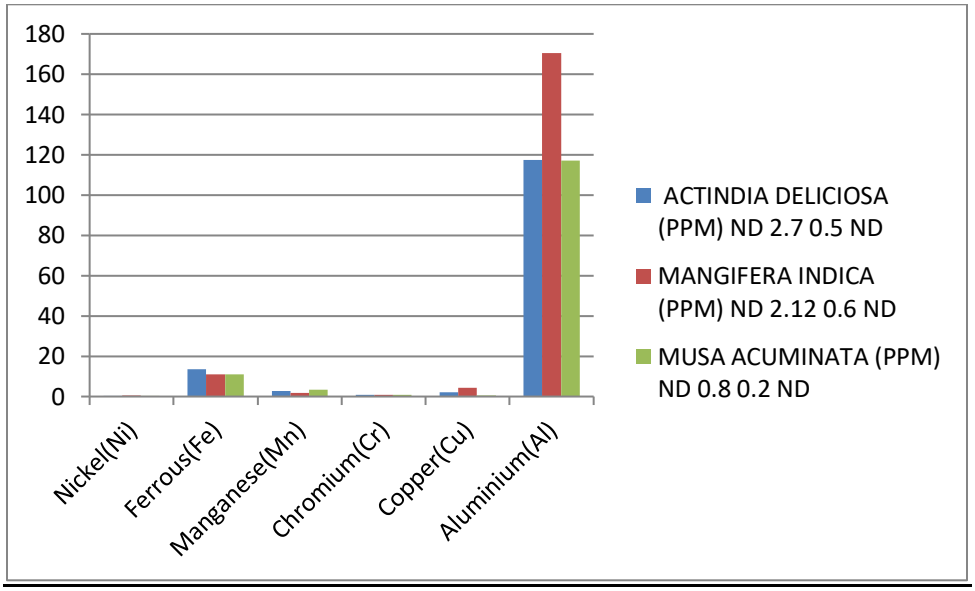
S.NO	NAME OF THE EXTRACT	WEIGHT TAKEN
1	*ACTINDIA DELICIOSA*	4.15
2	MANGIFERA INDICA	4.0965
3	MUSA ACUMINATA	4.095

LIST OF METALS AND ITS WAVELENGTH

S.NO	ELEMENTS	WAVELENGTH
1	**Arsenic(As)**	193.696
2	**Zinc(Zn)**	206.200
3	**Lead(Pb)**	220.802
4	**Cadmium(Cd)**	228.802
5	**Nickel(Ni)**	231.604
6	**Ferrous(Fe)**	238.204
7	**Manganese(Mn)**	257.610
8	**Chromium(Cr)**	267.716
9	**Copper(Cu)**	327.393
10	**Aluminium(Al)**	396.153

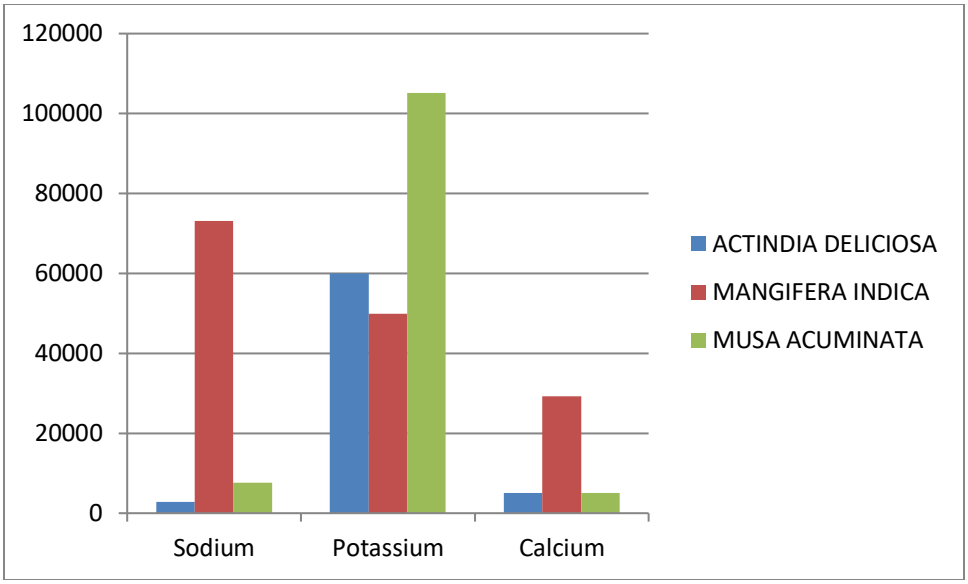
METAL CONCENTRATION IN THE FRUIT EXTRACT BY USING ICPOES:

COMAPRATIVE STUDY OF METAL ANALYSIS				
S.NO	**ELEMENTS**	*ACTINDIA DELICIOSA* **(PPM)**	MANGIFERA INDICA **(PPM)**	MUSA ACUMINATA **(PPM)**
1	**Arsenic(As)**	**ND**	**ND**	**ND**
2	**Zinc(Zn)**	**2.7**	**2.12**	**0.8**
3	**Lead(Pb)**	**0.5**	**0.6**	**0.2**
4	**Cadmium(Cd)**	**ND**	**ND**	**ND**
5	**Nickel(Ni)**	**0.3**	**0.4**	**0.3**
6	**Ferrous(Fe)**	**13.7**	**11.1**	**11**
7	**Manganese(Mn)**	**2.8**	**1.8**	**3.3**
8	**Chromium(Cr)**	**0.7**	**0.7**	**0.7**
9	**Copper(Cu)**	**2.2**	**4.2**	**0.6**
10	**Aluminium(Al)**	**117.5**	**170.5**	**117.2**



METAL ANALYSIS BY FLAME PHOTOMETER

s.no	Name	Sodium	Potassium	Calcium
1	*ACTINDIA DELICIOSA*	2753ppm	60000ppm	5000ppm
2	MANGIFERA INDICA	73200ppm	49900ppm	29250ppm
3	MUSA ACUMINATA	7625ppm	105200ppm	5000ppm



DISCUSSION:

As per the plant extracts the percentage yield in ascending order Musa acuminata(4.095%),Mangifera indica(4.0965%),Actindia deliciosa(4.15%)

As per the Phyto chemical screening the contents present in

Actindia deliciosa - Alkaloids(Alk),Carbohydrates(Carb),Tannins(Tan),Phytosterols(Phytos),Saponins(Sapo),proteins(pro),Mucilage(muci)

Mangifera indica -Alkaloids (Alk) ,carbohydrates(carb),Glycosides(Gly),Tannins(Tan), Phytosterols(Phytos),Saponins(Sapo),Proteins(pro),Mucilage(Muci).

Musa acuminata - Carbohydrates(carb),Flavanoids(Flav),saponins(Sapo),Proteins(Pro)

Zinc:

Zinc is an essential trace element for humans, animals, microorganisms and for plants. It is the second most abundant transition metal in organisms after iron. Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye.

It is the only metal which appears in all enzyme classes and found in nearly 100 specific enzymes. It is considered as of "exceptional biologic and public health importance", especially regarding prenatal and postnatal development

Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth.

Symptoms of mild zinc deficiency include depressed growth, diarrhea, impotence and delayed sexual maturation, alopecia, impaired appetite, and reproductive teratogenesis etc. The U.S. Food and Drug Administration (FDA) has stated that zinc damages nerve receptors in the nose, which can cause anosmia.

Excess zinc can be harmful, and cause zinc toxicity to occur at ingestion of greater than 225 mg of Zinc. Excessive absorption of zinc can suppress copper and iron absorption.

The U.S. Food and Drug Administration (FDA) has stated that zinc damages nerve receptors in the nose, which can cause anosmia.

Tolerable Upper Intake Level UL: 4mg -40mg

Our experiments revealed that zinc was present at musa acuminata < actinida deliciosa < mangifera indica *levels in* these extracts.

These levels of zinc are far lower than the toxicity level.

Calcium:

Calcium is essential for living organisms, in particular in cell physiology, where movement of the calcium ion into and out of the cytoplasm functions as a signal for many cellular processes. As a major material used in mineralization of bone, teeth and shells, calcium is the most abundant metal by mass in many animals.

Symptoms of hypocalcemia include numbness and tingling in the fingers, muscle cramps, convulsions, lethargy, poor appetite, and abnormal heart rhythms if left untreated, calcium deficiency leads to death.

Inadequate calcium intake causes osteopenia which if untreated can lead to osteoporosis. The risk of bone fractures also increases, especially in older individuals.

Excessive consumption of calcium carbonate antacids/dietary supplements (such as Tums) over a period of weeks or months can cause milk-alkali syndrome, with symptoms ranging from hypercalcemia to potentially fatal renal failure. Persons consuming more than 10 grams/day of $CaCO_3$ (=4 g Ca) are at risk of developing milk-alkali syndrome,

Tolerable Upper Intake Level UL of calcium is: 1000mg -3000mg.
The experimental results indicated that musa acuminata = actinida deliciosa < mangifera indica *were present.*
Therefore the levels of calcium present in these three tested samples are safe and give only beneficial effects.

Iron:
Iron is a necessary trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport.
The WorldHealth Organization (WHO) estimates that approximately half of the 1.62 billion cases of anemia worldwide are due to iron deficiency.

Large amounts of ingested iron can cause excessive levels of iron in the blood. High blood levels of free ferrous iron react with peroxides to produce free radicals, which are highly reactive and can damage DNA, proteins, lipids, and other cellular components. Thus, iron toxicity occurs when there is free iron in the cell, which generally occurs when iron levels exceed the capacity of transferrin to bind the iron

Tolerable Upper Intake Level UL: 40mg- 45mg
The experimental results indicated that musa acuminate < mangifera indica< actinida deliciosa were present.
These provide beneficial effects and do not cause any toxicity.

Copper:

Numerous antimicrobial efficacy studies have been conducted in the past 10 years regarding copper's efficacy to destroy a wide range of bacteria, as well as influenza A virus, adenovirus, and fungi.

Copper is also found in many superoxide dismutases, proteins that catalyze the decomposition of superoxides, by converting it (by disproportionation) to oxygen and hydrogen peroxide:

Deficiency of copper in animals has resulted in anemia, osteoporosis, delayed wound healing and the development of aortic aneurysms, and loss of hair color.

Acute copper toxicity, such as that following the ingestion of more than 15 mg of elemental copper, has been associated with nausea, vomiting, diarrhea, and intestinal cramps. Intravascular hemolysis has occurred with larger ingestions.

Tolerable Upper Intake Level UL: 1mg- 10mg
The experimental results indicated that musa acuminate < actinida deliciosa < mangifera indica were present.
The amounts are within the limits.

Chromium:
Humans require chromium in trace amounts, although its mechanisms of action in the body and the amounts needed for optimal health are not well defined Chromium is known to enhance the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body Few serious adverse effects have been linked to high intakes of chromium, so the Institute of Medicine has not established a Tolerable Upper Intake Level (UL) for this mineral . A UL is the maximum daily intake of a nutrient that is unlikely to cause adverse health effects. It is one of the values (together with the RDA and AI) that comprise the Dietary Reference Intakes (DRIs) for each nutrient

Tolerable Upper Intake Level: 200 mcg -5,000 mcg
The experimental results indicated that musa acuminate =mangifera indica = actinida deliciosa *were present.*
The quantities are in safe limits.

Nickel:
Nickel plays important roles in the biology of microorganisms and plants. Plant enzyme urease (an enzyme that assists in the hydrolysis of urea) contains nickel.

Nickel deficiency causes hyperglycemia (high blood sugar), low blood pressure, depression, liver disease, anemia, low stomach acid, sinus congestion, fatigue, low adrenals

Tolerable Upper Intake Level UL: 200 mcg -1,000 mcg
Most of the nickel absorbed every day by humans is removed by the kidneys and passed out of the body through urine or is eliminated through the gastrointestinal tract without getting absorbed. Nickel is not a cumulative toxicant; however, larger doses or chronic exposure may be dangerous for human health and may represent an occupational hazard due to their acute toxicity and carcinogenicity.

Tolerable Upper Intake Level UL: 200 mcg -1,000 mcg
The experimental results indicated that musa acuminata = actinida deliciosa< mangifera indica were present.
The amounts of nickel are well within the UL values therefore safe to consume.

Potassium:
Potassium levels influence multiple physiological processes Resting cellular-membrane potential and the propagation of action potentials in neuronal, muscular, and cardiac tissue.
Potassium Deficiency causes irregular and/or rapid heartbeat, palpitations,high blood pressure (hypertension), shortnessof breath, asthma, heart disease, chest pains,stroke, paralysis, muscle spasms / weakness,bladder weakness, edema (water retention),kidney disease, liver disease, endometriosis,frequent menstrual cycles, high blood sugar,weight gain, fatigue, impotence.

Tolerable Upper Intake Level UL: 300mg - 15,000mg
The experimental results indicated that mangifera indica < actinida deliciosa< musa acuminate were present.
Our estimation of potassium revealed that the amounts of potassium are well within the UL valus therefore safe to consume.

Sodium:
In humans, sodium is an essential nutrient that regulates blood volume, blood pressure, osmotic equilibrium and pH; the minimum physiological requirement for sodium is 500 milligrams per day.[50] Sodium chloride is the principal source of sodium in the diet, and is used as seasoning
Sodium deficiency leads to fatigue, depression, mental apathy, low bloodpressure, headaches, dehydration, confusion,dizziness, arthritis, kidney stones, seizures,In some cases: greater risk for LDL-related heart disease, high blood pressure, or edema.

Tolerable Upper Intake Level UL : 1,500mg - 2,300mg
Excess sodium intake leads to edema, hypertension, stroke, dizziness, gout,headaches, kidney damage, kidney stones, stomach problems, nausea, vomiting, coma
The experimental results indicated that actinida deliciosa < musa acuminata <mangifera indica were present.
The results proved that amounts of sodium are well within the UL valus therefore safe to consume.

Manganese:
The deficiency (hypomagnesemia) is common: and found in 2.5–15% of the general population. The primary cause of deficiency is decreased dietary intake: Other causes are increased renal or gastrointestinal loss, an increased intracellular shift, and proton-pump inhibitor antacid therapy
Health Risks from Excessive Too much magnesium from food does not pose a health risk in healthy individuals because the kidneys eliminate excess amounts in the urine.

Tolerable Upper Intake Level UL : 65mg -360mg
The experimental results indicated that mangifera indica <actinida deliciosa < musa acuminata were present.
Overdose from dietary sources alone is unlikely because excess magnesium in the blood is promptly filtered by the kidneys

Lead:
Lead is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the body. The component limit of lead (1.0 µg/g) is a test benchmark for pharmaceuticals, representing the maximum daily intake an individual should have. However, even at this low level, a prolonged intake can be hazardous to human beings.
Recommended not more than: The component limit of lead (1.0 µg/g)
Our experimental results indicated that musa acuminata < actinida deliciosa <mangifera indica was present.

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