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ELEMENTAL ANALYSIS, DITERMINATION OF ALKALOID, SAPONIN AND FLAVONOID OF THREE SELECTED SPECIES OF ZINZIBERACEAE FAMILY

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ABSTRACT: The present study reveals the chemical composition and mineral analysis of Curcuma amada, Alpinia nigra, Amomum gracile. They are belongs to the same family zingiberaceae. The result reveals; (a) alkaloid contains in Alpinia nigra (6.0mg/100gm), amomum gracile (0.6mg/100gm), Curcuma amada (4.280mg/100gm); (b) saponin contain in Alpinia nigra burtt(18.960mg/100gm), Amomum gracile (18.1mg/100gm) and Curcuma amada (21.22mg/100gm); (c) flavonoid contains in Alpinia nigra burtt (14.160gm/100gm), Amomum gracile (9.220mg/100gm) and Curcuma amada (8.400mg/100gm). The result of elemental analysis measure in ppb reveals; Alpinia nigra burtt contains iron (1.007 ± 0.001), Zn (0.408 ± 0.001), Cu (2.27 \pm 0.08), Mo (0.011 \pm 0.006), Cr (2.151 \pm 0.0307) and Mn (0.013 ± 0.002) , Amomum gracile was found to contains Fe (0.971 ± 0.046) , Zn (0.414 \pm 0.083), Cu (1.70 \pm 0.03), Mo (0.013 \pm 0.004), Cr (5.091 \pm 0.011) and Mn (0.015 \pm 0.003) and Curcuma amada was found to contains Fe (0.954 ± 0.003) , Zn $(0.1764 \pm 0.0.005)$, Cu (1.80 ± 0.02) , Mo $(0.016 \pm$ 0.008), Cr (1.643 \pm 0.001) and Mn (0.014 \pm 0.002) respectively. Vanadium cannot be detectable in all the three selected plant species for the study

INTRODUCTION: Phytochemicals are natural bioactive compounds found in plant and herbs. According to their functions in plant metabolism phytochemical ingredients are broadly classified secondary into primary and metabolites. Carbohydrates, amino acid. proteins and chlorophyll are includes in primary constituent while alkaloids, terpenoid, steroids, flavonoids and saponins etc. are includes in secondary metabolite ¹. Plants and herbs are used as medicine in many different ways since from the time of early man.



One medicinal plant is found to be used for different purpose; different medicinal plants have diff. properties². Various kind of medicinal plants are found all over the world. To the development of ancient materia medica those plants having medicinal properties gave a good contribution. Those plants that exert beneficial pharmacological effect on the animals and its environments or those possess therapeutic properties are treated as medicinal plants. Generally, medicinal plants are used as complex mixture made of single plant containing multicomponent mixture extract comprised of several closely related biologically active components.

On the other ways, it is used as pure, chemically defined active principle isolated from medicinal plants. Chemical compounds that contains in medicinal plants are used directly or indirectly prevent or treatments of various kind of disease ^{3, 4}.

Medicinal plants take parts important role in modern allopathic medicine, herbal medicine, homeopathy and aromatherapy. Among the indigenous medicinal plants some are used as spices and food plants and some of these are also added as food meant for pregnant mother for medicinal purposes ^{5, 6, 7}. A group of herbal healer of the Indian traditional health care system (Ayurveda) used traditionally many medicinal plants for thousands of year and purposed for their multilevel activities ⁸.

About 80% of the world's total population relies on traditional medicine, most of which involved the use of the plant and herb extracts for their primary health care system ⁹. Among the world India is the largest producer of medicinal plant and herb therefore appropriately called the botanical garden of the world ¹⁰. 95% of the drug prescriptions of India were plant based in the traditional systems of Unani, Ayurveda, Siddha and Homeopathy¹¹. Due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the used to discover new molecular structure as lead compounds from the plants kingdom, the revival of interest in herbal medicine is also increase ¹². Due to widely availability and less side effect herbal base drugs are suitable for treating a wide range of infection and diseases ¹³.

Various minerals are present in traditionally use medicinal plants, some of this mineral act as catalytic agents in many metabolic reactions and some other of these minerals takes part of body structural components. For a wide range of cell function these minerals are required in trace amount ¹⁴. The metabolic product of the plant cells and a number of trace elements form the active constituent of medicinal plants, and take part an important role in the cell metabolism ¹⁵. Iron, iodine, copper, manganese, zinc, molybdenum, selenium and chromium etc. are the trace elements that are necessary for human health ¹⁶. Both metallic and non-metallic elements in trace amount are required for their growth and good health by the human beings ¹⁷.

Curcuma amada roxb popularly known as mangoginger (Yai-Heinounam, Manipuri name) is having characteristic odour similar to raw mangoes and used as major ingredient in the pickles, candies, salads, sauces and chatneys ¹⁸. The Rhizomes of this plant are useful in vitiated condition of pitta, anorexia, dyspepsia, flatulence, colic, bruises wounds, chronic ulcers, skin diseases, pruritus, fever, constipations, hiccough, cough, bronchitis, sprains, gout, halitosis, and inflammations ¹⁹. Fried dried rhizomes of *Alpinia nigra burtt* are used in gout and colic. Young shoots are used as vegetables. Inflorescence is usually taken as appetizer. Rhizomes of *Amomum gracil* are used as spice.

The main aim of the present work was to study the quantitative analysis of some secondary metabolites and mineral content of three selected species of *Zinziberaceae* family, namely *Curcuma amada, Amomum gracil* and *Alpinia nigra burtt* to ascertain ethno-medicinal claims of this widely used medicinal plant.

MATERIAL AND METHODS:

Plant material: The Mature and healthy rhizomes of *Curcuma amada, Amomum gracil* and *Alpinia nigra burtt* were collected from Kangmong village, Imphal west district of Manipur during the month of March and April 2014. The collected plant material were washed two-three times by tape water and then by distilled water. They were cut separately into thin slice by knife and dried at room temperature at about one week. It is then converted into fine powder with the help of hand grinder. These powder plant materials were then kept in an airtight glass container for further use.

Chemical analysis

Analysis of minerals:

The minerals analysis was done by Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS).

Alkaloid determination: ²⁰

5gms of each plants powder sample were weight separately into a 250 ml conical flasks and 200 ml of 10 % acetic acid in ethanol were added, cover the contains of the conical flasks by aluminium foil and allow to stand 2 days then filter. After filtration, the extract was reduced to ¹/4th of its original volume on a water bath. To the reduced volume of the extract concentrate ammonium hydroxide was added in drops until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weigh.

Saponin determination: ²¹

5gms of each Plant samples were taken separately in a 250 ml conical flask 250 ml of 25% ethanol was added, the suspension was heated with continuous stirring on a water bath at about 60°c for 4 hrs. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol and then filtered. The combined extracts were reduced to 40 ml over water bath at 90° c. The concentrate mixture was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered into 250 ml conical flask while the ether layer was discarded. The purification process was repeated thrice; 60 ml of n-butanol was added. The combined n-butanol extracts was washed with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in percentage.

Flavonoid determination: ²²

5gms of each plants material were extracted separately in 250 ml conical flasks repeatedly with 150 ml of 80% aqueous methanol at room temp. The whole solution was filtered through Watman filter paper no. 42 (125mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed.

RESULT: Ouantitative estimations of some secondary metabolites beings of Curcuma amada, Amomum gracil and Alpinia nigra burtt are summarizes on Table 1. The element contents of these three selected medicinal plants are shown on table 2. Chromium was the most abundant mineral present ranging from 5.091 ± 0.011 ppb in Amomum gracil, 2.151 ± 0.07ppb in Alpinia nigra burtt and 1.64 ± 0.001 ppb in Curcuma amada. Copper are the nest most abundant minerals present ranging from 2.27 ± 0.08 ppb in *alpinia nigra burtt*, 1.80 ± 0.02 ppb in *Curcuma amada* and 1.70 ± 0.03 ppb in Amomum gracile. This is followed closely by Iron which was present 1.007± 0.001 ppb in Alpinia nigra burtt, 0.911± 0.046 ppb in Amomum gracil and 0.954 ± 0.00 ppb in *Curcuma amada*.

DISCUSSION: Phosphorous is vital for growth and repair of every cell membrane. It found in substantial amount in the nervous system and form part of the body's energy storage system. Out of the three important elements phosphorous was one that takes part plant's life possible ²³.

Zinc, as a vital mineral, take parts in many function of the body, synthesis of DNA, RNA, aiding enzymes in digestion, wound healing and energy metabolism. It involved in cell mediated, antibodymediated immunity and possess direct antioxidant activity ²⁴ This agreed with the findings of Padma S vankar *et-al* (2006), they reported that *Alpinia nigra burtt* possess good antioxidant property. This also agreed with the finding of Angel *et-al* (2013) they reported, *Curcuma amada* possess good antioxidant property.

Copper prevents damage to cells due to its antioxidant action and as a component of many enzymes it helps in production of energy from carbohydrate, protein and fat. It is also essential for formation of bone, connective tissues and red blood cells. However, in excessive levels it can be toxic ²⁵. In our study all the sample contains within the permissible limit. On the other hand, deficiency of copper results in anaemia and congenital inability to excrete copper resulting in Wilson disease ²⁶.

Iron, as a component of haemoglobin in blood, it transport oxygen from the lungs to different parts of the body. Iron is also part of many enzymes and is essential for growth, healing, immune function and synthesis of DNA.

Manganese helps in the formation of enzymes, it also necessary for their activation. It works as an antioxidant, helps develop bones and heals wounds by increasing collagen production. It is also related to carbohydrate and fat metabolism ²⁷. Deficiency of manganese in human being, causes the interruption of blood supply to a part of the heart, causing heart cells to die. It also causes disorder of bony cartilaginous growth in infants and children and may lead to immunodeficiency disorder and rheumatic arthritis in adult ²⁸.

Chromium is an important trace mineral that is necessary for normal functioning of insulin, a hormone that maintains blood sugar levels. It is also essential for metabolism of carbohydrate, proteins and fats. Molybdenum is required for the function of several enzymes and its content in food varies with the soil conditions.

 TABLE 1: PHYTOCHEMICAL COMPOSITION OF RHIZOME OF CURCUMA AMADA, ALPINIA NIGRA AND

 AMOMUM GRACILE EXPRESSED AS MG PER 100GM DRY WEIGHT.

Phytochemicals	Curcuma amada	Alpinia nigra	Amomum gracil	
Alkaloid	4.280	6.0	0.60	
Saponin	21.22	18.960	18.1	
Flavonoid	8.40	14.160	9.220	

TABLE 2: ELEMENTAL ANALYSIS OF CURCUMA AMADA, AMOMUM GRACILE AND ALPINIA NIGRAEXPRESSED IN ppb.

Elements	Alpinia nigra	Ammomum gracil	Curcuma amada
Iron	$1.007 \pm 0.001 \text{ ppb}$	$0.971\pm0.046~ppb$	$0.954 \pm 0.003 \text{ ppb}$
Zinc	$0.408 \pm 0.001 \text{ ppb}$	$0.414\pm0.083~ppb$	$0.1764 \pm 0.005 \text{ ppb}$
Copper	$2.27 \pm 0.08 \text{ ppb}$	$1.70\pm0.03~\mathrm{ppb}$	$1.80 \pm 0.02 \text{ ppb}$
Molebdenum	$0.011 \pm 0.006 \text{ ppb}$	$0.013\pm0.004~ppb$	$0.016 \pm 0.008 \text{ ppb}$
Chromium	$2.151\pm0.307~ppb$	5.091± 0.011 ppb	$1.643 \pm 0.001 \text{ ppb}$
Manganese	$0.013\pm0.002~\text{ppb}$	$0.015 \pm 0.003 \text{ ppb}$	$0.014\pm0.002~ppb$
Vanadium	ND	ND	ND

NB (ND---Not detectable)

CONCLUSION: The present study revealed that the element contents of the three selected species of *Zinziberaceae* family from Kangmong village, Imphal west District, Manipur were within the safe limits. It is therefore, concluded that these plants does not affect directly to the human health, when it takes orally or consumed as part of diet. This study, therefore, has provided some biochemical basis for the ethno medicinal use and also adds to the data base on medicinal plants which will be useful for researchers working in the field of ethnopharmacology.

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