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Heavy Metal Analysis and Physico-Chemical Properties of Seed Oil of Cucumis callosus from Arid Zone of Rajasthan

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Abstract: Valuable added products, dietary supplements and medicines can be obtained from *Cucumis callosus* seeds. These seeds are good source of energy, carbohydrates, proteins, fats and minerals such as calcium, iron, magnesium, phosphorous and potassium. The *Cucumis callosus* (commonly known as Kachri) seed and its oil were studied for the presence of heavy metals, especially metals like copper, zinc, magnesium, iron, calcium, potassium, and sodium. Copper, zinc and manganese play an important role in the breakdown of carbohydrates, fats and proteins into digestible forms and convert them into energy. Trace elements like Cu, Mn and Zn are essential in bone metabolism as cofactors of specific enzymes. The seeds of Kachri were found to contain moisture (2.358%) and high ash contents (4.801%). Ash obtained from it is composed of the inorganic matter such as minerals. The heavy metal analysis of seed oil of *Cucumis callosus* shows that they are rich in calcium (2417 mg/L), potassium (7589 mg/L), magnesium (4516 mg/L) and low in iron (164 mg/L), sodium (74 mg/L) and zinc (77 mg/L).

Key words: **Kachri seeds, Mineral analysis, Bone metabolism, *Cucumis callosus***

1. INTRODUCTION:

Heavy metal contamination and environmental imbalance due the industrial revolution and exponential growth in population cause numerous health problems. Mining activities can release toxic metals to the environment. Metal mining and smelting activities are regarded as major sources of heavy metals in the environment. In environments where these activities take place, it is indicated that large amount of toxic metals deposits are found in their water, soil, crops and vegetable [1,2]. These metals cause various diseases in human. Cadmium is one of the most dangerous metals due to its high mobility and the small concentration at which its effect on plants begin to appear [3]. Cadmium has been observed earlier in Japan due to dietary intake of cadmium by people. This was termed as 'Itai Itai' and the symptoms were painful osteomalacia (bone disease) combined with kidney malfunction. This incident of cadmium poisoning was due to irrigation of rice fields with contaminated water from cadmium, lead and zinc mines. The major threat to human health is the chronic accumulation of cadmium in kidneys leading to kidney dysfunction. Similarly, lead is known to be toxic to plants, animals, and microorganisms and its effects are generally limited to contaminated areas. Lead contamination in the environment poses serious human health problems like brain damage and retardation. The main root by which cadmium can enter into human body is food intake and tobacco smoking whereas lead contamination in soil and plants is generally due to automobile exhaust, dust, and gases from various industrial sources in addition to other sources such as industrial sites, leaded fuels, old lead plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Pb^{2+} is non-biodegradable and shows acute toxicity in humans when present in high amounts [4]. Excess of nickel in soil can affect the growth of plants by inducing leaf chlorosis and wilting and disrupting photosynthesis. An uptake of nickel above threshold limit can also cause many consequences in humans such as dizziness, cancer, respiratory failure, birth defects, asthma, chronic bronchitis, allergic reactions, and heart disorders. The major sources of nickel contamination in the soil include metal-plating industries, combustion of fossil fuels, and mining operations [5]. Further, nickel contamination of soil can also be due to power-plant ash which finally settles to the ground on precipitation.

The cucurbitaceous family has a tremendous genetic diversity, extending to reproductive and vegetative characteristics. They grow in tropical, subtropics, arid desert and temperate locations. The plants of the five varieties studied are annual, herbaceous, monoecious plants with climbing or creeping stems. After planting, they completely cover the soil surface within 4 weeks of growth, thus helping in weed control. Pollination is by insects. Flowering occurs about 4-5 weeks and fruits mature at 7-8 weeks after planting (Oil Crops, 2000). The fruits are indehiscent smooth berries, are very large and seedy and when sound, can be stored for over a year, or the seeds can be removed, washed and dried. For use, they are decorticated, ground into a nutritious oily meal and cooked. Most of the oil is made up of unsaturated fatty acids with high amounts of essential fatty acids, especially linoleic acid [6].

Cucumis callosus (Cucurbitaceae) commonly known as "Kachri" in Rajasthan (India) has been claimed in traditional literature as a valuable against a wide variety of diseases. The herb is distributed throughout India in arid zones. The herb is much branched very common prostate, perennial herb; leaves are cordate, suborbicular, deeply palmately 5-7 lobed; flowers are yellow; fruits are smooth, obovoid-ellipsoid, green variegated stripes and fruiting in August-November. Fruit is traditionally used to prevent insanity to strong memory and remove vertigo. The seeds are useful in bilious disorder [7, 8], diabetics, easy bowl syndrome, stomach pain, vomiting and constipation [9, 10]. Paste of root is applied on scorpion sting; decoction of root is given in indigestion, dropsy, and pulp of fruit used in abortion and to increase menses for women [11]. Hence, the present study was aimed at evaluating the free radical scavenging activity of the aqueous extract of seeds of *Cucumis callosus*.

Plant seeds are important source of oils of nutritional, industrial and pharmaceutical importance. The seeds of *Cucumis callosus* have significant antioxidant, anti-inflammatory and analgesic properties [12]. The native variety as a soft, white flesh, which is cut up, shredded, or mashed, and eaten or drunk with sugar. Seeds are salted, dried, and the kernels eaten as delicacy. Seed kernels are rich in protein and fat. Sometimes, it is used as substitute for almonds and pistachio. It is a source of edible oil with the nutritional value of melon seed oil and also a good source of potassium, vitamin A, and foliates.

Fruit

Seeds

Absorption of metals by plant root can be take place by both active and passive processes. Passive (non-metabolic) uptake involves diffusion of ion from the soil solution to the root endodermis. On the other hand active (metabolic) uptake takes place against the concentration gradient and requires metabolic energy thus can be inhibited by toxins. The mechanisms of absorption is different for different metals; for instance Pb uptake is generally considered to be passive while that of Cu, Mo and Zn is thought to be either active or a combination of both active and passive uptake [13].

The aim of the study is to analyse contents of heavy metals accumulated in seed oil of *Cucumis callosus* plant. For this purpose, the concentrations of Pb, Zn, Cd, Cu, Fe, and Ni were measured for the estimation of heavy metal pollution.

Pollutants must clearly be counted among plant growth. It is the outcome of many factors [14]. Various types of environmental pollutants such as poisonous gases, agricultural chemicals, solid wastes, radioactive materials, industrial effluents, sewage water etc. are known to pollute air, water, soil and bring about hazardous effects on plants, animals and human beings. High consumption, frequent disposal and replacement of disposable items are generating diverse types of metallic and polymer wastes which are discharged into the environment and thus poisoning the biosphere [15-19]. Heavy metals like Cd, Cu, Co, Ni, Zn and Cr are phytotoxic either at all concentrations or high concentrations level.

Toxic metals existing in biological system are magnified through the food chain. Toxic metals are affecting the environment. When concentrations of heavy metals are within limit, then it is useful to micronutrients for plants, human and animals. But it becomes toxic when their concentration exceeds a limit [20].

The Heavy metal contaminants within the soil also depend on their ability to travel through water system and their availability for biological uptake. Iron when exceeds limit typically damages cells in the heart and liver which can cause cancer, coma, liver failure, circulatory shock and long term organ damage. There are also many other activity that *Cucumis callosus* is helpful for traditional medicine describe mostly the use of the fruit juice as nasal drops for the treatment of jaundice in new born.

Risk of heavy metal contamination on vegetation by industrial waste, sewage sludge and exhaust from automobiles has been revealed Fe, Mn, Mg, Zn and Cr are beneficial to plant life in micro quantities, hence are trace nutrients. But they become toxic at higher concentrations [21-24]. Metals like mercury (Hg), cadmium (Cd), nickel (Ni), tin (Sn) etc. are in no way, good for living organisms.

World Health Organisation (WHO) [25] has recognized health hazards of metals in food chain even at low concentrations. Studies have been conducted to evaluate the transferred elements from soil to plant [26]. Naturally occurring chelating agents have been known to interact with the metal ions and increase their availability to the plants. The low molecular weight organic acids released as root exudates were reported to be efficient phytochelators of metals [27].

2. MATERIALS AND METHODS

2.1 *Sampling*: The seeds of *Cucumis callosus* plants were collected from arid region of Rajasthan, India. Seeds were dried in air. The damaged seeds were discarded and good seeds were selected that means in seeds in good condition were cleaned, de-shelled and dried at high temperature of 100- 105°C for 35 min. Seeds were grounded using grinder prior to extraction.

2.2 *Oil Extraction:* Extraction of oil from seeds was done by solvent extraction method. The clean and dried seed samples were crushed in mortar and oil was extracted from the crushed seeds by extraction with petroleum ether (60-80^{0}C) in a Soxhlet apparatus for 6 hrs. The solvent is removed under reduced pressure. The obtained oil was stored in cool place (refrigerator) until further investigation [28].

2.3 *Reagents:* All reagents were of analytical reagent grade. Double deionized water was used for all dilutions. HNO_3, H_2SO_4, H_2O_2, HF, $HClO_4$ and HCl were of superior quality. All the plastic and glassware were cleaned by soaking in dilute HNO_3 and were rinsed with distilled water prior to use. The working standard solutions of heavy metals used for calibration were prepared by diluting a stock solution of 1000 µg/L (Pb, Cd, Zn, Fe, and Ni).

2.4 *Mineral metal analysis*: One of the methods for determination of the total contents and speciation analysis of heavy metals of their environmental concentrations is atomic absorption spectroscopy [29, 30]. This method is simple and very selective. In this paper we present determination of heavy metals in seed oil of *Cucumis callosus* plant by atomic absorption spectroscopy method.

2.5 *Preparation of standard for metal:* In spectrophotometric measurements we are concerned with solution having very small concentration of the metal to be determined. It follows that the standard solution which will be required for analysis must also contain very small concentration of the relevant metal. Standards are prepared by dissolving 1gm of metal cadmium, nickel, iron lead and zinc dissolve in minimum quantity of aquaregia (1:3) HCl and HNO_3, made up to 1 litre in volumetric flask by adding deionized water. This is a stock solution which contains about 1000µg/L of required metal and then the working standard solution is prepared by suitable dilution of stock solution.

2.6 *Percentage Yeild:* 100 gm of the grounded seeds were taken and were set in the Soxhlet mechanical assembly and the oil was extracted utilizing oil ether as dissolvable. The get together was made to run for 8 hours. Anhydrous Sodium sulphate was added to expel any hint of dampness from the separated arrangement. The oil was isolated from the dissolvable utilizing refining get together. The percentage of oil content can be calculated as below:

% of oil = Wt of oil obtained in gm x 100

Wt of seed is taken in gm.

After the oil had been acquired and its level of oil content is determined the equivalent is exposed to physiological test, for example, acid value test, iodine value test and saponification value test, chemical analysis of seed oil.

2.7 *Digestion of seed oil:* For the seed oil samples analysis, seed oil was digested in 100 ml Pyrex glass beaker. For this we took 1g of seed oil added 10 mL Concentrate Nitric acid. Kept first for cold digestion for 24 hours and then heat at 50^{0}C for 4hours. The solution was finally boiled with 1:5 mixtures of concentrate acids HCl and HNO_3 in order to digest all organic matter [31] and then filtered after cooling. Finally volume of the extract was made up to 25 mL using double distilled water [32].

2.8 *Fatty acid analysis:* The fatty acids composition of *Cucumis callosus* plant oil was determined in two steps. In first step hydrolysis of oil was done and mixed fatty acids were obtained, and in second step this mixture of fatty acids was further derivatised to their methyl esters. The formation of methyl esters was confirmed by thin layer chromatography (TLC). The methyl esters so obtained were analysed by HPLC [33]. The physico-chemical properties such as Saponification value, acid value, iodine value and peroxide value of the *Cucumis callosus* seed oils were determined, using the method described by AOCS [34, 35].

2.9 *Acid Value*: 2 g of pure oil (250 ml) is weighed accurately using a conical transfer method Flask. Neutral ethanol (20 ml) was added by pipette and heated in a steam bath 3 minutes. The flask was then cooled and the subjects were diluted with 0.1N alcohol potassium Phenolphthalein hydroxide is used as an indicator for the solution. It has also driven space titration for years Aspect.

2.10 *Iodine value*: The oil (0.2 g) is accurately weighed using a transfer method to a 250 ml iodine flask and dissolved in chloroform (20 ml). Wij's reagent (20 ml) was added by pipette. The flask Holds for 1 hour and put in the dark with intermittent trepidation. Then 15% potassium iodide solution (10 ml) and 50 ml of distilled water were added to the flask and the mixture was stirred. Free iodine was titrated with 0.1 N sodium thiosulfate solution and fresh flour solution Indicator. It has also driven space titration for years.

2.11 *Saponification Value*: 2 ml of oil using the transfer method is exactly 250 ml flask below and freshly prepared 0.5N alcohol potassium hydroxide solution (25 ml). The sample and mixture through the pipette is refluxed to the water bath using an air conditioner an one hour. Then the flask is cooled and the condenser tip is washed with a little distilled water. Subjects were titrated with 0.5 N hydrochloric acid solution using phenolphthalein as indicated.

3. RESULTS AND DISCUSSION:

3.1 Physicochemical Properties:

The oil yield is 35.2% and different chemical properties like as acid value, saponification value, iodine value, protein content give structural stability. The oil contents and physiochemical properties of oil *Cucumis callosus* seed from arid zone are presented in Table1. In *Cucumis callosus* seeds, dietary fibre was low but it contains moisture and high ash content. Ash is composed of minerals which include iron, copper, calcium, magnesium, potassium, sodium, zinc etc. It was rich in calcium, potassium, magnesium and phosphorus.

Table 1: Macronutrient and micronutrient of *Cucumis callosus* seeds

Sample	*Cucumis callosus* Seeds
Carbohydrate (%)	22.874
Protein (%)	32.80
Fat (%)	37.167
Dietary Fiber (%)	24.74
Moisture (%)	2.358
Ash (%)	4.801
Iodine value (mg/g)	110.8
Saponification value (mg/g)	186.9
Acid value (mg/g)	2.73

3.2 Heavy Metal analysis:

Heavy metal analysis is done by atomic absorption spectroscopy. On analysis it was found that seed oil of *Cucumis callosus* is rich in Potassium (K), Magnesium (Mg), Calcium (Ca) and iron (Fe) as shown in table 2. According to the limits provided by WHO in food, heavy metals concentration are within the limit as shown in the graph.

Table 2: Mineral content of *Cucumis callosus* seeds

S. No.	Analyte	Sample concentration unit (mg/L) in *Cucumis callosus* seeds
1	Ca	2417
2	Cd	0.2
3	Zn	77
4	Cu	22.5
5	Fe	164
6	K	7589
7	Mg	4516
8	Mn	34.2
9	Na	74
10	Ni	1.2
11	Pb	0.9
12	As	0.002

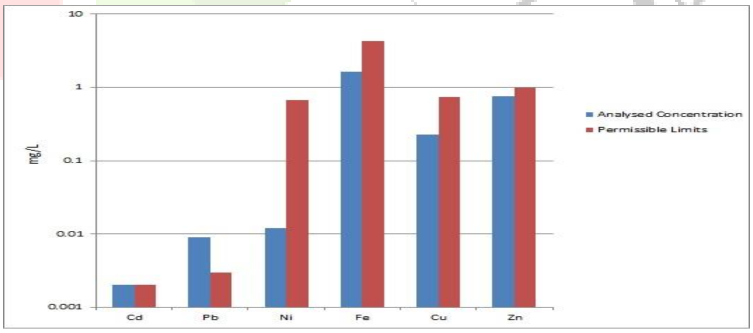

Figure1: Plot of heavy metal content in seed oil of *Cucumis callosus* vs their permissible limits

3.3 Fatty Acid Composition:

The non-saturated fatty acids were oleic acid (12.4%), Linoleic acid (52.9%) present. The saturated fatty acids were identified as palmitic acid (24.1%), stearic acid (5.5%). The unsaturated fatty acids are 67.6% higher than that of saturated fatty acid as shown in table 3.

Table 3: Fatty acid composition

S.No.	Fatty acid	Composition
1	Linoleic acid	52.9%
2	Oleic acid	12.4%
3	Stearic acid	5.5%
4	Palmitic acid	24.1%

4. CONCLUSION:

Based on the results of this study the conclusion can be made that seeds of *Cucumis callosus* are good source of calcium, magnesium, potassium and sodium. Calcium builds bones and teeth; activates enzymes throughout the body; helps regulate blood pressure; and helps muscles to contract, nerves to send messages, and blood to clot. Sodium and potassium help in the maintenance of normal blood pressure. Heavy metal analysis shows that these metals are within the permissible limits for human being as per shown in above graph and it is not harmful for them. The analysis of seeds for the nutritional composition has shown that they are good source of energy protein, fat and carbohydrates. The seeds provide opportunities to develop as medicines, value added products and dietary supplements.

Cucumis callosus seed oil is good source of essential Omega-9 (oleic acid) fatty acid. It is very important fatty acid because it increases HDL (good cholesterol) and decreases LDL (bad cholesterol), they help reduce plaque build-up in the arteries, so it prevents heart diseases. *Cucumis callosus* seed oil is also good source of essential Omega-6 (linoleic acid) fatty acid. Linoleic acid is the most important PUFA in human diet, as it is also prevents heart and vascular diseases. It seed oil is good for human health. The high acid values of the crude vegetable oils lead to difficulties in the biodiesel preparation, occurs the formations of soaps and stable emulsions.

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