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ORIGINAL ARTICLE **OPEN ACCESS**

Heavy Metals Investigation in Seed Oil of *Citrullus colocynthis* from Arid Zone of Western Rajasthan

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ABSTRACT

Citrullus colocynthis is herbaceous plant that is high in nutrients and plays an important part in overall health. This arid-zone fruit crop is neglected and underappreciated. Oil was extracted from the seeds by soxhlet extraction process using petroleum ether (40-60□) and the average yield was 17.82%. In this study, we examined physico-chemical properties, fatty acid composition and heavy metals. The physico-chemical properties of seed oil are acid value (2.89mg/g), saponification value (211.63mg/g), iodine value (123.71mg/g), unsaponified matter (2.07%), ash content (3.1%), peroxide value (1.36mg/g). The pH (5.92) indicates that the oil contains little free fatty acid. The heavy metal analysis performed by MP-AES and results are in order- Zn (78.73) > Cu (66.21) > Pb (8.27) > Ni (4.52) > Cd (2.43).The high amount of linoleic acid (67.26%) shows that this seed oil is a rich source of omega-6 fatty acid.
Keywords: *Citrullus colocynthis, Heavy metals, Linoleic acid.*

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INTRODUCTION

The Cucurbitaceae family is classified as dicotyledonous and belongs to the Anthophyta division. This family has over 100 genera and nearly 880 species. This plant family has a broad range of genetic variety and adaptability in tropical and subtropical zones, as well as deserts [1]. Citrullus colocynthis is a nutrient-dense herbaceous plant that aids in the enhancement of health. It is a tiny perennial herbaceous creeping plant with angular, rough stems. Leaves of Citrullus colocynthis contain 5 to 7 lobes and are alternately oriented. Summer flowers are yellow, monoecious, solitary, pedunculated, and monoecious. Citrullus colocynthis produces 15-30 spherical fruits per branch, which are usually referred to as "bitter apples". The pulp of the fruits is soft and spongy white. When ripe, the seeds are tiny, silky, and brownish. The leaves and berries are used as cures in traditional medicine to treat diabetes, arterial hypertension, and cancer [2,3]. Seeds from the Cucurbitaceae family were shown to have higher amounts of powerful phytochemicals such as sterols, which have been linked to immunological regulation, reproductive health, and therapeutic effects for a variety of diseases [4].Roots can also be used to treat ascites, jaundice, urinary tract infections, and rheumatism.

Food, cosmetics, medicines, oleochemicals, and other sectors employ fats and oils, as well as their various lipid components. Essential and fixed oils are the two types of oils. Essential oils are volatile and normally obtained from the plant's non-seed portions, whereas fixed oils obtained from the seeds [5].Perfumes, flavours, deodorants, antiseptics, and medications are all made with essential oils, whereas fixed oils are typically edible due to their nutritional value [6].Cucurbitaceae family seeds were chosen for this study because they produce a large amount of oil when compared to other plants. They are edible oils with a wide range of uses in food, medicine, and industry [7].Seed oils of Cucurbitaceae family are often used in cooking and may also be utilized as a feedstock for biodiesel manufacturing and as a skin moisturiser [6].

It can also be used to treat fevers, liver problems, jaundice, constipation, hypertension, dropsy, rheumatic ailments, and as a fungicide [9].Fatty acids are essential in the human diet because they regulate cholesterol metabolism, which is linked to cardiovascular disease [10].Plant development is influenced by a variety of variables, one of which is pollution [11]. Environmental pollutants like industrial effluents, agricultural chemicals etc. are causing harmful effects [12].

Metal pollution is a huge problem in India, and it is just getting worse. Many recorded incidents of poisoning of metal in foundries, smelters,mining, coalpower plants and agriculture demonstrate this. Heavy metal is a non-biodegradable substance. As a result, they have a proclivity to accumulate in living

things. Metal toxicity has a significant influence on plants, and as a result, it has an impact on the ecosystem, in which plants and animals play an important role. Plants grown in metal-polluted environments have a different metabolism, less growth, less biomass output, and more metal accumulation. Metals have an impact on a variety of physiological and biochemical processes in plants and humans [13].

The elements in the periodic table with the higher density and atomic mass are known as heavy metals. These elements are found in low concentrations on Earth (individual concentrations seldom reach 1000 mg/kg), although they are present throughout the earth's crust [14].

Scientists used to find individual chemicals that were responsible for the possible risk of environmental toxins, but today the combined effects of chemical combinations are far more important.Its combined toxicity impact is analogous to synergetic (stronger) or antagonistic (weaker) behaviour. It is dependent on a number of factors, including the impact of specific exposures [15]. The majority of research point to a synergistic interaction between metal mixtures and insecticides [16].

Metal-bearing solids at contaminated sites can come from a variety of anthropogenic sources, including metal mine tailings, improperly protected landfills for high metal waste, leaded gasoline and lead-based paints, fertilizer application, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition [17-19].

Toxic metals are amplified physiologically as they go up the food chain. Due to their toxicity, heavy metals remain in the environment, pollute food systems, and create a variety of health concerns. Chronic environmental exposure to heavy metals poses a serious hazard to living beings [20]. The capacity of heavy metal pollutants in the soil to transit through the water system and their availability for biological absorption are also important factors. Industrial waste, sewage sludge, and automotive exhaust all provide a risk of heavy metal contamination of vegetation [21].

Metals in the food chain are known to cause health problems, even at low amounts, according to the World Health Organization (WHO) [22]. Studies have been carried out to assess the components transported from soil to plant [23]. Metal ions have been shown to interact with naturally produced chelating agents, making them more accessible to plants. Organic acids with a low molecular weight that are formed as a result of root exudates have been proven to be excellent metal chelating agents. Phyto chelators are plants that act as chelators[24].

The Physicochemical properties were based on acid value, saponification value, iodine value, peroxide value, specific gravity, and fatty acid content of the oil extracted by using HPLC. The quantity of free fatty acid (FFA) present in oil or fat is referred to as the acid value. The quantity of base in milligrams necessary to neutralize the free organic acid contained in 1g of fat or oil is known as the acid value of an oil or fat [25].

The quantity of alkali required to neutralize the free fatty acids produced by full hydrolysis of the sample in milligrams is known as the saponification value of oil or fat. It's a measurement of the fatty acid's average molecular weight (chain length). The amount of peroxide in the oil is measured by its peroxide value. A solution of oil in acetic acid and chloroform is titrated with a potassium iodide solution [26].

The ratio of the weight of the sample to the weight of distilled water is known as specific gravity or relative density. It is expressed as the weight of the sample divided by the weight of distilled water. It does not have a unit. The study's major goal is to extract oil from melon, determine its fatty acid content, and describe the oil by assessing chemical parameters including saponification value, peroxide value, and iodine value, as well as the specific gravity of the extracted oil.

MATERIAL AND METHODS

A. Materials and Oil extraction

Seeds were harvested when they were fully matured in the western Rajasthan arid zone (India). Harvested mature bitter apple fruits were washed manuallyand dried in sunlight for 4-5 days, after which the seeds were dried (100g sample) at 90°C for 7 hours in a hot air oven to a consistent weight. To avoid moisture loss, the seeds were kept in an airtight container at room temperature. The seeds were then mashed into a paste with a mortar and instantly evaluated.

Using the Soxhlet extraction procedure, oil was extracted from powdered Citrullus colocynthis seeds using mild petroleum ether (40-60^{o}C). Using a rotary evaporator, the solvent was entirely evaporated under a vacuum by standard AOCS methods. Oil methyl esters were created using the trans-esterification technique. To seek for any unusual fatty acids, the direct analytical TLC test, Halphen test, 2,4DNP TLC test, alkaline picrate test and picric-acid TLC test were utilised.

B. Reagents

All of the reagents used were of analytical quality. All dilutions were done using double deionized water.

The purity of HNO_3, H_2SO_4, H_2O_2, HF, $HClO_4$, and HCl was excellent. Prior to usage, by soaking plastic and glassware in dilute HNO_3 and then washing them with pure water, they were cleaned. Heavy metal calibration working standard solutions were created by diluting a 1000 g/L standard solution (Pb, Cd, Zn, Cu, and Ni).

C. Seed Oil Digestion

1 g of oil was mixed with 5 mL of strong nitric acid in a beaker. By placing the beaker on a hot plate, the substance was drained to near-dryness. 5 mL concentrated nitric acid was added after cooling, and the beaker was covered with a watch glass and returned to the hot plate. The heat was turned on till the digestion was complete. After adding 1-2 m pure nitric acid to the beaker, it was warmed to dissolve the residue. Thesample was filtered and adjusted to a volume of 100 ml with double distilled water.

RESULT

A. Physico-Chemical Analysis

The AOCS approach was used to determine the physico-chemical characteristics of Citrullus colocynthis seed oils, such as peroxide value, saponification value, iodine value, and acid value.

A number of factors are included in the physico-chemical analysis, including physical state, colour, taste, and the percentage of loss on drying using the standard method. Sulfuric acid vapours were extinguished by placing the crucible on a heated plate. In a muffle furnace, the crucible containing the sulfated ash was heated to 600°C until the weight of the materials remained unchanged. Technique determined ash content and ash value [Table-1].

Table 1: Physico-Chemical Value

S.No.	Characteristics	Value
1.	Oil %	17.82
2.	Iodine Value	123.71 mg/g
3.	Unsaponified matter	2.07%
4.	Saponification Value	211.63 mg/g
5.	Ash Content	3.1%
6.	Refractive Index	1.2147
7.	Peroxide Value	1.36 mg/g
8.	pH Value	5.92
9.	Acid Value	2.89 mg/g

B. Heavy Metals Analysis

Utilising nitrogen as the plasma source gas Cadmium (Cd), Zinc (Zn), Copper (Cu), Nickel (Ni), and Lead (Pb) were evaluated in plants employing microwave plasma atomic emission spectroscopy (MP-AES). A nitrogen flame was used to evaluate the metal concentration. It is used to determine both major and minor elements at the same time employing several analytes. By employing microwave radiation to produce a plasma discharge employing nitrogen from a gas cylinder or atmospheric air, MP-AES avoids the need for a gas supply.

Table 2: Heavy Metal in Citrullus colocynthis seed oil

S.No.	Metals	Concentration(mg/L)
1.	Zn	78.73
2.	Cu	66.21
3.	Pb	8.27
4.	Ni	4.52
5.	Cd	2.43

Lead is a metal with an atomic number of 82, an atomic mass of 207.2. This element has a melting point of 327.4°C and a density of 11.4 g cm^{-3} and a boiling point of 1725°C, that belongs to group IV and period 6 of the periodic table. It is a naturally occurring bluish-gray metal that is commonly found as a mineral in combination with other elements such as sulphur (i.e., PbS, $PbSO_4$) or oxygen ($PbCO_3$). Its concentration in the earth's crust ranges from 10 to 30 mg kg^{-1}[27]. The brain, neurological system, red blood cells, and kidneys are all vulnerable to lead poisoning [28]. Depending on the degree and duration of lead exposure, a wide range of biological consequences can occur. Lead has no known physiological function in the

human body, and it can only affect people if they consume it through food, air, or water. Because lead can accumulate in both individual organisms and entire food systems, it is a particularly dangerous chemical.

Transition metal zinc has the following properties: period 4, group IIB, electronic configuration 30, atomic mass 65.4, density 7.14 g cm^{-3}, melting point 419.5°C, and boiling point 906°C. Zinc is found naturally in soil (approximately 70 mg kg^{-1} in crystal rocks) [27], but human inputs have caused Zn concentrations to rise unnaturally.The alteration in the nucleus of the root tip cells is one of zinc's cytotoxic effects on plants[29].

Cadmium has an atomic number of 48, an atomic weight of 112.4, a density of 8.65 g cm^{-3}, a melting point of 320.9°C, and a boiling point of 765°C. It is found near the end of the second row of transition elements. Cd is one of the major three heavy metal toxins, along with Hg and Pb, and it has no recognised biological role [30].Cadmium has been shown to influence numerous enzymes in the body. The kidney damage that leads to proteinuria is thought to be caused by Cd interfering with enzymes involved in protein reabsorption in kidney tubules [29].

Nickel is a transition metal with an atomic weight of 58.69 and an atomic number of 28.Nickel is an element that only exists at extremely low levels in the environment and is necessary in tiny quantities, but it may be harmful if the maximum tolerated quantity is surpassed. This can result in many types of cancer in the bodies of animals, particularly those who dwell near refineries [31].

Copper metal with an atomic number of 29, a weight of 63.5, a density of 8.96 g cm^{-3}, and melting and boiling temperatures of 1083°C and 2595°C, respectively. It belongs to period 4 and group IB of the periodic chart. In crystal rocks, the metal's average density and concentrations are 8.1 103 kgm^{-3} and 55 mg kg^{-1}, respectively [27].Cu is a necessary micronutrient, but in excess, it can be considered a poisonous element, inhibiting development [32].

C. Preparation of mixed fatty acid

Hydrolysis of oil and fats yielded the fatty acid combination. 2g of oil sample was saponified using 2-3 ml of 1N standard NaOH alcoholic solution and 10 ml alcohol as a solvent in a round bottom flask, and then heated gently for 1-2 hours while TLC was observed. Both saponified and unsaponified materials were present in the final combination. 30 ml double distilled water was added to the mixture to dilute it further. The saponified matter was extracted using a separating funnel and repeated washings with diethyl ether. The unsaponified materials in the top organic (ether) layer were collected in a separate beaker. Diethyl ether is recovered after evaporation with a rotatory evaporator. Dilute hydrochloric acid was used to acidify the lower aqueous solution, which included fatty acid salts (HCl-6N).Fatty acids were extracted from this combination by repeatedly washing it with diethyl ether, with the bottom aqueous layer discarded and the top mixed ether extract containing a fatty acid mixture collected in aflask that has been dried in the oven.

The MFA'S were rinsed with double distilled water and then dried over Na_2SO_4 after the excess ether was removed. MFA'S were found to be clean and pure.TLC was used to monitor the entire operation. HPLC was used to examine the methyl esters that were produced. TLC plates were made by covering a glass plate with approximately.025 mm of layer silica gel. In an iodine chamber, a mobile phase consisting of a combination of petroleum ether, diethyl ether, and acetic acid (70:29:1) was employed to make the spot visible.

The fatty acid mixture was subsequently derivatized in esters and quantified by HPLC and GC-MS for quantitative analysis. MFA's were re-extracted in a round bottom flask with extra methanol to produce fatty acid methyl ester (FAME) (1:6) over a pan of water (100°C) for roughly 1-2 hours applying 1 percent H_2SO_4 as a catalyst. After full trans-esterification (as monitored by a TLC plate), the assembly was removed and the flask was chilled at room temperature to drain excess solvent and cooled over an ice bath before adding 30 ml double distilled water. To eliminate the stirred wall and fatty acid methyl ester, diethyl ether was utilised (FAME). The mixed ether obtained in the top layer was deposited in a dry flask, and the lower aqueous layer was removed. Anhydrous FAME was produced when the solvent was evaporated and dried on Na_2SO_4, which was collected and kept at low temperature for subsequent analysis.

D. Fatty Acid Methyl Ester Separation on HPLC

For HPLC analysis, methyl esters derivatives are very useful. A modified approach was used by the researchers (with gradient elution). A binary Ultimate 3000 RS pump wavelength UV detector, and a column (900×6.4mm) loaded with µBondapack C-18 and rinsed with acetonitrile-water in the ratios of 67:33 (by volume) at initially, gradually shifting to 74:26 were all included in the Dionex HPLC with a degasser.

Table 3: *Citrullus colocynthis* seed oil fatty acid composition

S.No.	Fatty Acid	Composition %
1.	Palmitic	9.07
2.	Linolenic	0.16
3.	Linoleic	67.26
4.	Palmitoleic	0.05
5.	Erucic	0.19
6.	Oleic	12.17
7.	Arachidic	0.11
8.	Stearic	8.58

DISCUSSION

Low acidity and a pH of 5.92 indicate that the oil contains little free fatty acid (FFA). The acid value of oil is a measurement of the FFA concentration.The less acid value of the oil, the fewer fatty acids it contains, the less susceptible it is to rancidity. This means the oil will not get rancid as easily, and the low acid value also indicates that the oil is edible. The acid value is a crucial metric for determining the age, edibility, and appropriateness of oil for industrial usage.In terms of fat and oil categorization, the iodine value is a measure of unsaturation and an indicator of double bonds in the molecular structure. This bond indicates that the oil is cholesterol-free and can protect the heart. Peroxide value is connected to the oil's oxidation level as a result of hydroperoxide generation at double bond locations.The oil's low peroxide value suggests that it is resistant to oxidative rancidity and degeneration. As a result, one of the most important factors influencing lipid oxidation is double bond unsaturation in oils.

Heavy metals examination of the seed oil reveals a significant concentration of Cu and Zn, with negligible amounts of Pb, Cd and Ni. This provided a phytoremedy for Cu and Zn in the areas where it is produced. A routine analysis was carried out to determine the level of environmental degradation. To aid in the knowledge of metal cycling in the environment, a database bank should be created to investigate metal pollution.

Bitter apple seed oil was found to be mostly composed of unsaturated fatty acids, which are the primary fatty acid class which are the high content of linoleic acid C18:2 (67.26%) in C. colocynthis seed oil is followed by oleic acid C18:1 (12.17 %). The second group consisted of saturated fatty acids which are minor components palmitic acid C16:0 and stearic acid C18:0 were identified in quantities of 9.07% and 8.58%, respectively. Citrullus colocynthis oil is unsaturated (80% unsaturated fatty acids) and a linoleic acid source (omega-6).Linoleic acid is the most significant PUFA in the human diet since it reduces the risk of heart and vascular disease.

CONCLUSION

Based on the findings of this study, it can be concluded that Citrullus colocynthis seeds from the dry zone of western Rajasthan possess a high percentage of linoleic acid (67.26%). Linoleic acid is the most significant PUFA in the human diet since it reduces the risk of heart and vascular disease. Its seed oil is beneficial to human health. Zinc, copper, lead, cadmium, and nickel are abundant in *C. colocynthis* seeds. Copper is an essential nutrient. Nickel and Cadmium are present in very small amount, however they can be dangerous if the maximum tolerable amount is exceeded. The study was a success since it demonstrated that this seed oil is not as fatty as people believe and that it also has an unsaturated bond that protects the heart and is not harmful to health. Some of the physicochemical qualities of the oil revealed its use, such as its high saponification value and low acid value, which made it suitable for soap manufacture and cooking. Because of the therapeutic characteristics of the fatty acids found in this seed oil, the findings of the study advise individuals to continue consuming it.Many people have claimed that this seed oil promotes excessive cholesterol in the body, but scientific study has proven them false. Colocynthis seed oil was found to have certain beneficial fatty acids that have been believed to have therapeutic effects.

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