Determination of Heavy Metals and Physic1ochemical Characteristics in *Citrus limetta* Seed Oil from Jhunjhunu, Rajasthan INDIA

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ABSTRACT

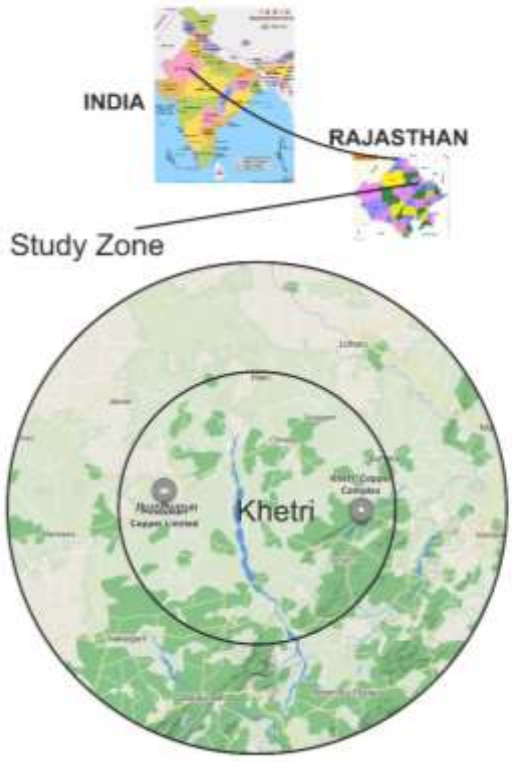
The seed oil of *Citrus limetta* cultivated in an around the particular area where largest copper mine of our country is situated in khetri, Jhunjhunu Rajasthan India is having various heavy metal and physicochemical characteristics not matching or differing from those seed oil of plant which are cultivated in fresh and natural ambiance. Seed samples of *Citrus limetta* cultivated in industrial waste nearby areas of khetri district Jhunjhunu were collected and oil extraction using soxhlet extraction process petroleum ether (40-60°C) used as solvent. The *Citrus limetta* seed and its oil were studied for the presence of heavy metals, especially metals like copper, zinc, magnesium, iron, chromium, lead, nickel and cadmium, which play an important role in the breakdown of carbohydrates, fats and proteins into digestible forms and convert into energy. Heavy metals analysis of the seed oil of *Citrus limetta* is performed by Atomic Absorbance Spectroscopy (AAS). The heavy metal found in seed oil of *Citrus limetta* are in the order– Fe (18.32 mg L-1) > Zn (8.20 mg L-1) > Al (6.28 mg L-1) > Cr (2.30 mg L-1) > Cu (1.59 mg L-1) > Mn (1.25 mg L-1) > Ni (0.92 mg L-1) > Pb (0.39 mg L-1). By HPLC analysis, Linoleic acid (Omega-6) (34.20%), Oleic acid (Omega-9) (27.10%) and Palmitic acid (26.30%) were major fatty acids found in *Citrus limetta* seed oil. There are also other acids found in small amount such as Stearic acid (4.70%), Linolenic acid (3.20%) and Palmitoleic acid (0.30%). The various physicochemical characteristics of *Citrus limetta* are as follows- Acid value (A.V.), Iodine value (I.V.), and Saponification value (S.P.) were 1.302 mg/g KOH, 103.09 g I_2/100g, 189.22 mg/g KOH respectively.

Key words: *Citrus limetta*, HPLC, Omega-6

INTRODUCTION

Citrus limetta is a member of the sweet lemons and belongs to the Rutaceae family. It is one of the most important fruit crops grown widespread throughout Rajasthan. It is also known as musami/mosambi, sweet lime, sweet limetta, and sweet lemon. Citrus limes are aromatic, juicy, and have low creating a palatable, mild, and sweet flavor. These are generally available in the late fall through winter. The most of the citrus fruits are available throughout the year, their plants are in the form of either bushes with average height 3-5 metre length. [1] Citrus species are grown for the juice of their fruits. In large-scale the sweet lime is mainly used to extract juice and the other materials like peels, pulps, and seeds are taken as a highly valuable by-product. [2] Citrus fruits are of high-financial worth in light of their different uses, for example, in the food industry, beauty care products, and traditional health care products. Most of the citrus seeds are known to be waste product from agro-industry, but these are main resource of oil. [3–6] Sweet lime has many powerful antioxidants which keep our metabolism high like Vitamin C, a nutrient that strengthens the immune system and keeps our skin smooth and elastic. These are also a good source of dietary iron and soluble which helps many health benefits lower cholesterol and lowering blood pressure, Prevents Gum and Teeth Diseases, Improve Immune System, is Good in Pregnancy, Good for Eyes, Skin, and Hair, Maintains

Sugar Levels, Useful for Respiratory Health our body hydrated and fresh and including improving digestive health and aids weight control, also having a property to purify blood and digestive system.



Map showing the study zones

Eating citrus regular things might assist with chopping down the danger of kidney stones in explicit individuals by raising citrate levels in urine. *Citrus limetta* may help boost brain function and protect the brain from neurodegenerative issues. *Citrus limetta* acts as a perfect detoxifying expert by flushing out toxic substances and neutralizing the perilous effects of strain and pollution. Sweet limetta in fact contains less acid than other limes and is thus praised for its mild and palatable flavour. It's fairly difficult to eat sweet lime as is a result of its overabundant seeds and toughness. In any case, sweet lime juice, on the other hand, is downright divine. There are more benefits of chewing mosambi seeds. It is very uncommonly known that musami seeds help to support our body and make it dynamic. It also helps with easing stomach issues, builds up the slight stomach related structure and paces up the emanation of stomach related juices. Due to its antioxidant properties, it thwarts the general deficiency and laziness in our body. sweet lime helps with various stomach related issues, fortifies hunger, fights scurvy, fortifies hunger, stays aware of electrolyte balance other than providing skin and hair stream from within. It also helps in averting the risk of cancer, boosts immunity. Anyway take up commonly as juice, sweet lime is certainly not an outcast fixing in baking and is used generally in present day cooking styles. In any case, take it in moderate amounts to avoid stomach related issues like nausea and vomiting. [7,8]

Linoleic acid has been shown to have critical action in inhibiting mammary carcinogenesis. [9] Oleic acid is one of the main constituent of olive oil which is under the category of mono-unsaturated fatty acids which may be responsible to increase the inhibitory growth impact on breast cancer cell lines SK-BR3 and BT-474. [10]

Heavy metals like Cu, Ni, Cd, Cr, Co, and Zn are phytotoxic either at high concentrations levels. Heavy metals are organically amplified through the food chain. Heavy metals are influencing the climate. When the level of heavy metals is within the limit, then it is helpful to micronutrients for plants, humans, and animals. It becomes harmful when their concentration level exceeds a limit. When iron exceeds the limit damages cells in the heart and liver which can cause liver failure, cancer, circulatory shock, coma, etc. [11-14]

As large number of agro industry dedicatedly use large amount of citrus fruits are responsible for a bulk of industrial waste, on yearly basis which can be taken into use for the production of *Citrus limetta* oil. It requires a detailed study for its synthesis and characterisation as an advancement in these directions. Our prime object is to start a specific study on finding out physicochemical properties of these seeds and seed oil specifically growing in khetri, Jhunjhunu Rajasthan India.

MATERIALS AND METHODS

Collection of Samples:

Seeds of *Citrus limetta* were collected from khetri, Jhunjhunu of Rajasthan, India. During the sample preparation process washed the *Citrus limetta* seeds were with water and oven-dried at 35◦C, until a constant weight was obtained. After the drying process, the seeds were stored in an airtight container at room temperature to prevent moisturization. The dried seeds were milled into fine particles through a grinder.

Oil Extraction:

Citrus limetta seed oil was extracted by continuous extraction in soxhlet apparatus with petroleum ether (40-60◦C) for six hours. Petroleum ether (40-60◦C) is used as solvent. [15] Complete the extraction and evaporate the solvent on water bath & remove the trace of the residual of solvent. The obtained oil was collected in vial and stored in cool place for further analysis. [16] The extracted oil was evaluated by standard methods. The analytical values of Seed oil and seeds were determined by standard American Oil Chemist Society (AOCS) methods. [17] Methyl esters of seed oil were prepared by using direct analytical TLC technique [18], 2,4-DNP TLC test [19], Halphen test [20], picric-acid TLC test [21], and alkaline picrate test [22] were also performed for determination of any unusual fatty acid present in oil.



Reagents and Chemicals:

All the Reagents and Chemicals Were Analytical grade which used. Double deionized water was used for all dilutions. Acids used in the present study were obtained from different sources. All acids like

HCl, H_2SO_4, H_2O_2, HF, $HClO_4$, and HNO_3 were of superior quality. All the glassware was washed by soaking in dilute Nitric Acid and then rinsed with distilled water earlier to use. The working standard solution of heavy metals used for calibration was processed by diluting a stock solution of 1000 µg/L (Pb, Cd, Zn, Fe, and Ni).

Mineral Metal Analysis:

The Atomic Absorption Spectroscopy technique is one of the simple and very selective methods for determination of heavy metals on their environmental concentrations. [23]

Preparation of Standard for Metal:

In Atomic Absorption spectrophotometric very minor concentration of the metal to be analyzed and Standard Solution of relevant metals contains a very small concentration of the metals.

Stock Solution Preparation:

Take 1gm of metal like cadmium (Cd), nickel (Ni), iron (Fe), lead (Pb), and zinc (Zn) and dissolve in a minimum quantity of aquaregia (1:3) HCl and HNO3, makeup to 1liter in volumetric flask by adding double deionized water. The stock solution contains about 1000µg L-1 of required metal and then the working standard solution is prepared by dilution of the stock solution. The Calibration curves were drawn by using the working standard of 0-40 µg L-1 metal ions.

Digestion of Seed Oil:

Citrus limetta seed oil was digested in a 100 mL Pyrex glass beaker. We take 1g of seed oil in a 100 ml Beaker and add 10 mL Concentrated Nitric acid. Keep in place for 24 hours for cold digestion after 24 hours heat the mixture at 50◦C for at least 4 hours then the solution was boiled with (1:5) concentrate HCl and HNO3 for digestion of all organic matter and after digestion cool, the mixture then filters and makeup to it 25 mL by using double distilled water.

Fatty acid Analysis:

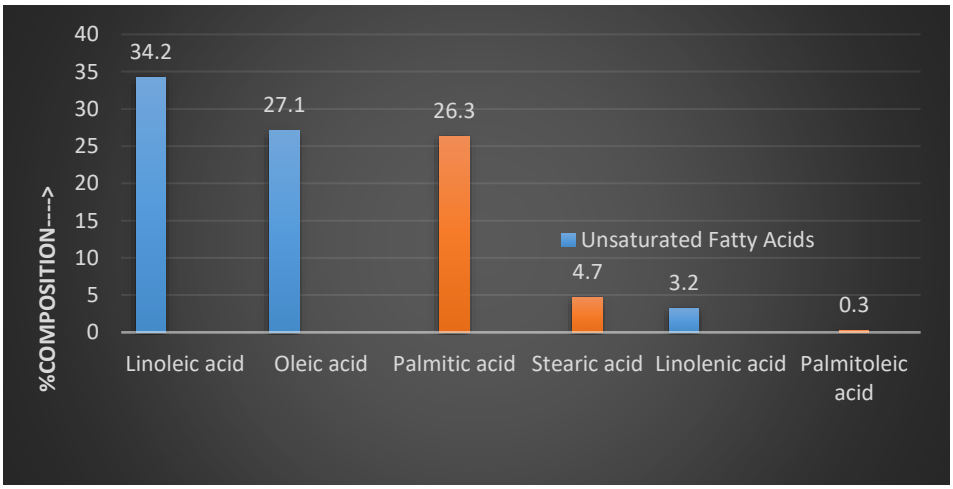
The fatty acids composition of *Citrus limetta* seed oil was determined by High-Performance Liquid Chromatography (HPLC). First, we hydrolyze the oil and mixed fatty acids were obtained after those mixtures of fatty acids were further derivative to their methyl esters, and the formation of methyl esters was confirmed by thin-layer chromatography (TLC) and the Methyl ester of Fatty acids was analyzed by High-Performance Liquid Chromatography (HPLC). [24]

RESULTS AND DISCUSSION

The Fatty acid composition of seed oils of *Citrus limetta* khetri, Jhunjhunu of Rajasthan are shown in table 1. Fatty acids are the primary component of oils and fats. The Mono-unsaturated fatty acids were identified as Linoleic acid (Omega-6) (34.30%), Oleic acid (Omega-9) (27.10%), Palmitic acid (26.30%), Stearic acid (4.70%), Linolenic acid (3.20%) and Palmitoleic acid (0.30%). The major Poly-unsaturated fatty acid was Linoleic acid (34.30%) present. Results show that seeds oil from *Citrus limetta* was found to be a rich source of polyunsaturated fatty acids, especially linoleic, Oleic, and Palmitic acids. *Citrus limetta* was the only species in the Rutaceae family characterized by the presence of (Omega-6) and (Omega-9) acids in seed oil. Table 4 shows the mineral content of the seed oil of *Citrus limetta*. Iron (18.32 mg L-1) is the major heavy metal present in *Citrus limetta* seed oil.

Table 1: Fatty acid composition in *Citrus limetta* seeds.

Fatty Acid	Obtained % by weight
Linoleic acid (Omega – 6)	34.30
Oleic acid (Omega-9)	27.10
Palmitic acid	26.30
Stearic acid	4.70
Linolenic acid	3.20
Palmitoleic acid	0.30



Plot showing comparison between Unsaturated and Saturated Fatty Acids

Physico – chemical properties:

The chemical properties of seed oil were obtained using the methods described by AOCS are given below in table 1.

Table 2: Analytical and Physicochemical characteristics of the seeds and oils

Seed Properties		Oil Properties	
Moisture content	58	Refractive Index	1.4659
Oil Content (% by w)	22.80	Acid value (mg/g KOH)	1.302
		Iodine value (g I_2/100g)	103.09
Protein Content (% by w)	5.86	**Saponification value (mg/g KOH)**	**189.22**
		Un-saponified matter (% w/w)	0.4

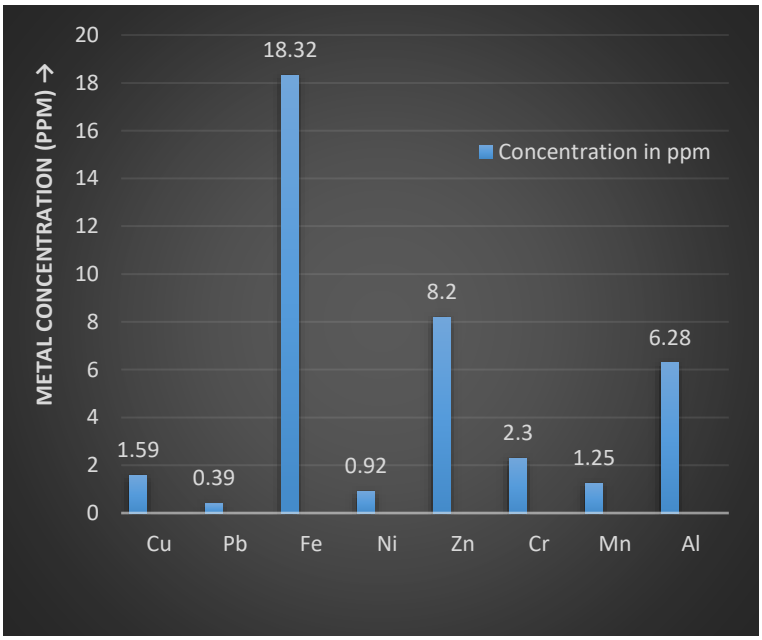
Table 3: AAS data for elements

S.No.	Element	Wave length of main resonance line λ (nm)	Type Flame*
1	Fe	243.3	AA
2	Cd	229.3	AA
3	Ni	232.0	AA
4	Zn	283.8	AA
5	Pb	214.1	AA

*AA Air Acetylene mixture

Table 4: Concentration of heavy metals analyte in seed oil of *Citrus limetta*

Metal	Concentration inmg/L or ppm
Cu	1.59
Pb	0.39
Fe	18.32
Ni	0.92
Zn	8.20
Cr	2.30
Mn	1.25
Al	6.28



APPLICATION

The present study of trace metal concentrations suggests that the concentrations of Cr, Zn, Ni, Cu, and Cd are likely from the Earth's crust while Fe is from multisource origin. The concentration of trace elements is acceptable for human health. It is also useful for industrial applications.

CONCLUSION

The present study has generated data on heavy metals in *Citrus limetta* seed oil, which are cultivated in and around the surrounding villages of the industrial waste nearby areas of khetri district Jhunjhunu, Rajasthan. The levels observed are comparable with the literature reported values from various regions of the globe. The *Citrus limetta* seed oil is a good source of essential Omega-6 and Omega-9 fatty acids, which are good for human health and useful for industries. *Citrus limetta* seeds generally discarded as an agro-industrial waste could emerge as a valuable commodity for production of useful oil and other value-added products. Seed oil is a good source of nutrients, therefore by-

products of oilseeds and parts of plants could be used as animal feedstock and as biomass in various applications.

The concentrations of Fe > Zn > Al > Cr > Cu > Mn > Ni, and Pb are measured for the estimation of pollution by heavy metals. Analysis of heavy metal concentrations in *Citrus limetta* seed oil indicates that, the contamination of Al, Fe, Ni, and Cu is likely from the earth's crust, whereas Mn, Pb, Cr, and Zn, is from multisource origin.

The physicochemical characteristics and fatty acids analysis of seed oil of *Citrus limetta* shows excellent source of Vitamin C, Fiber, Carbohydrates, Proteins. It also boasts powerful anti-inflammatory and antioxidants. Results showed that Linoleic acid (34.2%) and Oleic acid (27.1%) were the major polyunsaturated fatty acids. The results of the study showed that *Citrus limetta* seeds oil have the potential of being used as the source of edible oil and many industrial applications. *Citrus limetta* seeds may actually accelerate the energy level in our body.

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