ORIGINAL ARTICLE

Momordica charantia: A Potential Source of Eleostearic Acid from Western Rajasthan, India

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ABSTRACT *Momordica charantia* or bitter melon or bitter cucumber and by countless other names in other languages, is an extremely bitter fruit of the cucurbitaceae family. It is mainly distributed around the subtropical and tropical regions of the world. It has anti-diabetic properties also anti-inflammation and has cholesterol lowering effects. Momordica charantia seed oil is contains 9-cis, 11-trans, 13-trans-conjugated linoleic acid (9c, 11t, 13t-CLN) due to this characteristic this particular seed oil comes under a category of specific oil. The extracted oil mainly composed of poly-unsaturated fatty acid with high percentage of α-eleostearic acid (52.82%), Stearic acid (25.59%), Oleic acid (9.80%) and Linoleic acid (5.40%) the following percentage value were obtained by HPLC analysis. The various value which characteristics *Momordica charantia* are as follows-Acid value (A.V.), Iodine value (I.V.) and Saponification value (S.P.) were 1.852 mg/g KOH, 128.02 g, I_2/100 g, 184.02 mg/g KOH respectively. Out of various acids show obtain we observed that α-eleostearic acid has a tendency to subside the growth of melanin and fibroid cells. The dihydroxy derivate and α-eleostearic acid are major inducer of apoptosis in HL60 cells. (HL-60 cell line is an immortal human cell line from blood lymphocytes of a patient having acute leukemia). Thus α-eleostearic acid has anti-cancer, anti-diabetic, anti-inflammatory activities, inhibit tumor cell proliferation and prevents cardiovascular diseases. The accumulation of metals in seed oil of *Momordica charantia* were mainly Ca, Mg, V, Cr, Mn, Co, Cu, Zn and Mo as well as toxic like As, Cd, Hg and Pb present in trace amount. Samples of *Momordica charantia* seed oil cultivated around the western Rajasthan were collected and on digestion of oil for heavy metals. The following heavy metals were obtained concentration in ppm 6.54 for Cu, 108.32 for Fe, 8.98 for Ni, 0.79 for Pb and 18.20 for Zn by using AAS (Atomic Absorption Spectrometer).

Keywords: Digital technology, Digital nutrition platforms, Artificial Intelligence, Cloud based digital health solution, Hand-held device users, Personalized nutrition

Address for correspondence: *Momordica charantia*, α-eleostearic acid, Cucurbitaceae chemical components, Dihydroxy derivatives, Heavy metals, Trace elements, AAS

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INTRODUCTION

Cucurbits are among the most important plant families supplying humans with edible products and useful fibers. India is home to more than 8,000 species of medicinal plant from cucurbitaceae or gourd family consist of 98 genera and about 975 species of food and ornamental plants.[1]

Momordica charantia plant belonging to cucurbitaceae family having both pharmacological as well as nutritional properties.[2] The plant is climbing lifelong along with its fruits which resembles a cucumber or gourd. It is a fast growing vine and grows best in wet areas having optimum temperature between 12-24 °C. It prefers well-drained sandy loamy soil with high organic matter, pH [6.0-7.0] and water-retaining capacity. Although it cannot tolerate draught and water stress. It has fuzzy stems clothed with dark green, deeply lobed leaves and has yellow dioecious flowers.[3]

It varies from region to region depending on size, colour, presence or absence of ridges and bitterness of fruit bitter gourd fruit has medicinal value and are widely used for curing diabetes asthma, blood diseases and rheumatism. The plants

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are also used as laxative, contraceptive to treat various conditions such as scabies jaundice, leprosy, etc. It also possesses strong anti-cancer as well as strong cytotoxicity in various human cancer cell lines furthermore, Bitter melon has been used for people with sluggish digestion, dyspepsia and constipation. Bitter melon rarely does have negative effects.[4]

Bitter gourd seed oil Contains α-linolenic acid in its various isomer form position and geometric, which is found in required amt. of α-eleostearic acid which is under class of congugated fatty acid.

It's a long chain with conjugated double bonds [3, 4, 8]. The chemical and physiological properties of CLnA (conjugated α-linolenic acid) have several health benefits including anti-oxidant, anti-tumor, anti-atherosclerotic activities.[5] High content of α-eleostearic acid and phytosterols are present in bitter gourd seed oil from cultivated around western Rajasthan with numerous health beneficial properties. Seed oil of *Momordica charantia* is good drying oil for paint for making wrinkle varnish and for coating industry.[6]

The present study analysis the composition and the physical properties of the seed oil of cultivated around western Rajasthan and their use as a drying oil for manufacturing alkyl resins.

SEED MATERIAL

The seeds of bitter gourd set in location that gets 7-8 hours of sunlight everyday with temp. of soil from 16-20. The seeds were germinating in 9-10 days.

Bitter gourd will take almost 55-60 days to mature. The soil must rich in organic matter with pH (6.0-7.0) with sandy or loamy soil.

The seeds are soaked in water overnight before sowing for better germination. The seeds are also cleaned to remove the mucilaginous materials. The weight of cleaned seeds is approximately 150 g.

OIL EXTRACTION

The present studies involve extraction of oil from *Momordica charantia* seeds using Soxhlet apparatus (method of extraction

Figure 1: Showing the Study Zones

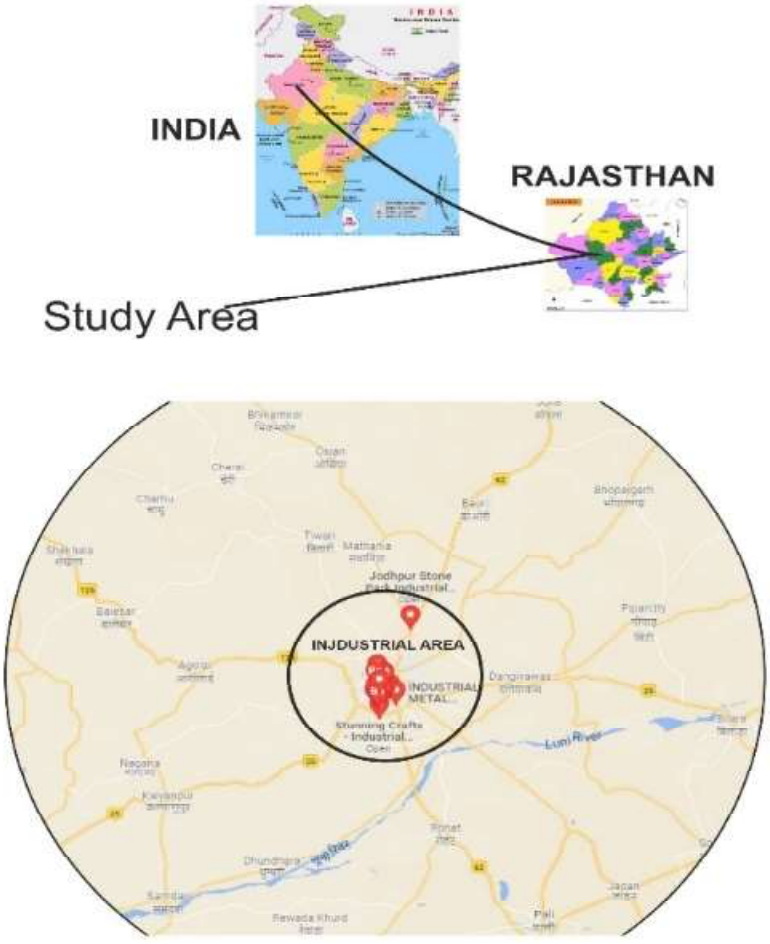


Figure 2: ***Momordica charantia***

Figure 3: Seeds of ***Momordica charantia***

of lipid from solid material). The cleaned seeds are dried in the oven at 104-108 for 6 hours and were grounded by using electric grinder and oil was separated from the crushed seeds by Soxhlet extraction with petroleum ether (60-80) in a Soxhlet apparatus for 6 hrs. The obtained oil was kept away in cool place until further investigation.

REAGENTS

All reagents were of A.R. grade and double deionized water was utilized for all dilutions like as H_2SO_4, HF, HNO_3, H_2O_2, HCl and $HClO_4$. All the glassware and plastic were cleaned by soaking in dilute HNO_3 and were washed with distilled water before using them. The working standard solution of heavy metals used for calibration were processed by diluting a stock solution of 1000 μg/L (Pb, Cd, Zn, Fe, and Ni).

MINERAL METAL ANALYSIS

Atomic Absorption Spectroscopy is one of the method for measurement of the total contents and to analyse evolution of the environmental concentrations of heavy metals.[7] This is a basic and exceptionally particular method in nature. Using this method, we present the measurement of heavy metals found in seed oil of *Momordica charantia*.[8]

PREPARATION OF STANDARD SOLUTION FOR METAL

In spectrophotometric determination with respect to the solution having trace concentration of the metal to be determined. It follows that trace concentration of the compatible metal is compulsorily required along with standard

solution which will be required for scanning. Standards are made by dissolving 1gm of metal Cadmium (Cd), nickel (Ni), iron (Fe), lead (Pb) and zinc (Zn) dissolve in little 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 1000 µg/L of compatible metal and after that, by appropriate dilution of stock solution, working standard solution is made. The calibration curves for metal ions were graphed by taking working standard of 0-40 µg/L as required for the calculations.

DIGESTION OF SEED OIL

100 ml Pyrex glass beaker was used to process *Momordica charantia* seed oil. For this, 1g of seed oil was added with 10 mL Conc. HNO_3 which was first kept for cold assimilation for a period of 24 hours and then heated at 50 for 4 hours. To process all the organic matter[9] the solution was then boiled with 1:5 ratio of conc. acids HCl and HNO_3, and then was separated after it cooled down. At last, volume of the extract was made up to 25 mL by using double distilled water.[10] From the calibration curves for these standard metal ions, concentration of metals in seed oil sample was determined.

PREPARATION OF FATTY ACID METHYL ESTER

The preparation of fatty acids methyl ester of *Momordica charantia* oil was processed mainly in two steps:

1. Initially, mixed fatty acids were obtained by performing hydrolysis of oil.
2. Secondly, this mixture of fatty acids was further derivatives to their methyl esters. The formation of methyl esters was confirmed by Thin Layer Chromatography. Thus obtained methyl esters were then analyzed by HPLC (High Performance Liquid Chromatography).

RESULTS AND DISCUSSION

Fatty Acids Analysis

The bitter gourd seed oil was obtained by the Soxhlet extraction method and prepared fatty acid methyl ester. Its acid composition was identified using HPLC (High-Performance Liquid Chromatography) with the use of a C18 column.[11, 12] The acid composition identified such as α-eleostearic acid (C18:3, 9c, 11t, 13t-CLnA), Stearic acid (C18:0) (25.59%); Oleic acid (C18:1) (9.80%); Linoleic acid (C18:2) (5.40%); Palmitic acid (C16:0) (3.02%); Arachidic acid (C20:0) (0.69%); Gadoleic acid (C20:1) (0.64%) respectively. Composition of fatty acid and its seven other fatty acids were identified. α-Eleostearic acid (52.82%) was determined as the major fatty acid among them (Table 2).

Physico-Chemical Properties

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

The Soxhlet extraction method is high yield and rapid process and its depend on various factors such as particle size, extraction time (number of cycles). The oil content of seed given as a percentage to the dry weight of the kernel is found to be 45%. Also the refractive index constant at 1.4661 for oil

Figure 4: Soxhlet Extraction Method

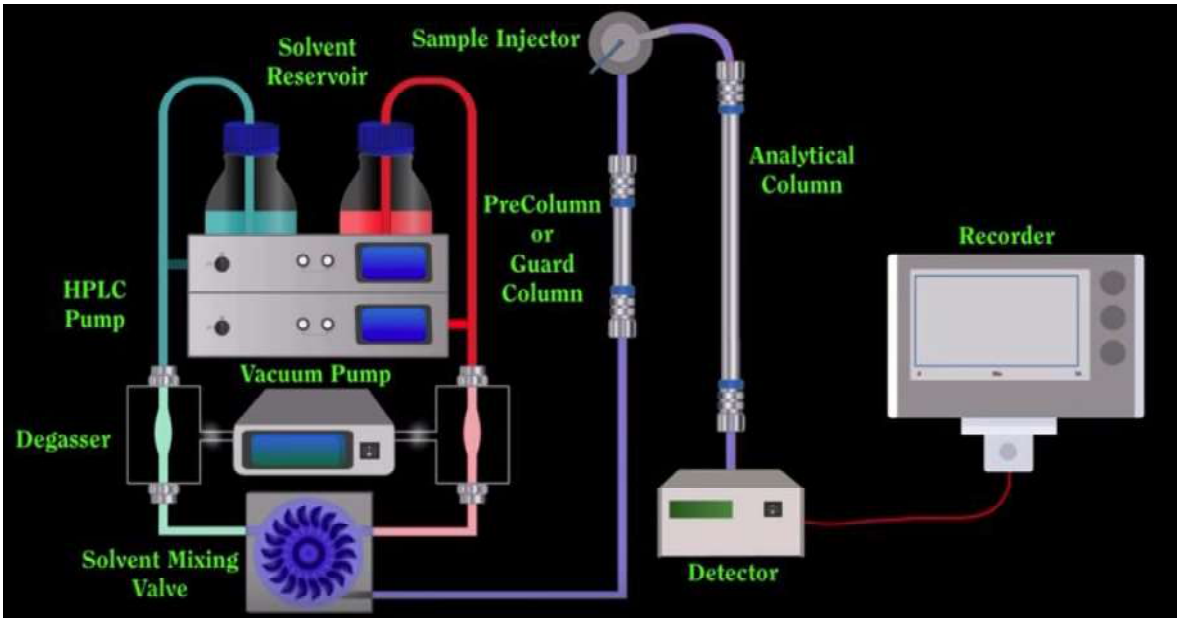


Table 1: Analytical and Physicochemical Characteristics of the Seeds and Oils

Seed Properties		Oil Properties	
Moisture content	53	Refractive Index	1.4661
Oil Content (% by w)	24.3	Acid value (mg/g KOH)	1.852
		Iodine value (g I_2/100 g)	128.02
Protein Content (% by w)	21.86	Saponification value (mg/g KOH)	184.02
		Un- saponifiable matter (% w/w)	1.16
Kernel to seed dry basis (% w/w)	45	Specific Gravity	0.93
Colour	Reddish brown		

Table 2: Fatty Acid Content in Plant Seeds

Fatty Acid		Obtained % by Weight
Octadecatrienoic acid (α-eleostearic acid)	(C18:3)	52.82
Octadecanoic acid(Stearic acid)	(C18:0)	25.59
Octadec-9-enoic acid (Oleic acid)	(C18:1)	9.8
Omega-6 (Linoleic acid)	(C18:2)	5.4
Hexadecanoic acid (Palmitic acid)	(C16:0)	3.02
Arachidic acid	(C20:0)	0.69
Gadoleic acid	(C20:1)	0.64
Unidentified minor acid		2.04

Figure 5: Plot Showing Comparison Between Unsaturated and Saturated Fatty Acids

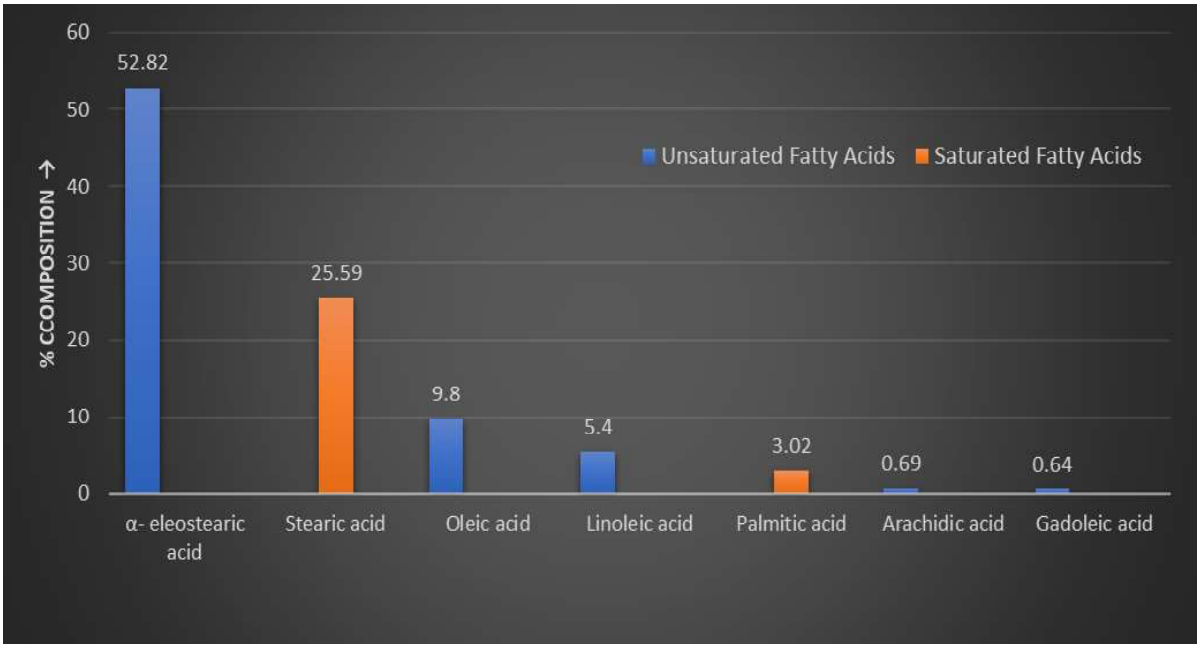


Table 3: AAS Data for Elements

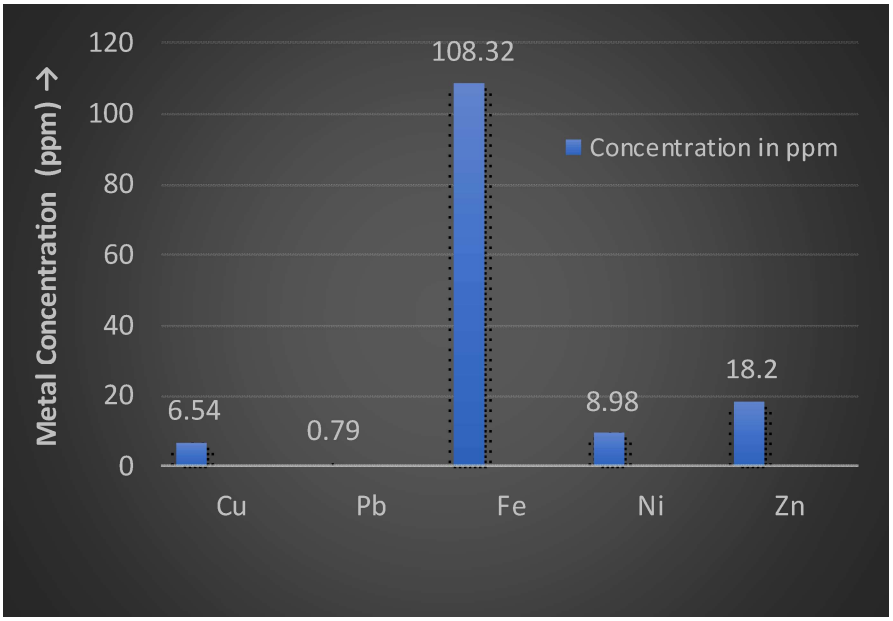
S. No.	Element	Wave Length of Main Resonance Line λ (nm)	Type Flame*
1	Cd	229.3	AA
2	Fe	243.3	AA
3	Ni	232	AA
4	Pb	283.8	AA
5	Zn	214.1	AA

Note: *AA Air Acetylene mixture.

Table 4: Concentration of Heavy Metals Detected in Seed Oil of Bitter Gourd Plant

Metal	Concentration in mg/L or ppm
Cu	6.54
Pb	0.79
Fe	108.32
Ni	8.98
Zn	18.2

Figure 6: Plot Showing Comparison Between Metals Concentration in ppm



sample. Refractive index is an important optical parameter to analyze the light rays transversing through materials medium.[13] The refractive index is useful in detecting the adulteration of oil that contains conjugated double bounds.[14]

The iodine value of bitter gourd seed oil was obtained 128.02 and thus the lower value than 140.10 for the same oil reported. The iodine value indicates high degree of unsaturation the saponification value of seed oil is 184.02 indicate high molecular mass of fatty acid. The lower amount of un-saponifiable matter (1.16%) indicate lower amount of hydrocarbon.[15]

Apparatus

The study of analysis of heavy metal measurement (Cd, Pb, Zn, Fe and Ni) was carried out in an air/acetylene flame using AAS. The operating condition as well instrumental parameters are given below Table 3.

In this experiment the instrument designed for flame absorption. The absorbance is directly related to concentration, in AAS method. It has an integrated aspirator burner having electric ignition, the burner fed with air and acetylene. For this, the hollow cathode is selected under analysis and operated at 50% of its current rating. The grinding monochromator is utilized between the fire and the detector. The wavelength of radiation permitted to go to the detector is chosen by selecting the component which consequently changes the monochromator. Five changes were basically made to set the monochromator at top outflow.

Signal strength change is attained by changing the flame position vertically with respect to the AA optical path by regulating the fuel oxidizer flow rates to the burner, and electronic adjustment on the control panel.

Concentration of heavy metals in the analyzed samples are given in Table 4.

Figure 7: Calibration Curve for Zn

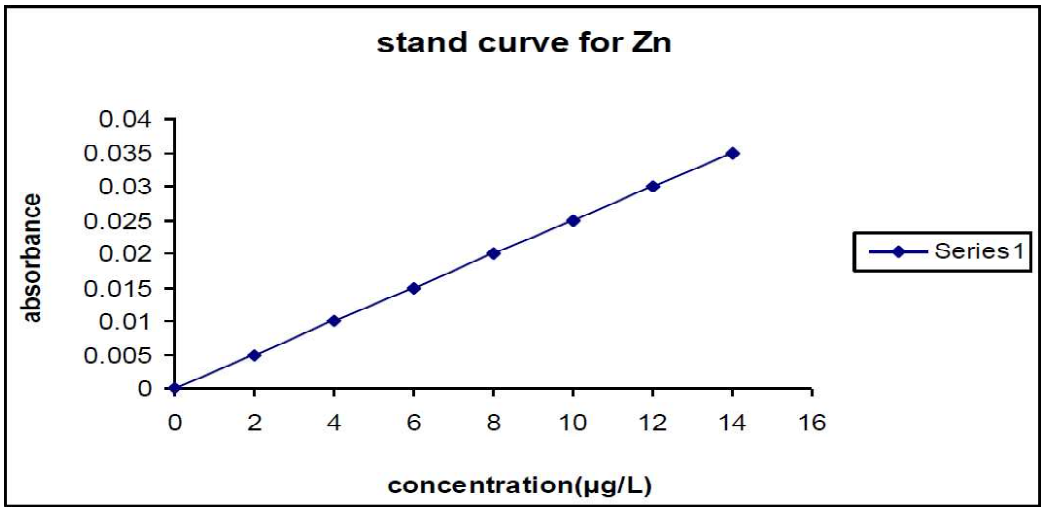


Figure 8: Calibration Curve for Fe

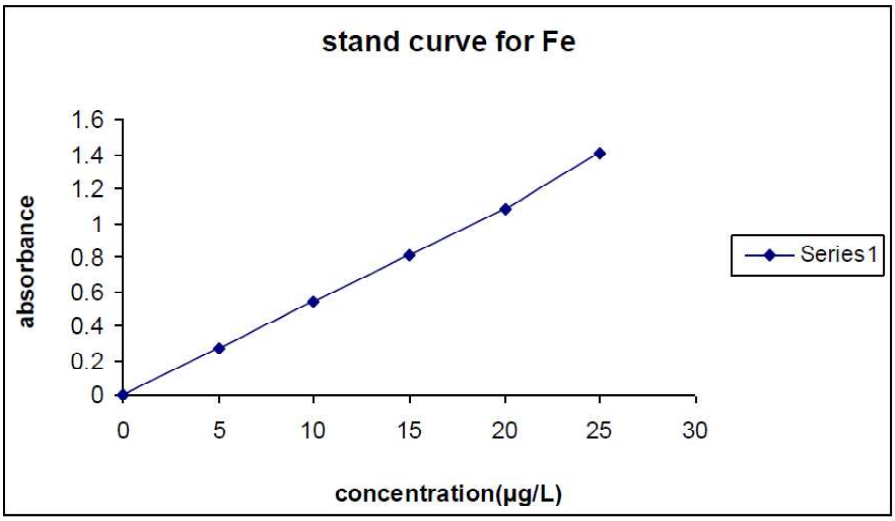


Figure 9: Calibration Curve for Ni

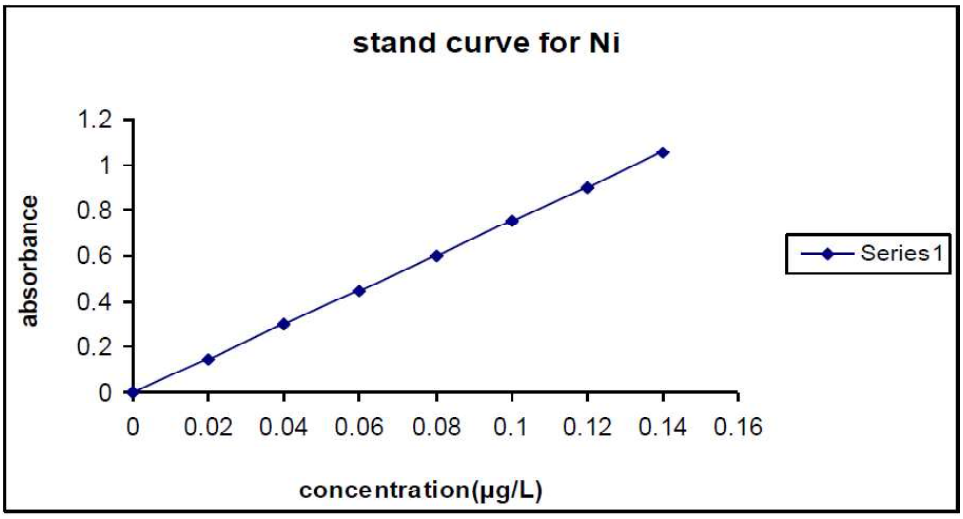


Figure 10: Calibration Curve for Pb

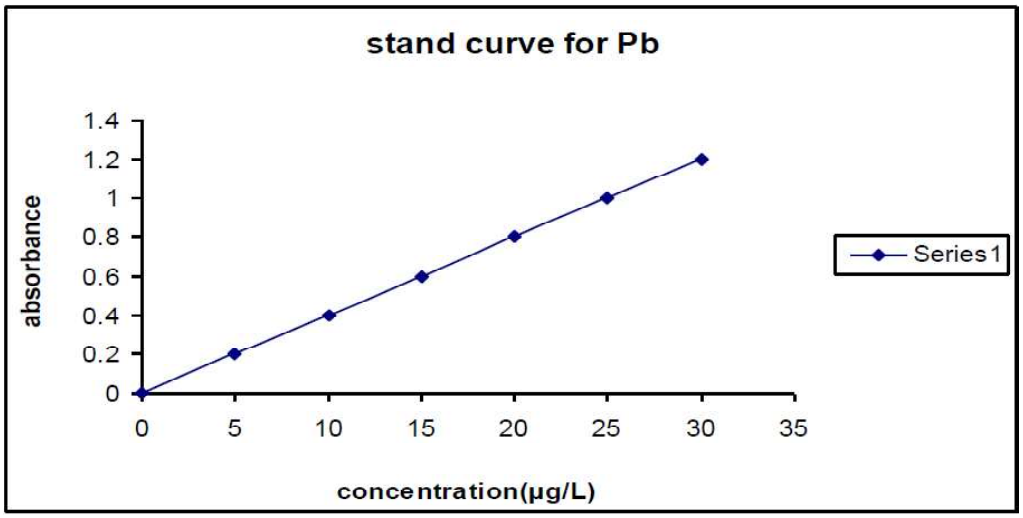
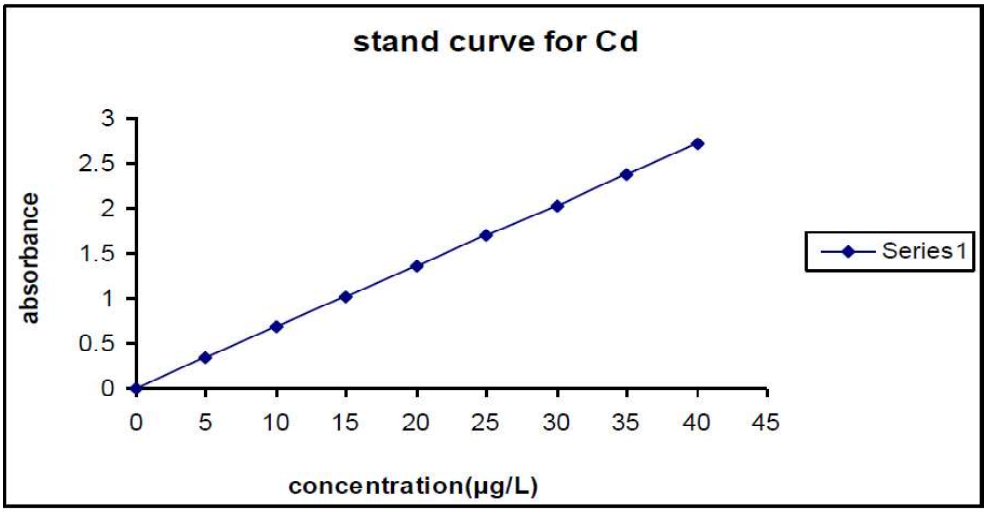


Figure 11: Calibration Curve for Cd



Calibration Curves for Metals

Using the calibration curve in atomic absorption measurements is a simple matter to calculate the concentration of compatible metal in test solution from the measured absorbance. The curve is plotted by aspirating samples of solution having known concentration of metal in to the flame, the graph is constructed by measuring the absorbance of each solution in which measured absorbance is plotted against the concentration of solution.

Concentration of Heavy Metals Detected in Seed Oil of Bitter Gourd Plant

Heavy metal concentration analyzed in seed oil sample is given below in Table 4. The concentration of the metals was determined on dry weight basis. On absorption of heavy metals showed that no single mechanism is accountable for metal uptake. Commonly, there are two mechanisms occurs- The first is 'adsorption' by which atoms, ions or molecules adhere to the surface of metal and other is 'absorption' which implicit insertion of metals in to the interior of crystals. Due to the aggregation of these heavy metals in vegetable contributed use of industrial waste water for their cultivation. From the result, it is found that concentration of Fe, Zn is significantly high.

CONCLUSION

Bitter gourd from cultivated around the western Rajasthan contains a high amount of total lipids. The oil of *Momordica charantia* is a potential source of α-eleostearic acid as it is obtained 52.82% by weight. It is good for human health and also a promoter of CDGSH Iron-Sulphur domain, a therapeutic target for CNS Injuries and Diseases.[16]

α-eleostearic acid, inducing apoptosis in HL60 cells is isolated by HPLC and identified as apoptosis.[17] The same acid is main or key fatty acid in bitter gourd seed oil which is having isomeric position like this (9Z,11E,13E). Some normal, cancer cell lines and growth of some fibroblasts (including those of HT29 colon carcinoma and HL60 leukemia) can be strongly inhibited by á-eleostearic acid.[18]

From the result, it is found that the average content of Ni is 8.98 ppm. The detected level of Cu was ranged from 6.54 ppm. This showed the effect of heavy traffic and anthropogenic activities by which the Cu metal may be assembled in the soil.[19] The high content of Fe is 108.32 ppm due to physical environment like pH of the medium, salinity, temp. and the presence of other metals impacts the process of uptake and aggregation of Fe in medicinal plants.[20] The concentration of Cu and Fe is more than the toxic level. Consequently, it is necessary to have some safety measures to control this environmental metal toxicity before using plants for medicinal and nutritional purposes.

The oil of *Momordica charantia* is of extremely good quality also encourages its medicinal use as it shown health beneficial effects. Also the oil is good for the paint and coating industry because it has high iodine value.[21]

It is known for its medicinal properties like anticancer, antivirus, anti-inflammation, antidiabetic, and cholesterol-lowering effects. Phenolic compounds are found in this which may have potential as antimutagen and antioxidant. It is nutrients rich plant-based food which contains bioactive compounds such as a polypeptide, vitamins, alkaloids, and minerals. Research has been conducted proving its hidden potential against viral disease, ulcer, and another microbial invasion.[22]

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