THEVETIA PERUVIANA (MILK BUSH) SEED OIL: HEAVY METALS AND FATTY ACIDS ANALYSIS FROM ARID ZONE OF RAJASTHAN

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Abstract

Heavy metal pollution is rapidly explored due to industrialization and urbanization. Heavy metal pollutants are matter of concern because of their potential impact on flora and fauna. For our study, we collected seeds of *Thevetia peruviana* plant from industrially polluted region in pali district, arid zone of Rajasthan. Heavy metals such as Zn, Mn, Co, Pb, Cd, Fe, Ni, Cu, and Cr were accumulated by plants from the industrial waste. The MP-AES technique was used to analyse heavy metals by digested seed oil. The physicochemical characteristics of *Thevetia peruviana* seeds oil were determined such as oil yield 61.20%, specific gravity 0.8830, moisture content 1.38%, saponification value 125 mg KOH/L, acid value 1.07 mg KOH/L, iodine value 76.12 g of I_2/100g and free fatty acid value 0.55 mg KOH/g. Preparation of FAME was carried out by trans esterification of *Thevetia peruviana* seed oil and then the fatty acid composition was determined using the GC-FID technique. The most predominant fatty acids in the *Thevetia peruviana* were Oleic acid 32.75%, Linoleic acid 31.46%, Palmitic acid 25.26% and least were Stearic acid 5.71% and Arachidic acid 1.36% respectively. The ratio of unsaturated fatty acids in *Thevetia peruviana* seed oil were high about 65.93 %, indicates that *Thevetia peruviana* was an excellent source for oleochemicals such as soaps, shampoos and biodiesel.

Keywords: *Thevetia peruviana*, FAME, GC-FID, Heavy metals, MP-AES.

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Introduction

Thevetia peruviana is an evergreen shrub of *Apocynaceae* family that is also known as Cascabela peruviana (L.) Lippold, lucky nut, bastard oleander, ayoyote, codo de fraile, tiger apple and exile oleander. This plant is native to tropical America, specifically Central America but has now naturalised throughout the world's tropical and subtropical regions. This plant have an ability to grow and develop in dumpsites and wastelands. When the plant is growing, it needed the least amount of water. It is widely available in Western Rajasthan; where it is primarily grown for ornamental purposes. The plant seed contains nearly 60% oil and the seed cake contains nearly 37% protein [1]. The plant has two varieties: yellow oleander (yellow flowers) and nerium oleander (purple flowers). Yellow oleander, also known as milk bush and it is an evergreen dicoted shrub. Due to the presence of cardiac glycoside in the oil, it'd be non-edible for humans. The seed oil has health beneficial properties and can used as an alternative protein source in the animal feed plan [2]. It is also an important component of industrial raw materials. *Thevetia peruviana* is commonly cultivated in different regions of the world due to its

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vast application and utility. Parts of the *Thevetia peruviana* plant such as seeds (fruits), bark, and leaves have been evaluated for anticancer activity [3]. The seed oil of *Thevetia peruviana* helps in the production of oleochemicals such as soaps, shampoos and biodiesel. Renewable fuels such as biodiesel and bioethanol can help to resolve energy crises because they are non-toxic and environmental friendly [4]. Renewable energies are viable and environmental friendly alternative to depleting fossil fuels [5]. In industrial areas, the soil is highly polluted due to heavy metals in industrial waste directly dumping in rivers and open lands. Due to this, plants accumulated these heavy metal pollutants through water and soil. Heavy metal pollution as a result of industrialization has become a major environmental concern in India and across the world [6-7]. Due to the high density and toxicity of heavy metals, even at low concentrations, these are considered as hazardous environmental pollutants [8]. Most wildly spread heavy metals in environment such as Pb (lead), Ni (nickel), Cd (cadmium), Co (Cobalt), Hg (mercury), Fe (Iron), Cu (copper), Mn (Manganese), Cr (chromium) and As (arsenic) were listed by EPA (Environmental Protection Agency) [9-10]. Non-biodegradability and toxicity of heavy metals make them hazardous for human health and environment [11-12]. Industrial processes, pharmaceutical wastes, mining and smelting of metal ores, battery industry, waste dumps leachate and fertilizers are the major sources of metal pollution in soil [13]. The most prevalent source of metal pollution is industrial liquid waste from diverse sectors such as dye, plastic, textile, paper, battery and paint, which is a major concern for India [14-15]. These industries dump their waste into rivers and nearby areas. As a result, heavy metals from industrially polluted soil reach the food chain via plants [16-17]. Plants absorb heavy metals from the industrial polluted land, which disturb seed germination, growth of plant, chlorophyll content, photosynthetic efficiency, root growth, nutritional quality and different enzyme activities [18-20].

Materials and Methods

Extraction of Seeds

Fresh fruits of *Thevetia peruviana* (milk bush) were collected from industrial area of pali district, arid zone of Rajasthan. The green external part of the fresh fruits were manually separated with the help of a knife. The seeds aquite from its compartments by using a small hammer. The plant seeds were properly washed with water and then rinsed with distilled water and then Sun dried the seeds. For maximum oil extraction increase the surface area of seed so the dried seeds were ground with mortar-pestle. For further processing the grinding seeds were placed in a glass container.

Extraction of Seed Oil

For oil extraction, the Soxhlet extraction method was used. In the thimble of Soxhlet extractor 200g of crushed *Thevetia peruviana* seeds were placed and solvent n-hexane was taken in round bottom flask. One end of the extractor was attached with the round bottom flask and other end was attached with a condenser. The round bottom flask was heated to 60°C using an electric mantle. Nearly 8 hour time was taken to accomplish an extraction [21]. After extraction the solvent was recovered using rotary evaporator to create vacuum and the residual oil was stored in refrigerator for further analysis.

Reagents

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AR/GR grade reagent chemicals such as KOH, NaOH, $Na_2S_2O_3$, H_2O_2, HNO_3, HF, H_2SO_4, $HClO_4$ and HCl were used for analysis. Double distilled water was used for dilution of all chemicals. All the glassware were cleaned with dilute HNO_3 and rinsed with deionized water before use.

Preparation of FAME

The oil of *Thevetia peruviana* was turned into FAME by trans-esterification process. The oil was heated in a round bottom flask in which a magnetic stirrer was put throughout the trans-esterification process [22-23]. The flask was filled with excess of methanol and NaOH as a catalyst. For the completion of reaction, keep this mixture for nearly 3 hours on the magnetic stirrer with hot plate. A mixture of FAME and glycerol was obtained which was separated by a separating funnel. The obtained FAME was analysed by GC-FID (Gas Chromatograph-Flame Ionization Detector).

Preparation of Solution for Heavy Metals

In a 250 ml beaker, 1g of oil was added to 10 ml of concentrated HNO_3. The oil sample was heated until a clear solution formed. The solution was treated with 5 ml concentrated HNO_3 (repeated this process for 3 times) and digested it until the volume was decreased to 1 ml. The internal walls of beaker were rinsed with double distilled water to maintain the beaker clean and prevent the loss of digested sample. After cooling the sample, 5 mL of 1% HNO_3 was added to it. Use Whatman filter paper to filter the digested solution. Then by adding double distilled water, it was quantitatively transferred to a 25 ml volumetric flask and was analysed by MP-AES (Microwave plasma atomic emission spectroscopy).

Preparation of Stock Solutions

Appropriate amount (shown in table 1) of Metal salts were dissolved in water to make stock solutions of 1000ppm. 2–3 drops of concentrated HNO_3 were added to the stock solutions to prevent metals from precipitating via hydrolysis. Diluted 1000 ppm stock solutions were used to make the desired working standard solutions.

S.No.	Metals	Metal salts	Molecular weight of salt (g/mol)	Atomic weight of metal	Weight of metal salt dissolve(in g)
1	Cu	$CuSO_4.5H_2O$	249.68	63.54	3.93
2	Fe	$FeSO_4.7H_2O$	278.01	55.84	4.98
3	Co	$[Co(NO_3)_2.6H_2O]$	291.03	58.93	4.94
4	Zn	$ZnSO_4.7H_2O$	287.54	65.38	4.40
5	Cd	$CdSO_4.H_2O$	226.49	112.41	2.01
6	Ni	$NiSO_4.7H_2O$	280.86	58.69	4.78
7	Cr	$[CrCl_3.6H_2O]$	197.64	52.00	3.80
8	Pb	$Pb(NO_3)_2$	331.00	207.2	1.60
9	Mn	$MnCl_2.4H_2O$	197.91	54.94	3.60

Table 1: Amount of metal salt use for stock preparation

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Results and Discussion

Physicochemical Parameters

The seed oil of *Thevetia peruviana* is light yellow in colour, pleasant smell and liquid at room temperature. The physicochemical properties of *Thevetia peruviana* were analysed by American Oil Chemical society (AOCS) method shown in Table 2.

S.NO	Physicochemical properties	Value
1	Oil (%)	61.20
2	Moisture (%)	1.38
3	Acid value (mg KOH/L)	1.07
4	Iodine value (g I_2/100g)	76.12
5	Saponification value (mg KOH/L)	125
6	Specific gravity	0.8830
7	Free fatty acid value(mg KOH/g)	0.55

Table 2: Physicochemical parameters value

Thevetia peruviana seeds have a high oil content of 61.20 percent, indicating that they are oil rich. The iodine value of the seed oil was found 76.12 g I_2/100g, indicates that the seed oil is a semi-drying oil. This value indicate that oil has the potential to be used in the manufacturing of varnish, shoe polish, wax and alkyd resin. The free fatty acid value and acid value of *Thevetia peruviana* were 0.55 mg KOH/g and 1.07 mg KOH/L respectively which were higher than Deka et al. [24] and lower than Ogunneye et al. [25]. Other physicochemical properties of *Thevetia peruviana* such as moisture 1.38 percent, specific gravity 0.8830 and saponification value was 125 mg KOH/L.

GC-FID Analysis

GC-FID technique was used to analysis fatty acids methyl ester compositions. Total 16 fatty acids were identified including both saturated and unsaturated fatty acids, showing in table 3 and table 4.

S.No.	Fatty acids	Retention time(min)	Lipid number	Composition
1	Caproic acid	5.94	C 6:0	0.16
2	Capric acid	7.35	C 10:0	0.18
3	Myristic acid	9.61	C 14:0	0.20
4	Palmitic acid	12.01	C 16:0	25.26
5	Arachidic acid	22.33	C 20:0	1.36
6	Heptadecanoic acid	13.80	C 17:0	0.15
7	Stearic acid	16.07	C 18:0	5.71
8	Behenic acid	26.99	C 22:0	0.62
9	Lingoceric acid	33.25	C 24:0	0.33
			Total	33.97

Table 3: Saturated Fatty acids composition in *Thevetia peruviana*

S.No.	Fatty acids	Retention time(min)	Lipid number	Composition
1	Palmitoleic acid	12.29	C 16:1	0.39
2	Oleic acid	16.78	C 18:1	32.75
3	Vaccenic acid	16.91	C 18:1	0.81
4	Linoleic acid	18.21	C 18:2	31.46
5	Gamma Linolenic acid	20.17	C 18:3	0.10
6	11-Eicosenoic acid	23.01	C 20:1	0.24
7	11-14 Eicosadienoic acid	23.93	C 20:2	0.18
			Total	65.93

Table 4: Unsaturated Fatty acids composition in *Thevetia peruviana*

Saturated fatty acids were reported 33.97% which included Caproic acid (6:0), Caproic acid (10:0), Myristic acid (14:0), Palmitic acid (16:0), Arachidic acid (20:0), Heptadecanoic acid (17:0), Stearic acid (18:0), Behenic acid (22:0) and Lingoceric acid (24:0). Among all saturated fatty acids Palmitic acid being the highest (25.26%). The unsaturated fatty acids were reported 65.93% which included Palmitoleic acid (16:1), Oleic acid (18:1), Vaccenic acid (18:1), Linoleic acid (18:2), Gamma Linolenic acid (18:3), 11-Eicosenoic acid (20:1) and 11-14 Eicosadienoic acid (20:2). Among all unsaturated fatty acids Oleic acid was highest (32.75%) in monounsaturated fatty acids and Linoleic acid (31.46%) was highest in polyunsaturated fatty acids.

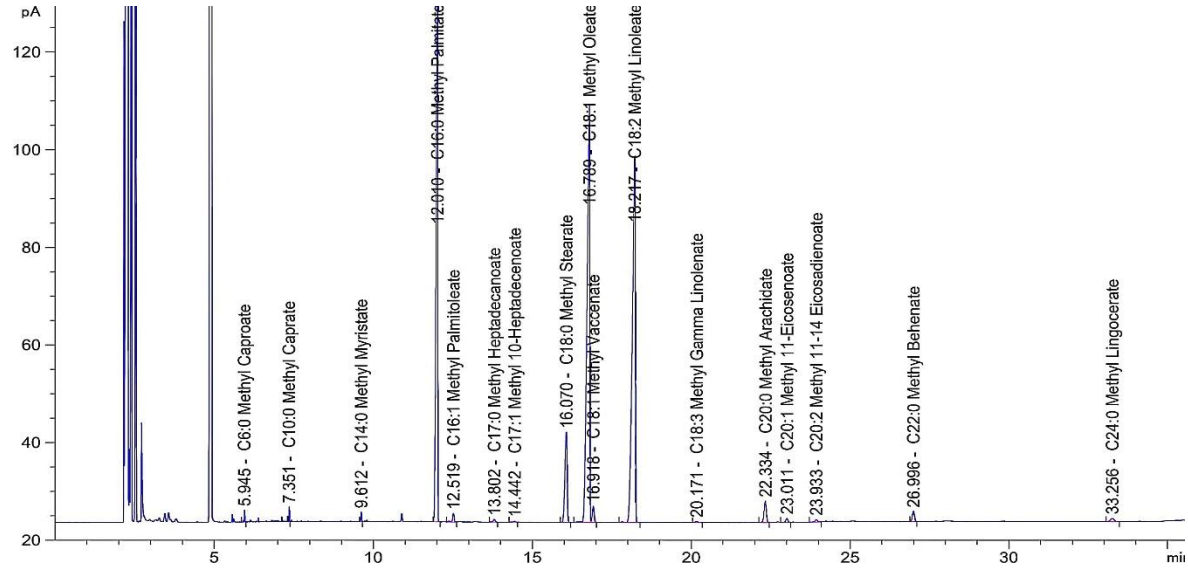

Figure 1: GC-FID of *Thevetia peruviana*

MP-AES Analysis

Agilent Technologies MP-4210 of Microwave plasma atomic emission spectroscopy (MP-AES) was used to analyse heavy metals of digested seed oil sample with 0.9 Calibration correction coefficient. In this research eight heavy metals were analysed as shown in Table 5.

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S.No.	Metals	Wavelength(nm)	Intensity	Concentration(ppm)	%RSD
1	Mn	403.076	12712.67	0.39	0.63
2	Fe	371.993	6095.83	1.60	3.00
3	Cu	324.754	7601.71	0.14	5.57
4	Ni	352.454	413.89	0.05	4.69
5	Cr	425.433	1627.67	0.05	0.38
6	Zn	213.857	124565.85	21.86	0.44
7	Pb	405.781	380.88	0.17	1.78
8	Co	340.512	143.66	0.02	9.17

Table 5: Heavy metals concentration of *Thevetia peruviana*

Conclusion

Results shows that *Thevetia peruviana* is an oil-rich shrub with high quantity of unsaturated fatty acids. The primary unsaturated fatty acids, oleic acid (ω-9) and linoleic acid (ω-6) present in *Thevetia peruviana* are beneficial to human health and valuable for industry. Oleic acid is advantageous for balancing body weight, reduces hypotension and lowering the risk of heart disease by managing cholesterol level. Oleic acid is also utilise in pharmaceuticals, surfactants, lubricants, detergents, cosmetics, coatings, soaps and ayurvedic medicines. *Thevetia peruviana* seed oil is a semi-drying oil that is use in oil paints and varnishes due to the presence of linoleic acid. Because of its skin-beneficial qualities, linoleic acid has become more popular in the cosmetics sectors. Plant parts and seed oil remnants can be used as animal fodder. The concentration trend of several heavy metals in digested seed oil was Zn > Fe > Mn > Pb > Cu > Ni = Cr > Co. The quantities of heavy metals in *Thevetia peruviana* seed oil were lie within the acceptable limit. Zinc concentration was found to be much higher but in standard permissible limits. Zn is the least poisonous among all metals and is a necessary component of human diet. It is necessary for regular brain activity and essential for the foetus growth and development.

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