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Detection of Heavy Metals in Seed Oil of *Calotropis procera* Plant From jodhpur (Rajasthan)

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ABSTRACT
The current study of Calotropis procera accumulates heavy metals which collected from industrial area of Jodhpur, Rajasthan. The investigation of physico-chemical parameters, heavy metals content and fatty acids composition in seed oil of Calotropis procera plant. C. procera seed oil was extracted using the soxhlet extraction method by using petroleum ether (40-60°C) as a solvent. Yield of oil achieved during analysis from C.procera seeds were24.13% by weight. High iodine content of 114.1 gI_2/100g was identified by analysis process. Higher iodine value is an indicator of unsaturation in oil and fats. Other physico-chemical parameter of seed oil sample found during study were moisture content 1.8 %, ash content 3.20 %,acidic value 4.54 mg KOH/g, specific gravity value at 0.8984 with respect to water, saponification value at 191.80 mg KOH/g and unsaponified matter 2.36 %.MP-AES (microwave plasma atomic emission spectroscopy) was used to determine heavy metal concentration which were:Zn 27.19 mg/l, Fe 2.00 mg/l,Pb 0.40 mg/l,Mn0.19 mg/l,Cr 0.14mg/l,Ni 0.13 mg/l, Cu 0.13 mg/l, Co 0.02 mg/l,Cd 0.01 mg/l identified in C.proceraseed oil sample. High Performance Liquid Chromatography(HPLC) technique was used to analyze the fatty acid content of seed oil sample.Fatty acids content found during study were linoleic acid 36.25%,oleic acid 33.22%,palmitic acid 13.79%,stearic acid9.25% and palmitoleic acid 1.78%.
Keywords: Calotropis procera, Heavy metals, FAME, MP-AES, HPLC

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INTRODUCTION

Calotropis procera is a soft-wooded perennial shrub in the *Apocynaceae* family with distinct and easily identifiable characteristics such as milky juice in stem, pod-like fruits and tufted silky-haired seeds.It's axero-phytic plant that flourish in semi-arid and arid conditions [1]. "Calotropis" is a Greek word that refers to flower, while "procera" is a Latin word that refers to the cuticular wax found on its leaves and stem [2]. It is found worldwide and commonly known as calotrope, Indian milkweed, apple of Sodom, wild cotton, giant milkweed, rubber tree, usher etc. Fruit morphology of two commonly found subspecies of *C.procera*, namely procera and hamiltonii, differs [3] and *C.procera*has a strong resemblance to the con-generic plant *Calotropis gigantea* (4).Seeds of *C.procera* generally drift away on wind currents toofar and distant locations thus *C procera* milk weed could be commonly found around farms and agricultural areas in sandy warm parts of Rajasthan (5-7).Since antiquity, it has been used for fuel, fibre, fodder and lumber (8).*C.procera* is a multi-purpose herb which is also used in traditional medicine system in South Asia, North Africa, South-East Asia and Middle East Asia [9]. Presence of heavy metals such as Ni, Cu, Fe, Pb, Cd, Mn etc. has been highlighted in *Calotropis procera* seed oil through various independent studies.

Release of heavy metals into the environment has been exponentially increasing as a result of metal extraction from ores, industrial waste and agricultural fertilizer usage. Heavy metals also enter the atmosphere through various natural and anthropogenic sources. The most common natural sources are volcanic activity, weathering of rocks, corrosion, erosion and also heavy uses of fertilizers, mining, electroplating, smelting, pesticides and phosphate as well as atmospheric deposition, bio-solids in agriculture, industrial waste, sludge dumping etc. [10-14].The environmental pollution and contaminated soil exert adverse effect on grown crops. Heavy metals are absorbed by plants roots and aerosol pollutants enter in the plant through the leaves. The aerosol concentration in plants depends upon the size of particles, weather conditions and source distance. Some of the particles cling to the plant leaves, while others are absorbed. For example lead (Pb) gets deposited at leaf surface in the form of precipitate, while copper (Cu), zinc (Zn) and cadmium (Cd) penetrate into the plant leaves [15-17].Due to rising urbanization and disruption of natural biogeochemical cycles the issue of heavy metal pollution is getting more and more serious thus rendering soil contamination by heavy metal as an issue of global importance.

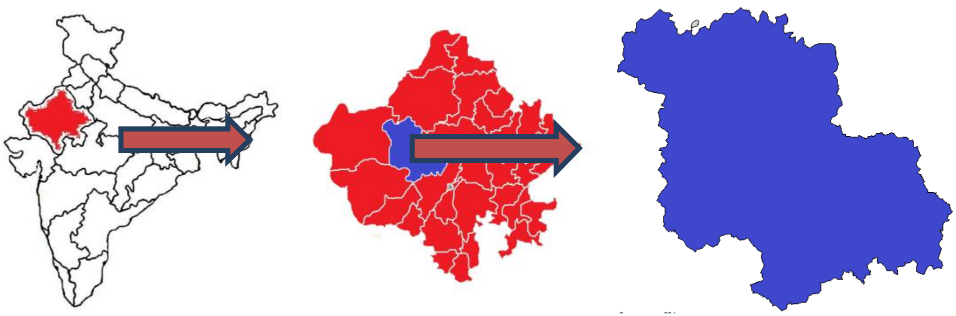
Heavy metals are classified as essential and non-essential depending on their involvement in biological systems. Heavy metals that are required in minute amounts by living organisms for crucial physiological and biochemical processes are referred to as essential heavy metals while heavy metals that aren't required by living organisms for any physiological or biochemical activity are known as non-essential heavy metals. Some of commonly known essential heavy metals are Zn,Fe,Ni, Cu and Mn [18-19] and non-essential heavy metals are Cd,Hg, As,Pb and Cr [20-26].

Concentration of heavy metals over a certain threshold is harmful to human health because they interrupt the regular functioning of biological systems. Excessive deposition of Heavy metal in soils and plants is hazardous to both the environment and human health as these Elements accumulate in living organism's tissues through natural food chain and their concentration rises as they migrate from lower to higher tropical level (Bio-magnification).Study of agricultural crops grown near industrially polluted area, wastelands and other locally grown weeds, herbs, plants etc is the way forward to answer concerns regarding heavy metals concentration into food chain.

MATERIAL AND METHODS

Raw Material and Sample Extraction:

Harvested ripe of *Calotropis procera* fruits from shrubs of *C.procera* (Family: *Apocynaceae*) were collected fromindustrial areas of Jodhpur (Fig 1).



INDIA RAJASTHAN JODHPUR

Figure1:- Map of study zone

Seeds were recovered from ripe fruits of *C.procera* and cleaned after hand picking and subsequently sun-dried for 5-10 days. 250g seed sample by weight was further dried at 105°C for 6 hours in a hot air oven to eliminate any moisture content from sample thus constant sample weight after oven drying the seed sample was achieved. Subsequently oven dried seed sample was crushed into fine powdered form with the help of pastel mortar**.**

Extraction of Seed Oil:

Using the soxhlet extraction procedure, oil was extracted from powdered *Calotropis procera* seeds using light petroleum ether (40-60°C) as solvent. Using a rotary evaporator, the solvent was entirely evaporated under vacuum. The oil was kept at 4°C until it was analysed.The conventional American Oil Chemist Society (AOCS) procedures were used to obtain the analytical values of seed oil [27].

Fresh fruit Dried Seeds Grinded Seeds

Figure2:- Fruit and Seeds

Digestion of Seed Oil:

1g oil sample and 5ml concentrated nitric acid was taken in a Borosil glass beaker. It was kept on a hot plate to obtain near dry seed oil sample. Sample obtained in semisolid state was left to Cool and another 5 ml of concentrated nitric acid was added. Beaker was covered with the help of watch glass and again kept on hot plate. Sample was heated continuously until digestion was complete. Further, 1-2 ml concentrated nitric acid was added and beaker containing residue sample was warmed to dissolve residue. Double distilled water was used for washing watch glass and beaker to recover residue sample. Sample thus received was filtered to make sample volume up to 100 ml [28].

Preparation of Mixed Fatty Acid:

Mixed fatty acid was prepared using oil hydrolysis process. All the glass wares used during oil hydrolysis process are clean and oven dried. 2g oil was taken in round bottom flask and2-3 ml of standardize alcoholic NaOH (1N) solution was added to flask containing oil and 10 ml ethanol was used as solvent. Subsequently solution was Refluxedat 70-80^{o}C for 1-2 hours and was monitored with TLC. The mixture contained both saponified and unsaponified matter. Mixture was diluted with 30 mL double distilled water.The saponified matter was extracted using a separating funnel and frequent washing with diethyl ether. The unsaponified matter in the top organic (ether) layer was collected in a separate beaker. Diethyl ether is recovered after evaporation with a rotatory evaporator. Dilute hydrochloric acid(HCl-6N) was used to acidify the lower aqueous layer, which contains fatty acid salts. Acidified fatty acid salts were repetitively washed with diethyl ether and two layers were thus obtained. Lower layer was discarded and upper layer containing mixture of fatty acid and ether was collected in flask. Through subsequent evaporation, excess ether was evaporated and mixed fatty acid (MFA)was obtained. MFA's thus received were further washed with deionized water and dried over Na_2SO_4. Subsequently pure MFA'S were collected. TLC was used to monitor this entire process.

TLC plates were made by covering a glass plate with approximately 0.025 mm of layered silica gel. The mobile phase was a 70:29:1 combination of petroleum ether, diethyl ether and acetic acid and the spot was visible in the iodine chamber.

Preparation of Fatty Acid Methyl Ester:

Fatty acid methyl esters (FAME) were obtained after further processing mixed fatty acids (MFA'S).This process is known asesterification and esterification process was also monitored through TLC.MFA was added with excess of methanol (1:6) and few drops of 1% H_2SO_4 as catalyst and refluxed for 2 hours.Again solution was cooledand 30 mldouble distilled water was added.Diethyl ether was used to extract fatty acid methyl ester (FAME). The bottom aqueous layer was discarded and the mixed ether extracted in the top layer was collected in a dry flask.FAME was obtained when the solvent was evaporated and dried on anhydrousNa_2SO_4. FAME thus obtained was stored in nitrogen atmosphere at low temperature for further analysis [29].For quantitative examination of FAME was done byHPLC (High Performance Liquid Chromatography).

Physico-Chemical Analysis:

Physico-chemical parameters like saponification value, acid value, unsaponifiable matter, specific gravity, iodine value and moisture content for seed oil sample of *Calotropis procera* were determined using the AOCS methods. A number of factors are included in the physico-chemical analysis, including physical state, taste,colour and percentage weight loss on drying according to the standard procedure.

Heavy Metals Analysis:

Microwave plasma atomic emission spectroscopy (MP-AES)technique is used for analysis of heavy metals such as Iron (Fe), Chromium (Cr), Zinc (Zn), Cadmium (Cd), Copper (Cu), Nickel (Ni), Cobalt(Co), Lead(Pb)and Manganese (Mn) in digested seed oil. Nitrogen gas was used as a source of plasma for detection of metal content. Use of nitrogen plasma for metal detection is highly reliable as its range is in billions, so it can detect elements present in minute quantities. Nitrogen Plasma can be used to determine major and minor elements simultaneously using multi-analyte. MP-AES uses microwave radiation to create a plasma discharge with nitrogen gas either sourced from a gas cylinder or taken from ambient air thus obviating the necessity for a gas supply.

RESULTS AND DISCUSSION

Physico-Chemical Analysis Results:

The Physico-chemical properties results obtained by AOCS method in study are summarized in Table 1.

S. No.	Characteristics	Value
1	Oil (%)	24.13
2	Moisture Content (%)	1.80
3	Iodine Value (g I_2/100g)	114.10
5	Saponification Value (mg KOH/g)	191.80
6	Specific Gravity	0.8984
7	Density (g/dm^3)	0.8957
8	Acid Value (mg KOH/g)	4.54
9	Ash (%)	3.20
10	Unsaponifiable Matter(%)	2.36

Table 1: Physico-chemical parameters value

Calotropis procera seed oil may be utilized as a fuel after esterification as evident from % oil content derived from oil seeds. Use of *C.procera* FAME will not harm the engine because of favorable density and viscosity characteristics. The high Iodine value of 114.1(g I_2/100g) obtained in *C.procera* seed oil indicates that *C. procera* seed oil has a high unsaturated fatty acid content. Higher is the degree of saponification, greater is the potential of oil to make soap. It indicates large scale industrial application of *C.procera* seed oil.

Heavy Metal Analysis Results:

Microwave plasma atomic emission spectroscopy (MP-AES) with nitrogen as the source gas for plasma was used to investigate heavy metals in *Calotropis procera*. Table 2 shows the heavy metal concentrations found in *Calotropis procera* seed oil samples.

S. No	Analyte	Heavy Metal concentration in *C.procera* seed oil
1	Zn	27.19mg/L
2	Fe	2.00 mg/L
3	Pb	0.40mg/L
4	Mn	0.19mg/L
5	Cr	0.14mg/L
6	Ni	0.13mg/L
7	Cu	0.13 mg/L
8	Co	0.02 mg/L
9	Cd	0.01 mg/L

Table 2: Heavy metal content of *Calotropis procera* seeds oil

The concentrations of all metals were calculated on a dry weight basis. Zn (27.19mg/L) and Fe (2.00mg/L) was found in large concentrations in the seed oil whereas Pb (0.14mg/L), Mn (0.19mg/L), Cr (0.14mg/L), Cu (0.13mg/L), Ni(0.13mg/L) and Cd (0.01mg/L)was found in negligible amounts. Thus indicating a huge Phyto-remediation potential Zn and Fe polluted waste lands.

Fatty Acid Analysis Results:

Fatty acids were identified in seed oils of *Calitropis procera* as shown in Table 3.

S.No.	Fatty acid %		Composition
1	Linoleic acid	C 18:2	36.25
2	Stearic acid	C 18:0	9.25
3	Palmitic acid	C 16:0	13.79
4	Palmitoleic acid	C 16:1	1.78
5	Oleic acid	C 18:1	33.22

Table 3: Fatty acid composition of *Calotropis procera*

The seed oil contains 23.04% saturated fatty acids, 34.00% monounsaturated fatty acids and 37.25% polyunsaturated fatty acids. Unsaturated fatty acids content was relatively higher than saturated fatty acids in C procera seed oil. In saturated fatty acids palmitic acid content was highest at 14.79% and stearic acid content was 8.25%.High saturated fatty acid consumption is implicated in heart disease. In monounsaturated fatty acids percentage of oleic acid was highest at 32.22% and palmitoleic acid content was 1.78%. Oleic acid is an omega-9 (ω-9) fatty acid that is one of the healthiest sources of fat in the diet.

It helps to decrease chest pain, high blood pressure, and cholesterol. Linoleic acid content was 37.25%which is highest in all fatty acids as well as polyunsaturated fatty acids.

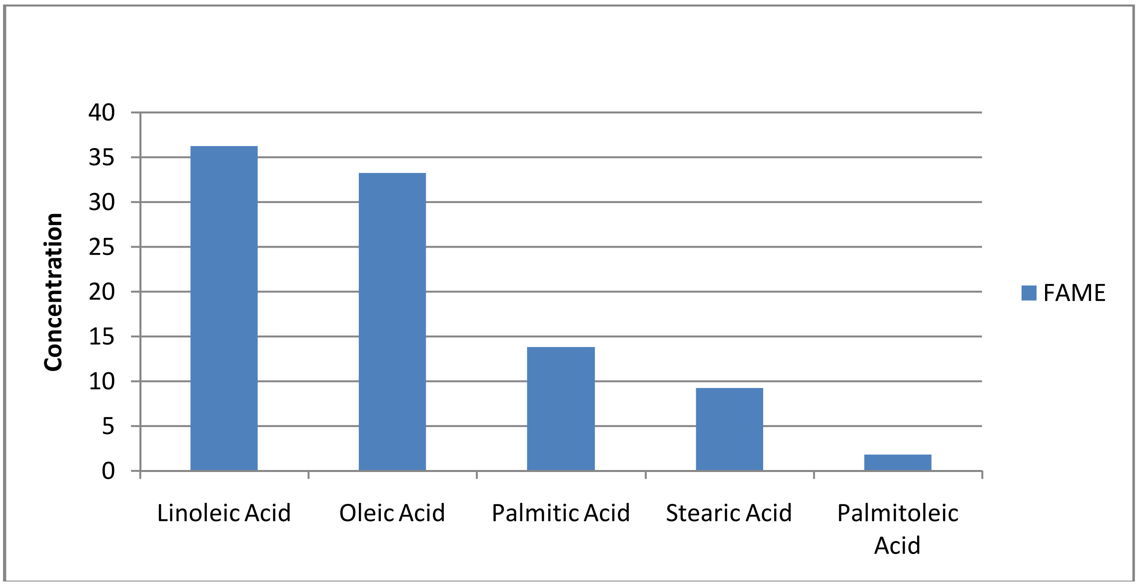


Figure 4:- Concentration of fatty acids

CONCLUSION

C. procera seed oil cultivated on degraded lands with minimal soil fertility especially in arid zones. *C.procera* is also a developing source of oil in saline soils due to its high seed oil content. *C.procera* absorbs heavy metals(Fe, Cu, Mn, Zn, Cd, Cr) so it can be used for phytoremediation. Phytoremediation is a possible remediation approach for soils that have been polluted with inorganic contaminants. The *Calotropis procera* seed oil is a good source of essential Omega-6 and Omega-9 amino acids which is good for health and useful fordietary supplements, healthy diet, industrial uses and medical uses. Calotropis procera has a high content of polyunsaturated fatty acids, so it can be used for paint-varnishes.

REFERENCES

1. Al-Rowaily, S. L., Abd-ElGawad, A. M., Assaeed, A. M., Elgamal, A. M., Gendy, A. E.-N. G. el, Mohamed, T. A., Dar, B. A., Mohamed, T. K., &Elshamy, A. I. (2020). Essential oil of Calotropisprocera: Comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. *Molecules*, *25*(21), 5203.
2. Hassan, L. M., Galal, T. M., Farahat, E. A., & El-Midany, M. M. (2015). The biology of *Calotropis procera* (Aiton) WT. *Trees*, *29*(2), 311–320.
3. Dhileepan, K. (2014). Prospects for the classical biological control of *Calotropis procera* (Apocynaceae) using coevolved insects. *Biocontrol Science and Technology*, *24*(9), 977–998.
4. Kaur, A., Batish, D. R., Kaur, S., & Chauhan, B. S. (2021). An overview of the characteristics and potential of *Calotropis procera* from botanical, ecological, and economic perspectives. *Frontiers in Plant Science*, *12*, 1188.
5. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Simons, A. (2009). Agroforestree Database: a tree reference and selection guide. Version 4. *Agroforestree Database: A Tree Reference and Selection Guide. Version 4.*
6. H.S.Al-Zahrani, 2002. Effects of salinity stress on growth of *Calotropis procera* seedlings, Bulletin of Pure and Applied Sciences, 21B (2): 109-122
7. Al-Yemni, M. N., Sher, H., El-Sheikh, M. A., & Eid, E. M. (2011). Bioaccumulation of nutrient and heavy metals by *Calotropis procera* and Citrulluscolocynthis and their potential use as contamination indicators. *Scientific Research and Essays*, *6*(4), 966–976.
8. Batool, H., Hussain, M., Hameed, M., & Ahmad, R. (2020). A review on *Calotropis procera* its phytochemistry and traditional uses. *Data Agric. Res*, *2*(2), 29–31.
9. Al Sulaibi, M. A. M., Thiemann, C., & Thiemann, T. (2020). Chemical constituents and uses of *Calotropis procera* and Calotropisgigantea–a review (Part I–the plants as material and energy resources). *Open Chemistry Journal*, *7*(1).
10. Modaihsh, A. S., Ai-Swailem, M. S., &Mahjoub, M. O. (2004). Heavy metals content of commercial inorganic fertilizers used in the Kingdom of Saudi Arabia. *Journal of Agricultural and Marine Sciences [JAMS]*, *9*(1), 21–25.
11. Chehregani, A., &Malayeri, B. E. (2007). Removal of heavy metals by native accumulator plants. *International Journal of Agriculture and Biology (Pakistan)*.
12. Fulekar, M. H., Singh, A., &Bhaduri, A. M. (2009). Genetic engineering strategies for enhancing phytoremediation of heavy metals. *African Journal of Biotechnology*, *8*(4).

13. Sabiha, J., Mehmood, T., Chaudhry, M. M., Tufail, M., &Irfan, N. (2009). Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. *Microchem. J*, *91*, 94–99.
14. Wuana, R. A., &Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices*, *2011*.
15. Kabata-Pendias, A. (1992). Trace Metals in Soils of Poland. *Trace Substances*, *25*, 53.
16. Pilc, L., Rosada, J., &Siepak, J. (1999). The influence of dust emission from the "Głogów" Copper Foundry on heavy metal concentrations in agrocenoses. *Pol. J. Environ. Stud*, *8*(2), 107.
17. Rosada, J.; Nijak, K. Concentration of copper and lead in vegetables cultivated in the region of the Glogow copper foundry in the years 1996–1998. Progress in Plant Protection 1999, 39, 541–543
18. Cempel, M., &Nikel, G. (2006). Nickel: a review of its sources and environmental toxicology. *Polish Journal of Environmental Studies*, *15*(3).
19. Göhre, V., &Paszkowski, U. (2006). Contribution of the arbuscularmycorrhizal symbiosis to heavy metal phytoremediation. *Planta*, *223*(6), 1115–1122.
20. Mertz, W. (1981). The essential trace elements. *Science*, *213*(4514), 1332–1338.
21. Kärenlampi, S., Schat, H., Vangronsveld, J., Verkleij, J. A. C., van der Lelie, D., Mergeay, M., &Tervahauta, A. I. (2000). Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution*, *107*(2), 225–231.
22. Suzuki, N., Koizumi, N., & Sano, H. (2001). Screening of cadmium-responsive genes in Arabidopsis thaliana. *Plant, Cell & Environment*, *24*(11), 1177–1188.
23. Cobbett, C. (2003). Heavy metals and plants: Model systems and hyperaccumulators. *New Phytologist*, 289–293.
24. Peng, K. J., Luo, C. L., Chen, Y. H., Wang, G. P., Li, X. D., &Shen, Z. G. (2009). Cadmium and other metal uptake by Lobelia chinensis and Solanumnigrum from contaminated soils. *Bulletin of Environmental Contamination and Toxicology*, *83*(2), 260–264.
25. Sánchez-Chardi, A., Ribeiro, C. A. O., &Nadal, J. (2009). Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew Crocidurarussula inhabiting the protected wetland of Doñana. *Chemosphere*, *76*(3), 387–394.
26. Dabonne, S., Koffi, B., Kouadio, E., Koffi, A., Due, E., &Kouame, L. (2010). Traditional utensils: potential sources of poisoning by heavy metals. *British Journal of Pharmacology and Toxicology*, *1*(2), 90–92.
27. W.E., 1973.Link Official and Tentative Methods of the American Oil Chemists Society.3rd ed. AOCS, I.L. Champaig, USA
28. Novozamsky, I., van der Lee, H. J., &Houba, V. J. G. (1995). Sample digestion procedures for trace element determination. *MicrochimicaActa*, *119*(3), 183–189.
29. Roy, B. C., Goto, M., Hirose, T., Navaro, O., &Hortacsu, O. (1994). Extraction rates of oil from tomato seeds with supercritical carbon dioxide. *Journal of Chemical Engineering of Japan*, *27*(6), 768–772.

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