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Analysis of Heavy Metals and Physico-Chemical Properties of *Salvadora Persica* Seed Oil

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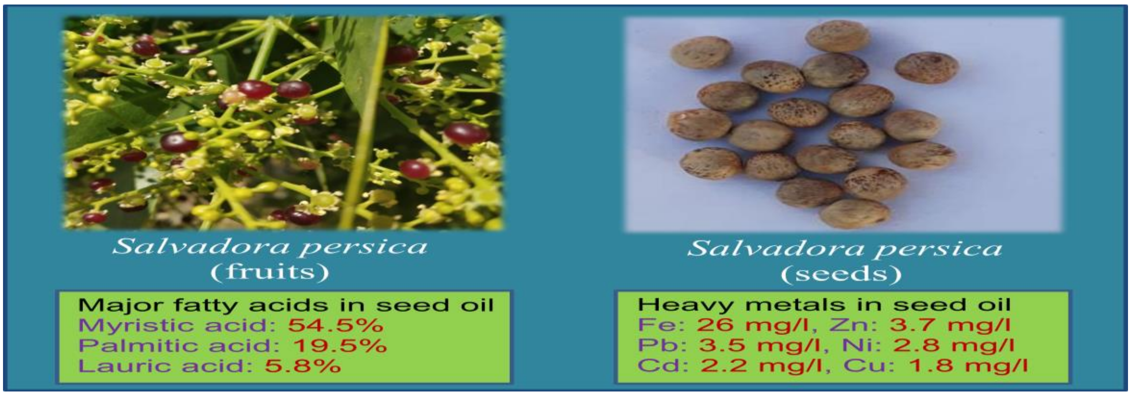
ABSTRACT: The accumulation of heavy metals in plants pose a serious problem for plants, animals and humans as these metals are non-biodegradable and persistent pollutants that cannot be eliminated by incineration. Another risk of heavy metal pollution comes from alterations in species diversity, vegetation cover and biomass, therefore, study of heavy metal accumulation in plant parts including seed and seed oil is important in order to know the contamination in surrounding and safe use of the plant parts. Since, plant depends on soil, water and air for their nutritional and other requirements, so, any contamination in plant is an indication of contamination in soil, water and air in surrounding of plant. In this study, physico-chemical properties, fatty acid composition and heavy metal content in seed oil of *Salvadora persica* is determined. Interestingly a high percentage of saturated fatty acids (87.3%) is found, which indicates the potential of this oil to be used in soap and detergent industry. The concentrations of heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) in seed oil of *Salvadora persica* is determined using atomic absorption spectroscopic method. The concentrations of metals Cd (2.2 mg/l), Cu (1.8 mg/l), Fe (26 mg/l), Pb (3.5 mg/l), Ni (2.8 mg/l) and Zn (3.7 mg/l) were found in seed oil sample. Presence of toxic metals Cd and Pb above the threshold concentrations prescribed for plants indicates the ability of plant to uptake metal from soil, water and air. This ability of the plant can be further utilized in phytoremediation and as a bioindicator for determination of pollution levels in urban areas.



Graphical Abstract

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Keywords: Heavy metal accumulation, Fatty acid composition, Bioindicator, Phytoremediation, Physico-chemical properties, *Salvadora persica*, Seed oil.

INTRODUCTION

Salvadora persica is a small green tree or shrub which belongs to the family Salvadoraceae found in Asia and Africa.[1,2] In India, it is mainly found in Kota, Banswara (Loharia), Bikaner (Shivbari), Jaipur (Amer), Baran, Sirohi (Abu Road) and Jodhpur region of Rajasthan. It is deep rooted and high salt tolerance plant[3-6] and therefore, its natural habitats are near mangroves, desert flood plains, saline lands, thorn shrubs, swamps, grassy savannah, seasonally wet sites and drainage lines in arid zones.[7,8]This tree is also known as Chotapilu, Arak, Jhak, *Salvadora indica*, Toothbrush tree and Mustard tree. It has a number of proven medicinal applications as well as traditional uses and almost all parts have been found to be pharmaceutically and industrially important.[9,10] The generic name of *Salvadora persica* plant was given by Dr Laurent Garcin in 1749 in the honour of an apothecary of Barcelona, Juan Salvadory Bosca. The origin of the plant is believed in Persia as the specific name persica indicates this. Till date numerous pharmaceutically useful chemical compounds have been isolated from different parts of this plant. For example, Salvadorocine, a new indole alkaloid, has been isolated from the leaves of *Salvadora persica*.[11] In addition to alkaloids, many organic compounds such as benzyl nitrile, isothymol, eucalyptol, eugenol, thymol, isoterpinolene, and β-caryophyllene have been identified in volatile oil extract of *Salvadora persica* leaves.[12] Glucotropaelin[13], amino acids[14-16], Flavanoids and flavanoid glycosides[17-19], and glucotropeolin[13] have also reported in literature. Some parts of plant have been traditionally used by common people as tooth brush, tinder, fodder and source of oil.

In 18th century, industrial revolution and exponential growth in population causes exploitation of natural resources. The exploitation of natural resources is still in continuation and causing environmental imbalance. The heavy metal toxicity and accumulation in plants is a mere result of human interference in natural cycle. As humans bring these heavy metals from remote areas for industrial applications and throw them after use as waste in nature. These waste materials get distributed in soil, water and air and contaminate them. The plants depend on soil, water and air for their nutritional and other requirements. Therefore, on uptake of nutrients from contaminated soil and water, toxins and heavy metals are also taken by plants. Thus, these heavy metals reach in plants and pose a serious problem for mankind

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as the major risks from heavy metal pollution results from the fact that these metals are non-biodegradable and persistent pollutants that cannot be eliminated by incineration and progressively accumulating in humans and plants. Another risk of heavy metal pollution comes from alterations in species diversity, vegetation cover and biomass.[20]A report of World Health Organization[21] has recognized health hazards of metals in food chain even at low concentration and outlined that agricultural plants are responsible for the movement of potentially toxic trace elements from soil to human beings. This fact has been further confirmed in studies demonstrating heavy metal accumulation in the soil due to their non-biodegradable nature and transfer from soil to plants.[22]

The reason for the transportation of heavy metals from soil to plants is naturally occurring chelating agents which are known to interact with the metal ions and increase their availability to the plants.[23,24] Shortfalls in available technologies and recent concerns regarding the environmental contamination prompted researchers to development of appropriate technologies by utilizing plants.[25] This new technology is known as phytoremediation which is very effective, environmental-friendly, cost effective and affordable solution to extract or remove metal pollutants from contaminated soil. In this, plants are used to clean up the contamination from soil, sediments, and water.[26] Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body. Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radio-nuclides from soil. One of phytoremediation categories, phytoextraction, can be used to remove heavy metals from soil using its ability to uptake metals (Fe, Mn, Zn, Cu, Mg, Mo, and Ni) which are essential for plant growth. Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se, Hg) can also be accumulated. In literature, many research groups have carried out studies in order to study the accumulation of heavy metals in plants.[27-30]For example, Salamon Ivan and Fejer Jozef studied accumulation of heavy metals in plants like poppy seeds and reported that poppy seed is able to accumulate many toxic elements including cadmium.[31] Similarly, Sobukola *et al.* have studied the levels of abundance in sixteen different fruits and vegetables by using atomic absorption spectrometry and found similar result as reported in literature.[32]

As we have seen that heavy metals get accumulated in plants from organic waste and other means.[33] These heavy metals contaminants can restrict the growth of plants, vegetables

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and crops as well as can enter in human food cycle and can cause numerous harmful effects. On other hand, we have seen the effectiveness of plants to remove toxic metals from contaminated site. Therefore, in continuation of our efforts to study the heavy metal accumulation in plants, we decided to study the presence of heavy metals in seed oil of *Salvadora persica*. In this article, physico-chemical properties of seed oil of *Salvadora persica* along with fatty acid composition and concentration of heavy metals is presented.

MATERIALS AND METHODS

Sampling: The seeds of *Salvadora persica* were taken out from dried fruits. The fruits were collected from Jodhpur region of Rajasthan and dried in sunlight.

Extraction of oil: The oil was extracted from dried seeds using solvent extraction method. The seed samples were crushed in mortar to make fine powder before extraction. The fine powder of seeds was wrapped in filter paper and oil was extracted using petroleum ether in a Soxhlet apparatus at 60-70ºC for 6 hours. The resulting solution was concentrated on a rota vapour and solvent was removed under reduced pressure. The obtained oil was dried and stored in paraffin tight sample vials in refrigerator at 4ºC.

Chemicals and Reagents: For the purpose of this study, all reagents used were of analytical grade. The acids such as HNO_3, H_2SO_4, HF, HCl, $HClO_4$ and other reagent like H_2O_2 were of superior quality. All the vessels and glasswares used were cleaned by soaking in dilute HNO_3, rinsed with deionised water and dried properly before use. The standard samples of heavy metals (Pb, Cd, Fe, Ni and Zn) were prepared with a concentration of 1000 μg/l. The calibration curves were prepared by diluting these stock solutions using deionised water for the estimation of metals in oil sample.

Heavy metal analysis: The atomic absorption spectrometer is used for the determination of heavy metals in seed oil sample.[34] Initially, hollow cathode lamp of desired metal put up in the operating position followed by adjusting current and selection of appropriate resonance line. The operating conditions were also adjusted to give a fuel lean air-acetylene flame. The prepared solutions were aspirated into the flame and their absorbance data were recorded. The standard solution of the least concentration was taken first followed by the solutions with gradually increasing concentrations. The test sample was taken at the last.

Preparation of standard solution of metal: The standard solution of a desired metal was prepared by dissolving 1 gram of metal in minimum amount of aqua regia (HCl and HNO_3 in a molar ratio of 1:3). The final volume of the solution was made up to 1 litre in volumetric

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flask by adding deionised water. The stock solution was used for preparing standard solutions of various concentrations by dilution. For the purpose of calibration curves, standard solutions of 0-40 μg/l were used. For this study standard solutions of cadmium, copper, iron, lead, nickel, and zinc were prepared.

Digestion of seed oil: The seed oil sample was digested in 100 ml Pyrex glass beaker. In beaker containing 1 gram seed oil, 10 ml of conc. HNO_3 was added and the solution was kept for 24 hours at room temperature for cold digestion. After this, the solution was heated at 50ºC for 4 hours followed by boiling the left over in HCl and HNO_3 solution (1:5) in order to digest all organic matters. The solution was filtered after cooling and final volume of the solution was made up to 25 ml using deionised water.

Fatty acid analysis: The fatty acid analysis was done by making methyl esters of fatty acids present in oil sample. This was done in two steps: (i) Mixed fatty acids were obtained by hydrolysis of oil sample and (ii) Mixed fatty acids obtained in step (i) were further derivatized to their methyl esters. The formation of methyl esters was monitored and confirmed by thin layer chromatography (TLC). Finally, the obtained mixture of methyl esters of fatty acids were analyzed by HPLC.[35] The physico-chemical properties such as saponification value, iodine value and peroxide value of these fatty acids obtained from seed oil of *Salvadora persica* were determined using the standard methods as described by AOCS.[36,37]

RESULTS AND DISCUSSION

First of all, after extracting oil from seeds of *Salvadora persica*, physico-chemical properties of the oil was measured. The obtained results are summarized in Table 1. The peroxide, iodine and saponification value of seed oil of *Salvadora persica* has been found as 1.1, 22 and 189, respectively. On saponification of seed oil of *Salvadora persica* a small amount of matters (4.9%) remained unsaponified, which is also shown in Table 1 (entry 4). These values are in close agreement with the reported values for seed oil of *Salvadora persica*.[38]After determining the physico-chemical properties, fatty acid composition present in the seed oil sample of *Salvadora persica* was estimated by making methyl ester of fatty acids. The result of fatty acid analysis is summarized in Table 2 along with their composition. The seed oil of *Salvadora persica* contains six saturated fatty acids namely Myristic acid (C14:0), Palmitic acid (C16:0), Lauric acid (C12:0), Stearic acid (C18:0), Arachidic acid (C20:0) and Behanic acid (C22:0) in 54.5, 19.5, 5.8, 4.5, 1.7 and 1.3%, respectively. It also

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contain some unsaturated fatty acids such as Oleic acid (C18:1), Eicosenoic acid (C20:1), Linoleic acid (C18:2) and Linolenic acid (C18:3) in low percentage (Table 2; entry 7-10). The saturated fatty acids constitutes 87.3% and among these Myristic acid (C14:0), Palmitic acid (C16:0) together makes 74% contribution. These fatty acids are very useful and can be utilized in soap and detergent industry.

Table 1: Physico-chemical properties of seed oil of *Salvadora persica*.

Entry	Parameters	Obtained values
1	Peroxide value (meq./kg)	1.1
2	Iodine value (g/100g)	22
3	Saponification value (mg of KOH/g)	189
4	Unsaponified matter (%)	4.9

Table 2: Fatty acid composition of seed oil of *Salvadora persica*.

Entry	Fatty acids	% of fatty acids
1	Myristic acid (C14:0)	54.5
2	Palmitic acid (C16:0)	19.5
3	Lauric acid (C12:0)	5.8
4	Stearic acid (C18:0)	4.5
5	Arachidic acid (C20:0)	1.7
6	Behanic acid (C22:0)	1.3
7	Oleic acid (C18:1)	5.5
8	Eicosenoic acid (C20:1)	2.1
9	Linoleic acid (C18:2)	2.6
10	Linolenic acid (C18:3)	1.4

After exploring the physico-chemical properties in seed oil of *Salvadora persica*, the content of heavy metals Cd, Cu, Fe, Pb, Ni and Zn was determined using atomic absorption spectrometer. For the purpose of this analysis, acetylene and air was used as source and oxidizer in flame. The oil sample was digested before use by using HNO_3/ HCl mixture. The result obtained for heavy metals in seed oil of *Salvadora persica* is summarized in Table 3.

Table 3: Composition of heavy metals in seed oil of *Salvadora persica*.

Entry	Metal	Wavelength used	Weight of metal (mg/l)

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1	Cd	228.8	2.2
2	Cu	324.8	1.8
3	Fe	243.3	26
4	Pb	283.3	3.5
5	Ni	232.0	2.8
6	Zn	213.9	3.7

The heavy metals (Cd, Cu, Fe, Pb, Ni and Zn) are found in concentration varying between 1.8 to 26 mg/l of seed oil. High amount of iron (Fe) showed that the seeds of *Salvadora persica* can be used as a rich source of iron for animals. On the other hand, the concentrations of cadmium and lead are higher than the threshold limits prescribed for humans, plants and animals. However, high concentrations of these metals also indicate the ability of the plant to uptake these toxic metals and accumulate them and therefore, can be used for the treatment of highly contaminated soil in industrial and urban areas. High concentration of lead also indicates that *Salvadora persica* plant can uptake toxic metals by foliar absorption as main source of lead is vehicular emission. Thus, *Salvadora persica* can be recommended as a bioindicator for the determination of pollution levels in the urban areas. However, the presence of other metals can be accounted to the uptake from soil and translocation of metals within the plants.

CONCLUSION

A high level of contamination of metals like cadmium and lead is found in the seed oil of *Salvadora persica* which indicates that this plant can uptake toxic metals from soil as well as by foliar absorption and therefore, can be used for soil remediation and air purification in contaminated areas. It may also be used as a bioindicator for the determination of pollution levels in the urban areas. Further, the presence of high percentage of saturated fatty acids (87.3%) in the seed oil of *Salvadora persica* make it useful in soap and detergent industry as well as in paint industry. This oil may also be used as an alternative of coconut oil for industrial utilization. The seeds of *Salvadora persica* may be directly used as an alternative source of iron in animal diets. Thus, this article has highlighted the importance of *Salvadora persica* plants for its use in phytoremediation and industrial applications.

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