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Ecballium elaterium Seed oil: Heavy Metals and Physicochemical Analysis from Arid zone of Rajasthan

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ABSTRACT

The physicochemical characteristics including fatty acids and heavy metals analysis of seed oil of Ecballium elaterium has importance of arid zone of Thar Desert. These seeds are good source of energy, carbohydrates, proteins, fats and minerals such as calcium, iron, magnesium, phosphorous and potassium. The Ecballium elaterium 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. Trace metals like Cu, Mn and Zn are essential in bone metabolism as cofactors of specific enzymes. Results showed that linoleic acid (47.14%) and punicic acid (21.98%) were the major polyunsaturated fatty acids. Heavy metals analysis of the seed oil of Ecballium elaterium is performed by Atomic Absorbance Spectroscopy (AAS). The heavy metal found in seed oil of Ecballium elaterium are in the order–Fe (23.46 mg L^{-1}) > Cr (3.40 mg L^{-1})>Zn (0.97 mg L^{-1})>Cu (0.23 mg L^{-1}) >Pb (0.09 mg L^{-1}) > Ni (0.08 mg L^{-1}) >Cd (0.03 mg L^{-1}).

Keywords: *Ecballium elaterium*, Seed oil, Heavy metal, Linolic acid, Punicic acid.

INTRODUCTION

The plant of *Ecballium elaterium* is known as squirting cucumber. It belongs to Cucurbitaceae family. The plant is perennial, fleshy, rough hairy with 30-100 cm long stems. The flowers are greenish-yellow and the fruits are large juicy berry, 3-4 cm, and ovate-oblong. Although plants belonging to Cucurbitaceae family that contain cucurbitacins are mostly known for their toxicity [**1, 2**]. Ripe fruits of the cucurbitaceae plant called *Ecballium elaterium* are known for their anti-tumoral, anti-protease, anti-inflammatory, analgesic, and antimicrobial properties. Conversely, the administration of *E. elaterium* at certain doses can have cytotoxic effects [**3**]. The juice contains a broad spectrum of biologically active substances [**4**]. Several biological activities of *E. elaterium* and its components, including anticancer activity, have also been reported [**5**]. The fruit juice of the plant contains several bioactive ingredients such as cucurbitacins. These tetracyclic triterpenoids compounds such as A, E, D, I are of interest medicinally because of their cytotoxic, antitumor and anti-jaundice properties. *E. elaterium* may be a great natural source for the development of new drugs and may provide a good and cost effective means of treating cancers and other diseases in the developing India. Diseases like Cancer remains one of the most dreaded causing an astonishingly high death rate, second only to cardiac arrest. A high number of new drugs derived from plant secondary metabolites have been

applied for the treatment and prevention of cancer [6]. A high ratio of polyunsaturated fatty acids can have beneficial effects on cancer prevention and treatment [7]. Several recent studies have revealed that the extracts of *Ecballium elaterium* contain a wide range of active ingredients such as phenolic compounds, flavonoids, alkaloids, sterols, amino acids, vitamins, tocopherols and fatty acids justifying their usage in food systems [8, 9]. Linoleic acid has been reported to have significant activity in inhibiting mammary carcinogenesis [10]. The diluted aqueous extract of the fruits is a traditional anti-inflammatory and analgesic for chronic sinusitis. It also possesses other uses especially the treatment of fever, liver disorders, jaundice, constipation, hypertension, dropsy, rheumatic diseases and as fungicidal [11]. The fruit juice from this plant has been reported to be rich in lipids, proteins, sugars, and minerals [12] and contain several bioactive compounds, such as triterpenoides (cucurbitacins), carbohydrates, tannins, gum and peptides [13]. Thus *E. elaterium* can be considered as a promising source of plant-derived anticancer agents. Oleic acid, the main monounsaturated fatty acid of olive oil, enhances the growth inhibitory effects of trastuzumab in breast cancer cell lines SK-BR3 and BT-474 [14].

Plant growth is the outcome of many factors among which pollutants must clearly be counted [15]. A wide range of environmental pollutants such as poisonous gases, agricultural chemicals, solid-wastes, radioactive materials, industrial effluents, sewage water etc. are known to pollute air, water, soil and bring about hazardous effects on plants, animals and human beings. High consumption, frequent disposal and replacement of disposable items are generating diverse types of metallic wastes which are discharged into the environment and thus poisoning the biosphere [16-20]. Heavy metals like Cd, Cu, Co, Ni, Zn and Cr are phytotoxic either at all concentrations or high concentrations level. Toxic metals are biologically magnified through the food chain. Toxic metals are affecting the environment. When concentrations of heavy metals are within limit, then it is useful to micronutrients for plants, human and animals. It becomes toxic when their concentration exceeds a limit.

The Heavy metal contaminants within the soil, is dependent on their ability to travel through water system and their availability for biological uptake. Iron when exceeds limit typically damages cells in the heart and liver which can cause cancer, coma, liver failure, circulatory shock and long term organ damage. There are also many other activity that *E. elaterium* is helpful for traditional medicine describe mostly the use of the fruit juice as nasal drops for the treatment of jaundice in new born.

Risk of heavy metal contamination on vegetation by industrial waste, sewage sludge and exhaust from automobiles has been revealed Fe, Mn, Mg, Zn and Cr are beneficial to plant life in micro quantities, hence are trace nutrients. But they become toxic at higher concentrations [21-24]. Metals like mercury (Hg), cadmium (Cd), nickel (Ni), tin (Sn) etc. are in no way, good for living organisms. World Health Organisation (WHO) [25] has recognized health hazards of metals in food chain even at low concentrations. Studies have been conducted to evaluate the transferred elements from soil to plant [26]. Naturally occurring chelating agents have been known to interact with the metal ions and increase their availability to the plants. The low molecular weight organic acids released as root exudates were reported to be efficient phytochelators of metals [27].

MATERIALS AND METHODS

Collection of samples: *E. elaterium* seeds were collected from arid zone of Rajasthan, India. *E. elaterium* seeds washed with water. The collected seeds were oven-dried at 35°C, until a constant weight was obtained. The seeds were stored in an airtight container at room temperature to prevent any gain of moisture. The dried seeds were ground into fine powder using a Laboratory mill.

Oil extraction: Seeds of *E. elaterium* were dried in air. Oil from the seeds of *E. elaterium* was extracted by continuous extraction in soxhlet apparatus for six hours using petroleum ether (40-60°C) as solvent according to the method described [28]. At the end of the extraction, the solvent was separated by a rotary evaporator. The obtained oil was stored in cool place (refrigerator) until further

investigation [29]. The extracted oil was assayed using standard methods. The analytical values of seed and seed oil were determined according to the standard American Oil Chemist Society (AOCS) methods [30]. Methyl esters of oil were prepared using direct analytical TLC test [31], 2,4DNP TLC test [32], Halphen test [33], picric-acid TLC test [34], and alkaline picrate test [35] were also performed for indication of any unusual fatty acid.

Reagents and chemicals: All reagents were of analytical reagent grade. Acids used in the present study were obtained from different sources. Double de ionized water was used for all dilutions. HCl, H_2SO_4, H_2O_2, HF, $HClO_4$ and HNO_3 were of superior quality. All the plastic and glassware were cleaned by soaking in dilute HNO_3 and were rinsed with distilled water prior to use. The working standard solutions of heavy metals used for calibration were prepared by diluting a stock solution of 1000 µg L^{-1} (Pb, Cd, Zn, Fe, and Ni).

Mineral metal analysis: One of the methods for determination of the total contents and speciation analysis of heavy metals of their environmental concentrations is atomic absorption spectroscopy [36]. This method is simple and very selective.

Preparation of standard for metal: In spectrophotometric measurements we are concerned with solution having very small concentration of the metal to be determined. It follows that the standard solution which will be required for analysis must also contain very small concentration of the relevant metal. Standards are prepared by dissolving 1gm of metal cadmium, nickel, iron lead and zinc dissolve in minimum quantity of aqua regia (1:3) HCl and HNO_3, made up to 1liter in volumetric flask by adding deionised water. This is a stock solution which contain about 1000µg L^{-1} of required metal and then the working standard solution are prepared by suitable dilution of stock solution. The calibration curves for metal ions were drawn by taking working standard of 0-40 µg L^{-1} as require for the calculations.

Digestion of seed oil: *Ecballium elaterium* seed oil was digested in 100 mL Pyrex glass beaker. For digestion take 1g of seed oil add to 10 mL Concentrate Nitric acid. Keep it first for cold digestion for 24 h and then heat at 50°C for 4 h. The solution was finally boiled with 1:5 mixtures of concentrate acids HCl and HNO_3 in order to digest all organic matter and then filtered after cooling. Finally volume of the extract was made up to 25 mL using double distilled water.

Fatty acid analysis: The fatty acids composition of *Ecballium elaterium* plant oil was determined in two steps. In first step hydrolysis of oil was done and mixed fatty acids were obtained and in second step that mixtures of fatty acids were further derivative to their methyl esters. The formation of methyl esters was confirmed by thin layer chromatography (TLC). The methyl esters so obtained were analyzed by high pressure liquid chromatography [37].

RESULTS AND DISCUSSION

The Fatty acid composition of seed oils of *E. elaterium* from arid zone are presented in table 1. Fatty acids are the primary component of oils and fats. The Mono-unsaturated fatty acids were identified as linoleic acid (47.14%), punicic acid (21.98%), oleic acid (15.13%), stearic acid (4.71%), palmitic acid (3.98%) and myristic acid (0.08%). The major Poly-unsaturated fatty acid was linoleic acid (47.14%) present. results show that seeds oil from *E. elaterium* was found to be a rich source of polyunsaturated fatty acid, especially linoleic and punicic acids. *E. elaterium* was the only species in the Cucurbitaceae family characterized by the presence of punicic acid in seed oil. Table 2 represents the mineral content of seed oil of *Ecballium elaterium*. Iron (23.46 mg L^{-1}) is the major heavy metal present in *Ecballium elaterium* seed oil.

Table 1. Nutritional profile of *Ecballium elaterium*

S. No.	Fatty acid		% Composition
1	Linoleic acid	C 18:2	47.14
2	Punicic acid	C 18:3	21.98
3	Oleic acid	C 18:1	15.13
4	Stearic acid	C 18:0	4.71
5	Palmitic acid	C 16:0	3.98
6	Myristic acid	C 14:0	0.08

Table 2. Heavy metals content of seed oil of *Ecballium elaterium*

S. No.	Analyte	Concentration of heavy metals (Mg L^{-1}) in *Ecballium elaterium* seeds oil
1	Fe	23.46
2	Cr	3.40
3	Zn	0.97
4	Cu	0.23
5	Pb	0.09
6	Ni	0.08
7	Cd	0.03
8	Co	0.01

APPLICATION

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

CONCLUSION

The *E. elaterium* seed oil is good source of essential Omega-6 fatty acid, which is good for human health and useful for industries. Byproducts of oilseeds and parts of plant could be used as animal feedstock and as biomass in various applications. The seed oil is good source of nutrients. The concentrations of Fe, Cr, Zn, Cu, Pb, Ni and Co are measured for the estimation of pollution by heavy metals.

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