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Analysis of Heavy Metals and Physico-Chemical Properties of Ricinus Communis Seed Oil from Arid Zone of Rajasthan

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Abstract: ***Ricinus Communis, the castor oil plant. It is a species of flowering plant in the spurge family, Euphorbiaceae. It is the sole species in the monotypic genus, Ricinus, and subtribe, Ricininae. Its seed is the castor bean, which, despite its name, is not a true bean. It is widespread throughout tropical regions. Castor seed is the source of castor oil, which has a wide variety of uses. The Ricinus Communis seed oil was extracted by soxhelt using petroleum ether (60-80°C). The seeds contain 47% oil that is rich in triglycerides, mainly ricinolein. They also contain ricin, which is also present in lower concentration throughout the planet. Analysis of Heavy metals (Fe, Cr, Zn, Cu, Cd, Ni, Pd, Co) determined in seed oil. A Heavy metal is a member of a loosely defined subset of elements that exhibit metallic properties. The levels of heavy metals were determined by microwave plasma atomic emission spectroscopy (MP-AES). The Heavy metals found in seed oil in following order : Fe(5.17) > Cr(3.61) > Zn(0.56) > Cu(0.09) > Cd(0.03) > Ni(0.02) ~ Pb(0.02) > Co(0.01) mg/L respectively. The present found provide us a clue for the selection of plant species, which show natural resistance against toxic metals and are efficient metals accumulators. The composition of fatty acid of Ricinus Communis seed oil is unusual, that up to 90% of the total fatty acids consist of ricinoleic acid (12-hydroxy 18:1). The fatty acids composition of have been analysed as their methyl ester (FAME) by High Performance Liquid Chromatography (HPLC). It contains a relatively percentage of total lipid content 42.6% (Per dry weight), high Iodine value (84.1 mg/g). The seed oil moisture content, acid value and free fatty acid percentage (%FFA) were 0.2%, 4.88 mg/g and 3.4% respectively. The poly unsaturated fatty acid (PUFA) contents were 97.5% of the total fatty acid composition. Recinoleic acid comprises over 85.2% while other fatty acid present were linoleic (6.1%), oleic (5.5%), palmitic (1.3%), stearic (1.2%) and linolenic (0.5%) respectively.***
Keywords: Ricinus Communis, Heavy metals, PUFA, ricinoleic acid, Ricininae.

I. INTRODUCTION

The common names of Ricinus communis are castor bean, in Hindi it is Endi and in Sanskrit it is Eranda. The name Ricinus is derived from the Latin language and means ‘tick’, as the mottled seeds of the plant looks like the insect. Castor is native to eastern Africa, southeastern Mediterranean basin and India. But it grows well in all tropical regions. This plant has been under cultivation for more than 6000 years and it was widely used for cosmetics and oil lamps in ancient Egypt. Castor is a tall branched shrub which reaches a height of up to 4 m. The stem is hollow, erect and grayish green in colour when tender, and as it ages, the stem changes to brownish red colour. The leaves are seen in spirals with coarse toothed segments. The flowers are seen in terminal inflorescences which are green and sometimes red in colour. Male flowers have prominent stamens and spikes which are around 15 centimeters long. Fruits are greenish or reddish purple, spiny capsules which contain oval, large shiny bean like seeds with brownish mottling on them. Castor plant is cultivated and grown in the wild throughout the tropical, warm temperate and subtropical regions. Ricinus communis grows as a wild plant almost all over the country [1]. Castor oil is a pale yellow viscous liquid with mild odour and boils at 313˚C (595˚F). It has a density and molecular weight of 961 kg/m3 (0.961 g/cm3) and 933 g/mol respectively and is normally obtained by solvents extraction or mechanical pressing of dried castor seeds. The high content of ricinoleic acid with its three functional groups of hydroxyl, carboxylate and carbon-carbon double bond (alkene) is the main reason why castor oil has versatile application possibilities in the chemical industry [2]-[4]. It has been noted that castor oils differ depending on the geographical location in which the plant is grown and the agricultural modifications which have been made during growth [5]. The castor oil that is obtained from the cold pressing method has low acid value, low iodine value, lighter color and slightly higher saponification value compared to the solvent-extracted oil [4]. Castor oil is widely used as a catharsis, and also for lubrication and illumination. The oil as such or after modification finds extensive applications in industry. Castor oil is used to make soap, sulfated castor oil or Turkey Red Oil which is used to manufacture of detergent, and other forms of the oil are important for the treatment of leather,

industrial lubricants, and other industrial uses [6]. Castor oil is also regarded as one of the most valuable laxatives in medicine [7]. The oil extracted from castor bean seed has been used in medicines, cosmetics, biodiesel, plastics and lubricant production [8]. Castor oil also used in products like paints, enamels and varnishes, oiled fabrics, linoleum, patent leather, fly-paper, typewriting and printing inks, polishes, waxes, cutting, dielectric and condenser oil, softening agent for gelatin in rayon sizing, nitrocellulose-baking finishes, hydraulic brake fluids, urethane foams and rubber substitutes.

II. MATERIALS AND METHODS

A. *Materials*

Seeds were collected at maturity from arid and semi arid region of western Rajasthan (India). Harvested ripe castor fruits were manually cleaned and sun-dried for 4-5 days, until fruit capsules split open to discharge encased seeds. This was followed by seed pod removal and tray-winnowing to separate shells from beans (cotyledons). Castor beans were further dried (per 100g sample) at 80˚C to constant weight for 9 hrs in a hot air oven. The beans were then ground to a paste using mortar and analyzed immediately.

B. *Extraction Of Oil*

Oil extraction was performed from grounded seeds of R. Communis with light petroleum ether (40-60^{o}C) using soxhlet extraction technique. The solvent was removed completely under vacuum using rotary evaporator. The analytical values of seed and seed oil were determined according to the standard American Oil Chemist Society (AOCS) methods [9]. Methyl esters of oil were prepared using trans-esterification technique [10], Direct analytical TLC test [11], 2,4DNP TLC test [12], Halphen test [13], picric-acid TLC test [14] and alkaline picrate test [15] were also performed for indication of any unusual fatty acid.

C. *Reagents*

All reagents were of analytical grade. Double deionised water was used for all dilutions. HNO_3, H_2SO_4, H_2O_2, HF, $HClO_4$ and HCl 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 (Pb, Cd, Zn, Fe, and Ni).

D. *Digestion Of Seed Oil*

For the seed oil samples analysis, seed oil was digested in 100 ml Pyrex glass beaker. For this 1g of seed oil is digested with 10 mL of concentrated nitric acid. It is kept for cold digestion for 24 hours and then it is heated at 50˚C for 4 hours. The solution was finally boiled with minimum quantity of 1:5 mixtures of concentrated 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.

E. *Heavy Metals Analysis*

Iron (Fe), Nickel (Ni), Chromium (Cr), Lead (Pb), Zinc (Zn), Cadmium (Cd) and Copper (Cu) in plant samples were analyzed using microwave plasma atomic emission spectroscopy (MP-AES**)** equipped with nitrogen as the source gas for the plasma. Nitrogen flame was used for determination of metal content. It is used for simultaneous multi-analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gas.

Table 1: Heavy metals content of Ricinus communis seeds oil

S. No.	Analyte	Sample concentration unit (mg/L) of Ricinus communis seeds oil
1	Fe	5.17
2	Cr	3.61
3	Zn	0.56
4	Cu	0.09
5	Cd	0.03
6	Ni	0.02
7	Pb	0.02
8	Co	0.01

F. Preparation of mixed fatty acid

The fatty acid mixture was obtained by hydrolysis of oil and fats. First of all took 2 gm of oil sample is an oven dried round bottom flask then saponified with 2-3 ml of 1N standard alcoholic NaOH solution and 10 ml alcohol as solvent and refluxed over water bath at 70-80^{o}C for 1-2 hours monitored with TLC. The final mixture contained both saponified and unsaponified matter. Mixture was further diluted with 30 mL double distilled water. The saponified matter was removed by repetitive washing with diethyl ether in a separating funnel. The upper organic (ether) layer contained unsaponified matter which was taken in another beaker. After evaporation by rotatory evaporator diethyl ether is recollected. The lower aqueous solution, contained salt of fatty acids was acidified with dilute hydrochloric acid (HCl-6N). Fatty acid were extracted by repetitive washing of diethyl ether from this mixture of the lower aqueous layer discarded and the upper combined ether extract which contained mixture of fatty acid was collected in oven dried flask .

After evaporation of excess ether, these mixed fatty acid (MFA'S) were further washed with double distilled water and dried over Na_2SO_4 clean and pure MFA'S were collected. The whole procedure was monitored with help of TLC. The methyl esters so obtained were analyzed by HPLC. TLC plates were prepared by coating approx .025 mm of layer silica gel on glass plate. The mobile phase used in a consisting of mixture of petroleum ether; diethyl ether; acetic acid (70:29:1) the spot were made visible in iodine chamber.

The mixture of fatty acid were further derivatives into their esters and further quantized in HPLC and GC MS (gas chromatography, mass spectrometer) for quantitative analysis. For the preparation of fatty acid methyl ester (FAME), mixed fatty acid (MFA'S) were refluxed in a round bottom flask with excess methanol (1:6) on a water bath (100^{o}C for the approx 1-2 hours using 1% H_2SO_4 as catalyst. After completion of complete trans-esterification (monitored by TLC plate) removed assembly and flask was cooled at room temperature evaporate excess solvent and cooled over ice-bath and 30 ml double distilled water was added in it. Stirred wall and fatty acid methyl ester (FAME) were extracted with diethyl ether. The lower aqueous layer was discarded and upper combined ether extracted was collected in another over dried flask. After evaporate the solvent and dried over Na_2SO_4 anhydrous FAME obtained which were collected and stored at low temperature for further analysis.

G. Physio-Chemical Analysis

The physico-chemical properties such as saponification value, acid value, iodine value and peroxide value of Ricinus communis seed oils were determined, using the method described by AOCS.

The physico-chemical analysis includes number of parameters such as physical state, color, taste, the percentage of loss on drying as per standard method. Crucible was placed on hot plate until fumes of sulfuric acid ceased to evolve. The crucible with sulfated ash was then heated in a muffle furnace at 600^{o}C till the weight of the content became constant. Ash content as per method [16], ash value (water, alcohol and acid soluble or insoluble ash) as per method [17, 18].

Table 2: Physico-Chemical Parameters Value

S. No.	Characteristics	Value
1	Lipid Content	42.6
2	Moisture Content (%)	0.2
3	Iodine Value (Mg/G)	84.1
4	Acid Value (Mg/G)	4.9
5	Free Fatty Acid	3.4
6	Peroxide Value (Meq/Kg)	10.2
7	Saponification Value (Mg/g)	192
8	Unsaponifiable Matter(%)	3.4
9	Viscosity (centipoise)	332
10	Refractive Index	1.47
11	Average Molecular Weight	890
12	pH	6.16

H. Fatty Acid Methyl Ester Separation On Hplc

Methyl esters derivatives of mixed fatty acids are of particular value for analysis by means of HPLC. A modified method (with gradient elution) was used. The equipment Dionex HPLC with a degasser included a binary ultimate 3000 pump, Ultimate 3000 RS variable wavelength UV detector and a column (900×6.4mm) was packed with µBondapack C-18 and was eluted with acetonitrile-water in the proportions 67:33 (by volume) initially and is gradually changed to 74:26. The fatty acid composition is given in Table-3.

Table 3: Fatty acid composition of the oil from seed of Ricinus communis

S. No.	Fatty acid %		Composition
1	Ricinoleic acid	C 18:1	85.2
2	Linoleic acid	C 18:2	6.1
3	Oleic acid	C 18:1	5.5
4	Palmitic acid	C 16:0	1.3
5	Stearic acid	C 18:0	1.2
6	Linolenic acid	C 18:3	0.5

III. RESULT AND DISCUSSION

Heavy metals analysis in the seed oil shows the presence of Fe, Cr and Zn in high amount where as Cu, Cd, Ni, Pb and Co in trace amount. This gave phyto remedy for Fe, Cr and Zn where it is cultivated. Routine analysis carried out to extent of detioration of the environment. There should be a creation of database bank to study the metal pollution to assist in the understanding of metal cycling in the environment.

Fatty acid analysis shows the potential source of Omega-9 fatty acid (Ricinoleic acid C 18:1), which is a rare fatty acid and it is used in cosmetics as well as in Ayurvedic healing oil and as a laxative. It is also source of poly unsaturated fatty acids like Linoleic acid and Linolenic acid. It is having high iodine value and low moisture content which is due to cultivated in arid zone of Rajasthan.

The acid value (4.88mg/g) of the castor obtained by the present study is in the same range with the value obtained by Ogunniyi (2006); (10 mg/g), who discovered that solvent-extracted oil was high in acid value. The oil shows quite a high saponification value (327.4 mg/g), which was almost two times the values obtained by Jumat et al. (2010); (182.9 mg/g) and Ogunniyi (2006), (177-182 mg/g). The difference in the saponification value may be due to the quality of the oil and unfavourable environmental conditions [5, 19]. The percentage of crude lipids extracted from castor beans, their physical and chemical properties are shown in Table 2. It shows that castor seeds contain a relatively high percentage of total lipids content; 43.3% which is in the same range as reported by Gupta et al. (1951); (35.7% - 51.9%) for the African castor oil [20]. Koutroubas et al. (1999) reported that oil content was affected by both locations and castor oil genotypes. The seed oil content depends on the genotype but is also affected by the environmental conditions, cultural practices and time of harvesting [21].

The high viscosity of the oil is due to hydrogen bonding of its hydroxyl groups (Ogunniyi 2006).These values are favourable with ASTM standards and general specifications for industrial grade castor oil (WHC, 2012) [22]. Low acid value and pH value of 6.16 suggest low free fatty acid (FFA) content in the oil. Acid value is a measure of the FFA content in oil. Iodine value on the other hand, is a measure of the oil's unsaturation level, while peroxide value is related to its oxidation lavel as a result of hydroperoxide formation at double bond positions. Double bond unsaturation in oils therefore is one of the most important parameters that influence lipid oxidation. In castor oil though, the double bond on C-9 of ricinoleic acid is believed to be protected against hydroperoxide formation by the hydroxyl group on C-12 (ICOA, 2013) [23].

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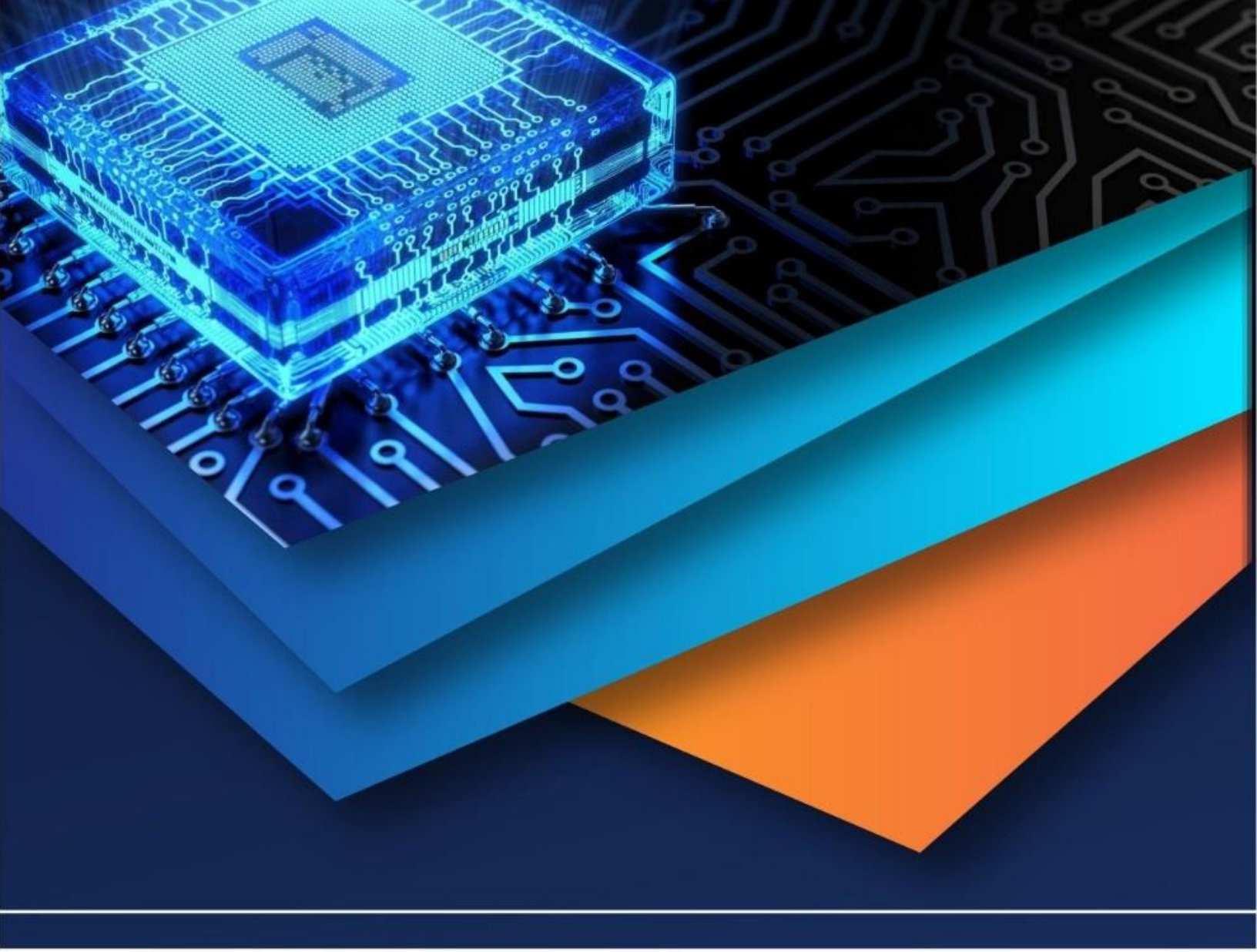

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