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Heavy Metals Analysis and Physico-Chemical Properties of *Brassica Campestris* Seed Oil from Western Rajasthan

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ABSTRACT:
Brassica campestris is an annual, erect and branched plant of the *Brassicaceae* family and a significant oil seed crop that plays a vital role in the agricultural sector. It is being consumed in enormous quantities across the world due to its nutritious values. Heavy metals impair crop growth and yield by interfering with other essential nutrients and impeding their uptake in plants. For our study, an industrially contaminated region in western Rajasthan was selected to collect seeds during the rabi season. *Brassica campestris* seed oil was extracted using an appropriate petroleum ether solvent in a Soxhlet extractor and then digestion of the seed oil was performed. Physicochemical characteristics such as percentage content of oil 39.32%, moisture 4.78%, ash content 3.88%, unsaponifiable matter 0.95%, saponification value 168.35 mg KOH/g, acid value 1.14 mg KOH/g and iodine value 107.27 g of I_2/100g of the seed oil were analysed. An Agilent 4210 MP-AES was used for the analysis of the concentration of heavy metals in the liquid digested *Brassica campestris* seed oil sample at different wavelengths. The transesterification process was used to prepare FAME and then GC-FID was used to scrutinise the quantitative analysis of the fatty acid methyl ester compositions. Seven major fatty acids were identified in Brassica campestris seeds oil which are erucic acid 45.28%, oleic acid 13.95%, α-linolenic acid 12.46%, linoleic acid 11.20%, eicosenoic acid 6.04%, palmitic acid 4.88% and stearic acid 1.35%. Erucic acid and oleic acid were the predominant fatty acids in it, so it can be utilised in lubricants, surfactants and a healthy human diet, but high and prolonged intakes of erucic acid may have a toxic effect on the heart.

Introduction

Brassica campestris is an upright, annual and branched plant of the *Brassicaceae (Cruciferae)* family with distinctive and identifiable properties. This species of mustard family is also known as Indian mustard, sarson, chinese mustard, kalamahore, oriental mustard, sarsapa, leaf mustard or field mustard. It is one of the most widely used herbs in the world [1]. The word "brassica" indicates the cabbage plant like appearance and "campestris" refers that it mainly grows in plain lands. One of the older references to its origin and cultivation originates from Central Asia, while the evolution of this crop took place in many countries throughout the globe [2]. *Brassica campestris* cropping is most common and predominant in sub-tropical and temperate climates in India during the winter season. Mustard has been a potential crop in the rabi (winter) season due to its wider adaptability and suitability to exploit residual moisture [3]. The leaves, seeds and stems of *Brassica campestris* are edible and play an important role in the human diet. Its seed oil is used for cooking purposes and the seed cake is used as cattle feed. It is also a valuable crop for the production of edible oil, industrial oil and biodiesel but depending on the fatty acid composition, it can also be utilised in a number of non-food and non-fuel industrial products. This plant possesses nutraceutical potentials such as anti-carcinogenic, anti-inflammatory, anti-cardiovascular and anti-ulcer properties, so it is used to cure many chronic and non-communicable diseases [4-6]. It is a rich source of several phytochemicals including antioxidants, phenolic compounds, vitamins, secondary metabolites [7]. The phytochemicals present in this crop also offer strong broad-spectrum support for protecting against the ubiquitous cancer-causing substances [8]. It is a popular crop and is being consumed in enormous quantities across the world due to its nutritional values [9]. It is a tolerant plant to heavy metals, grows rapidly and provides a large amount of biomass above ground level, so it is also known to be a beneficial plant for phytoremediation. [10-11].

Heavy metal contamination is a major problem in the environment, especially in growing medium-sized cities in developing countries like India. Once liberated into the environment through the air, drinking water, food or countless varieties of man-made chemicals and products, heavy metals enter into the body via inhalation, ingestion and skin absorption. If heavy metals enter and accumulate in body tissues more quickly than the body can remove them through its detoxification pathways, then a gradual build-up of these toxins occurs. Plants and animals take up metals from contaminated soil and water. Animals also take in metal pollutants from metals deposited on parts of plants exposed to contaminated environments. Environmental contamination has a complex influence on the growing plant in metallurgical work areas because the roots of the plant absorb heavy metals from the soil and aerosol pollutants penetrate from the atmosphere into the plant through the surface of the leaves.

Food crops, including cereals, seeds oil, vegetables and spices comprise the majority of the average human diet and are the vital sources of necessary vitamins, minerals and nutrients required by human beings to maintain good health. But due to the industrial revolution, agrarian soil has been heavily contaminated with various pollutants, including heavy metals. Cultivation of agricultural food crops in this heavy metal-contaminated soil results in the accumulation of these metals in the edible parts of the plants, which are directly consumed by humans [12]. The heavy metal toxicity of food crops is a huge concern because they have prolonged biological half-lives, are non-biodegradable and some are toxic even at extremely low concentrations [13]. Human activities such as industrial production, agriculture, mining and transportation release large amounts of heavy metal pollutants into the biosphere. The smelting of metal ores, fertilizers, the burning of fossil fuels, municipal wastes, pesticides and sewage are the primary causes of metal pollution [14].

Materials and Methods

Collection of Seeds

The seeds of *Brassica campestris* were collected from the industrial arid and semi-arid region of western Rajasthan when the colour of the leaves, stem and silique turned from green to pale or light yellow and also when the lower silique had a dried appearance and was fully mature. Its siliques have two carpels and a false septum; during maturity the two carpels are split and seeds come out easily. The collected seeds were dried in the sun light for ten to fifteen days. For maximum oil extraction, seeds were ground to a fine powder by using mortal-pestle and then the grinded seed powder was placed in a tight glass container for further processing.

Extraction of the Seed Oil

The Soxhlet extraction procedure was performed to extract oil from the powdered seeds of *Brassica campestris* by regularly washing them with hot petroleum ether (40–60) [15]. Grounded *Brassica campestris* seeds were placed in the thimble of a Soxhlet extractor, which had a condenser attached to one end and a round bottom flask attached to the other, in which petroleum ether was taken as a solvent. Using an electric mantle, the round bottom flask was heated up to 60°C for nearly 8 hours in order to accomplish an optimum extraction [16]. After extraction, a rotary evaporator was used for the complete removal of the solvent by creating a vacuum. The AOCS (American Oil Chemist Society) methods were implemented to assess the analytical values of seeds and seed oils [17].

Digestion of the Seed Oil

In a Borosil glass beaker, 1g of the *Brassica campestris* seeds oil sample was taken and then 5 ml of concentrated nitric acid was added to it and the mixture was placed at room temperature for 24 hours for cold digestion. Further, the beaker was heated on a hot plate to acquire a sample of almost dry seed oil by vanishing its moisture. The mixture formed in semi-solid condition was let to cool and re-poured 5 ml of concentrated nitric acid into it. The beaker was covered with a watch glass and again stored on the hot plate. The sample was continuously heated until complete digestion was performed. The remaining residues were boiled in the aqua regia solution of HCl and HNO_3 (3:1) to digest all organic matters. Deionized water was used to rinse the watch glass and beaker in order to retrieve sample residue. The sample that was obtained was diluted with deionized water in order to make the final volume up to 100ml [18].

Preparation of FAME

The oil of *Brassica campestris* was converted to FAME (Fatty acid methyl esters) by the transesterification process. The oil was heated up in a round bottom flask

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that was filled with excess of methanol and Sodium hydroxide as a catalyst and also a magnetic stirrer was placed during the whole process [19-20]. An amalgam mixture of FAME and glycerol was procure and a separating funnel was used to separate it. The below aquifer layer was discarded and in a dry flask the mixed ether extract from the top layer was collected. FAME was procured when the solvent evaporated and then thin layer chromatography (TLC) was used to confirm the formation of methyl esters. The obtained FAME was kept in a low-temperature nitrogen atmosphere for further analysis [21]. Thus, the quantitative analysis of the FAME obtained was scrutinise by HPLC (High-Performance Liquid Chromatography) and GC-FID (Gas Chromatography with Flame- Ionization Detector).

Analysis of Physicochemical Properties

Physicochemical characteristics including specific gravity, free fatty acid value, pH, unsaponifiable matter, ash content, acid value, moisture content, iodine value, density, flash point, kinematic viscosity and saponification value of *Brassica campestris* seeds oil were determined by the AOCS (American Oil Chemical society) methodology. According to standard procedures, the physicochemical analysis includes some factors such as the physical state, the percentage of oil, colour, taste, and the percentage of weight loss after drying.

Analysis of the Heavy Metals

The microwave plasma-atomic emission spectrometer was used for the analysis of heavy metals such as Zinc (Zn), Nickel (Ni), Copper (Cu), Cobalt (Co), Manganese (Mn), Chromium (Cr), Iron (Fe), Lead (Pb) and Cadmium (Cd) in the digested *Brassica campestris* seeds oil. This is a fast, sequential and multi-element analysis technique that uses microwave-induced nitrogen plasma as a source of excitation and is similar to inductively coupled plasma atomic emission spectrometry [22]. The Agilent 4210 MP-AES was used for heavy metal analysis and the liquid digested seed oil sample was aspirated and mixed with nitrogen gas and air. The nebulizer pressure was 140–240 kPa (optimized for each element) and the ignition temperature of the mixture over a flame varies from 2100 to 2800 ^{0}C. MP-AES was developed to improve analytical performance and productivity while reducing operating costs by eliminating the requirement of flammable and expensive gases in the basic elemental analytical techniques [23].

Results and Discussion

Physicochemical Assessment Results

The seeds oil of *Brassica campestris* is golden or brownish yellow in colour with a typical pungent aroma and flavour. The physicochemical characteristics of *Brassica campestris* seeds shown in Table 1 were analysed using the AOCS methodology.

Table 1: Physicochemical Characteristics

S. No.	Components	Value
1.	Oil Content (%)	39.32
2.	Moisture (%)	4.78
3.	Protein (%)	29.80
4.	Ash Content (%)	3.88
5.	Carbohydrate (%)	19.49
6.	Unsaponifiable Matter (%)	0.95
7.	Saponification Value (mg KOH/g)	168.35

8.	Iodine Value (g of I_2/100g)	107.27
9.	Acid Value (mg KOH/g)	1.14

The physicochemical components of *Brassica campestris* are summarised in Table 1, namely the saponification value, iodine value, acid value and percentage content of oil, moisture, protein, ash, carbohydrate and unsaponifiable matter. It could be concluded that its seeds contained higher amounts of both protein and oil. Also observed that it contained an adequate percentage of ash, moisture content and total carbohydrates. The saponification value, iodine value and acid value indicate that it can be utilised for soap making, polishing industry and medical purposes.

Heavy Metals Analysis Result:

Microwave plasma atomic emission spectroscopy 4210 (MP-AES) of Agilent Technologies with nitrogen as the source gas for plasma was used to analyse heavy metals in digested seed oil of *Brassica campestris* with a 0.9 calibration correction coefficient and at different wavelengths.

Table 2: Heavy metals concentration of *Brassica campestris*

S. No.	Element	Concentration (mg/L)
1.	Zn (Zinc)	28.63
2.	Fe (Iron)	13.16
3.	Cu (Copper)	1.14
4.	Mn (Manganese)	0.87
5.	Ni (Nickel)	0.32
6.	Co (Cobalt)	0.01
7.	Cr (Chromium)	0.02
8.	Pb (Lead)	0.28
9.	Cd (Cadmium)	0.17

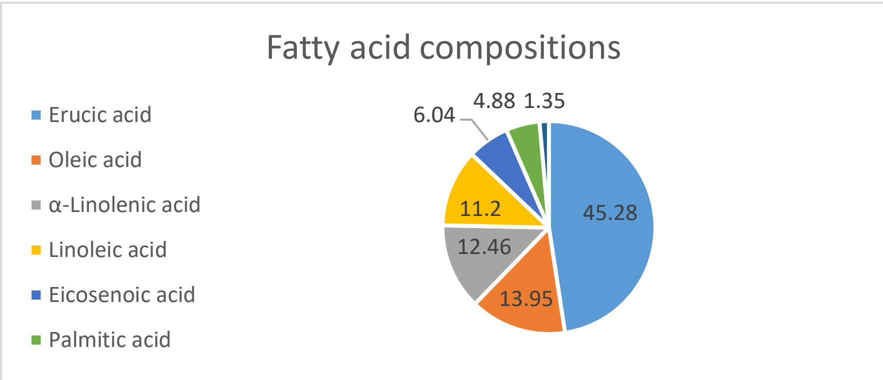
Fatty Acids Analysis Result:

The GC with flame ionization detector was used to scrutinise the quantitative analysis of the fatty acids methyl ester compositions. The compositions of both unsaturated and saturated fatty acids identified are shown in Table 3.

Table 3: Fatty acid compositions of *Brassica campestris*

S. No.	Lipid No.	Fatty Acids	Composition (%)
1.	C 22:1	Erucic acid	45.28
2.	C 18:1	Oleic acid	13.95

3.	C 18:3	α-Linolenic acid	12.46
4.	C 18:2	Linoleic acid	11.20
5.	C 20:1	Eicosenoic acid	6.04
6.	C 16:0	Palmitic acid	4.88
7.	C 18:0	Stearic acid	1.35



In *Brassica campestris* seeds oil, the seven major fatty acids were identified. A significant amount of long-chain monounsaturated fatty acids, mainly erucic acid, were found in its seed oil. Its seed oil contains 65.27% monounsaturated fatty acids, which include erucic acid (22:1), oleic acid (18:1) and eicosenoic acid (20:1). Amide derivatives of erucic acid are used as lubricants and surfactants. Oleic acid (ω-9) is a fatty acid that is a healthy source of fat in the normal human diet. Polyunsaturated fatty acids were reported 23.66%, which included α-linolenic acid (18:3) and linoleic acid (18:2). Linolenic acid is very popular in the beauty industry, but its presence in oil can make it tasteless and rancid. It contains a low 6.23% amount of saturated fatty acids, which include palmitic acid (16:0) and stearic acid (18:0). Erucic acid was the most important fatty acid in *Brassica campestris* oil and its high and prolonged intakes can have a toxic effect on the heart.

Conclusion

Brassica campestris is a significant oil seed crop consumed by the general population of the studied area in western Rajasthan. The results of the present investigation suggest *Brassica campestris* seed oil as a reliable and inexpensive source of fibre, ash and protein that is distinguished by an exceptionally high content of unsaturated fatty acids and resilient functional properties. The physicochemical properties indicate that its seeds provide opportunities to develop as pharmaceuticals, cosmetics, value-added products, lubricants, dietary supplements and surfactants. It is a tolerant plant to heavy contaminants, grows swiftly and has the ability to transfer or immobilise heavy metals (Pb, Zn, Cu, Fe, Mn, Ni, Cd,) in soil to reduce their adverse effects on ecosystems, so it can be an effective plant for phytoremediation. Furthermore, the results revealed that the fatty acid composition of *Brassica campestris* is not appropriate for prolonged edible usage. In broad terms, it possesses a high amount of erucic acid and low to moderate amounts of oleic (ω-9), linoleic (ω-6) and linolenic acids. Thus, there is a need to ameliorate its fatty acid profile.

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