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## Phytochemical, antioxidant investigations and fatty acid composition of *Lepidium sativum* seeds

V. Kumar<sup>1,3</sup>, V. Tomar<sup>2,3</sup>, S.A. Ranade<sup>1,3</sup>, H.K. Yadav<sup>1,3</sup> and M. Srivastava<sup>2,3\*</sup><sup>1</sup>Genetics and Plant Molecular Biology Division, CSIR-National Botanical Research Institute, Lucknow-226 001, India<sup>2</sup>Phytochemistry Division, CSIR-National Botanical Research Institute, Lucknow-226 001, India<sup>3</sup>Academy of Scientific and Innovative Research, Ghaziabad-201 002, India\*Corresponding Author Email : [m.srivastava@nbri.res.in](mailto:m.srivastava@nbri.res.in)

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### Abstract

**Aim :** The aim of the present study was to characterize *Lepidium sativum* seed for phytochemicals, fatty acid composition and antioxidant properties.

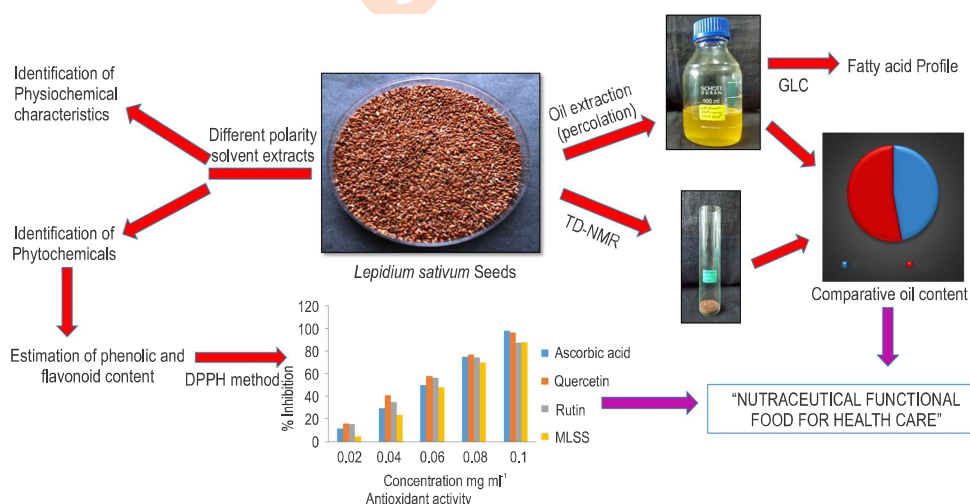
**Methodology :** Extraction of phytochemicals in different solvents, estimation of phenolics and flavonoids, physio-chemical properties, antioxidant assay by DPPH method was carried out. Seed oil content was determined by modified cold percolation extraction method and TD-NMR, followed by fatty acid compositional analysis using GLC.

**Results :** The perusal of data revealed that the total oil content varied from 21.36% to 24.38%, and the major fatty

acids identified were linolenic acid (24.447%) followed by oleic acid (24.413%) and palmitic acid (12.278%). Physio-chemical and phytochemical characteristics were estimated for useful functional properties and markers viz. alkaloids, sugar and proteins. *L. sativum* seeds exhibited high antioxidant potential (0.063mg ml<sup>-1</sup>) compared to standard compounds such as ascorbic acid, rutin and quercetin.

**Interpretation :** This study signifies the value of seed phytochemicals for its utilization in natural products as functional foods.

**Key words:** Antioxidants, Fatty acids, *Lepidium sativum*, Phytochemicals, TD-NMR



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## Introduction

*Lepidium sativum* L. (Family: Brassicaceae) commonly known as "Garden cress", a native of the Mediterranean region, is a small, smooth, erect, annual herb. It is widely cultivated in temperate and to some extent in sub-tropical climates throughout the world as a culinary and medicinal herb (Gokavi et al., 2004). In India, *L. sativum* is mainly cultivated in the states of Gujarat, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh (Kumar and Yadav, 2019). It is a fast-growing, edible plant botanically related to watercress and mustard with a similar peppery, sharp flavor and aroma. Nowadays, due to the availability of commercial antioxidants which are unsafe and toxic there is increased interest in the therapeutic potential of plants for the development of healthy fats and nutraceutical foods. The seed has diuretic, tonic, aphrodisiac, carminative, galactagogue and emmenagogue properties (Raval, 2016). In addition, health drink and food products incorporated with seed and its fractions are traditionally known and acceptable. Seed oil is reported to modulate the functions of immune competent cells (Doke and Guha, 2014). It is an important medicinal plant according to the Indian System of Medicine and Health (ISMH) and a source of several bioactive compounds (Chopra et al., 1956; Manohar et al., 2012; Kadam et al., 2012). Garden cress is recognized for several ethnopharmacological properties (Vohora and Khan, 1977) and it is used for the treatment of several disorders, including diarrhea, dysentery, liver diseases and damage (Manohar et al., 2012; Kadam et al., 2012; Wadhwa et al., 2012; Patel et al., 2000; Ghada et al., 2014).

The antioxidant activity of garden cress extracts has formed the basis of many applications, such as pharmaceuticals, alternative medicine, natural therapies and nutraceuticals. Seeds are rich source of mucilage as hydrocolloids, essential and non-essential amino acids and dietary fiber with water holding capacity for encapsulation and food formulations. Paste of seeds are effective against chapped lips and sun burns and when chewed treat soar throat, cough, asthma, headache and induces abortion (Gokavi et al., 2004; Kadam et al., 2012; Singh et al., 2015; Umesha et al., 2015). They are natural antioxidants, antimicrobial, chemo-protective agents, wound healing, hypoglycemic, laxative, immunomodulatory, reduced ulcerative colitis and used for bleeding piles, increasing lactation and birth control (Agarwal et al., 2013; Reddy et al., 2014; Sat et al., 2013; Sharma et al., 2011). Although, there are some available records on the medicinal properties of *Lepidium* seeds, however, its characterization in terms of functional application has not yet been explored in detail. Thus, the main objective of the present study was to characterize *L. sativum* seeds for useful phytochemicals, fatty acid composition and antioxidant properties for nutritional supplements/foods.

## Materials and Methods

**Collection of plant material:** *L. sativum* seeds were procured from local market of Lucknow and were grown in CSIR-NBRI

Lucknow field. Mature healthy plants were collected at flowering, fruiting stage and submitted in the Institute's Herbarium (Voucher specimen No. 302622). Seeds were dried in shade, ground to powder and sieved with 40 mesh (420 $\mu$ ) to remove coarse grits and ungrounded materials. The sieved fine powder was used for further studies.

**Solvent extraction of seeds and identification of phytochemicals:** Two gram of powdered seed was extracted with 100 ml different polarity gradient solvents (non polar to polar), i.e., hexane, chloroform, acetone, ethanol, methanol and water, respectively for 24 hrs. The extracts were filtered through Whatman filter paper no.42 and the process was repeated thrice. Different polarity solvent extracts were assayed for the presence or absence of primary and secondary metabolites like carbohydrates, proteins (Harborne, 1973), alkaloids (Kokate, 1991), flavonoids, tri-terpenoid, glycosides, steroids, phenolics and tannins (Trease et al., 1989).

**Physico-chemical properties of different extracts:** Physico-chemical properties of *L. sativum* seed extracts, like refractive index using Refractometer, color by Tintometer, conductivity with Conductivity meter, pH with pH meter and density with Pycnometer were determined by standard methods (Khandelwal, 2001).

**Quantitative analysis of total phenolic and flavonoid content:** A 0.5 ml of stock solution (1mg ml<sup>-1</sup> in methanol) was taken into 25 ml volumetric flask, added 10 ml water and 1.5 ml of folin ciocalteu reagent. The mixture was kept for 5 min and then added 4 ml 20% Na<sub>2</sub>CO<sub>3</sub> which was made up to 25 ml with distilled water. This was allowed to stand for 30 min and the absorbance was recorded at 765 nm (Ayurvedic Pharmacopoeia of India, 1989). Total phenolics content was calculated as Gallic acid (mg ml<sup>-1</sup>) using equation based on the calibration curve:  $y = 163.3x + 0.032$ ,  $r^2 = 0.993$ , where y is the absorbance and x is the Gallic acid equivalent (mg ml<sup>-1</sup>). In addition, the flavonoid content was estimated by adding 0.5 ml of sample (methanolic extract), 0.5 ml of 2% methanolic AlCl<sub>3</sub> solution. Standard solutions were prepared in similar way by using 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution (Quercetin solution). The yellow colour indicated the presence of flavonoids. Blank was prepared by using 0.5 ml 2 % methanolic AlCl<sub>3</sub>. All the solutions were made up to 5 ml with methanol and the absorbance of standard and sample solutions were recorded at 420 nm against blank after 1 hr (Ayurvedic Pharmacopoeia of India, 1989). Total flavanoids content was calculated as Quercetin (mg ml<sup>-1</sup>) by the equation:  $y = 46.32x + 0.027$ ,  $r^2 = 0.997$ , where y is the absorbance and x is the Quercetin equivalent (mg ml<sup>-1</sup>).

**Antioxidant assays-DPPH free radical scavenging activity:** The antioxidant activity of methanolic extract of seed powder was assayed on the basis of free radical scavenging effect of stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical by the modified method of Liyana and Shahidi (2005). DPPH (0.0053 g) was prepared in 100 ml methanol and this solution was kept in dark for

30 min. A 3 ml of this was solution was mixed with 1 ml of sample and the absorbance measured at 517 nm using UV-visible spectrophotometer (Thermo scientific, evolution 201). Ascorbic acid, Rutin and Quercetin were used as standard. The inhibition percent was calculated by the formula given below:

$$\text{Inhibition of DPPH (\%)} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}] \div \text{Absorbance (control)}}{\times 100}$$

The concentration of the extract providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph of inhibition percentage plotted against extract concentration.

**Oil extraction from seed:** Oil was extracted from 25 g seeds, anhydrous sodium sulphate and glass wool in definite ratio of 1:2:2 by the modified cold percolation method (Kalia *et al.*, 2002)]. Fine powder mixture (40 mesh) was put in column size 12×2 cm which already consists of cotton and anhydrous sodium sulphate. Hexane (SD-fine grade) was then poured as extracting solvent to obtain solvent oil mixture. The procedure was repeated thrice to collect the total oil mixture, the solvent was than finally evaporated through Rota-evaporator (IKA- Model no. RC 2 B SO22) to obtain oil.

**TD-NMR analysis:** Percent oil content in *Lepidium* seeds was determined (5 g in 40 mm glass tube) by NMR method (Hutton *et al.*, 1999; Jarret *et al.*, 2011), using a non-destructive Time-domain nuclear magnetic resonance (TD-NMR) Bruker (Madison, WI, USA) mq10 minispec NMR operating at a resonance frequency of 9.95 MHz and maintained at 40°C. For each signal acquisition, spin-echo parameters consisted of a 90°

pulse of 10.44  $\mu$ s and reading at 50  $\mu$ s followed by 180° pulse of 21.38  $\mu$ s (pulse spacing = variable) and reading at 7 ms.

**GLC analysis of seed oil:** Fatty acid methyl esters (FAME) of seed oil were prepared following the method of Morisson and Smith (1964) and fatty acids were estimated by gas-liquid chromatography (Shimadzu, GC-14B, Shimadzu Corporation, Japan) fitted with a fused silica capillary column (BP 21: 30 m length, 0.30 mm i.d., 0.50  $\mu$ m film thickness) using standard methyl esters references (Sigma Aldrich). The column, injector and detector temperature were maintained at 170°C, 230°C and 250°C, respectively, using nitrogen as the carrier gas.

**Statistical analysis:** Analysis was performed in triplicate. Average value and standard deviation was calculated and used.

## Results and Discussion

The study confirmed the occurrence of various phytochemicals including primary and secondary metabolites such as carbohydrates, proteins, alkaloids, flavonoids, triterpenoid, glycosides, steroids, phenolics, tannins along with previously reported phytochemicals (Ahmad *et al.*, 2015). Alkaloids and proteins were present in acetone, ethanol, methanol and water extract, while triterpenoids were present in acetone and methanol. Phenolics were also present in the methanol and water extract. Glycosides were present in hexane, chloroform and methanol extract, Carbohydrates were present in all extract, except acetone, while flavonoids were present in methanol extract only (Table 1). The quantitative estimation of phenolics and flavanoids content using Gallic acid and Quercetin

**Table 1:** Identification of phytochemicals/secondary metabolites in polarity gradient solvents of *Lepidium sativum* seed

Secondary metabolites	Functional groups	Assay method	Seed extract					
			H	C	A	E	M	DW
Alkaloids		Dragendorff	-	-	++	++	-	+
		Hager's	-	-	++	++	+	-
Carbohydrate		Molish's	+	-	-	+	++	++
		Benedict	-	-	-	-	+	-
	Reducing sugar	Fehling's	-	+	-	-	-	++
	Non-reducing sugar	Selivenoff's	-	-	-	-	-	-
Phenolics	Tannins	FeCl <sub>3</sub>	-	-	-	-	++	+
Tannin	Phenolics and tannins	Gelatine	-	-	-	-	-	-
Tri-terpenoids	Phenolics	Lead acetate	-	-	-	-	-	-
	Tri-terpenoids	Lebermann	-	-	+++	-	++	-
		Salkowski	-	-	-	-	++	-
Sterols	Steroidal glycosides	Killer Killiani	-	-	-	-	+	-
Proteins	Proteins	Xantho Proteic	-	-	+	++	++	+++
		Millon's	-	-	+	+	+	++
	Amino acids	Ninhydrin	-	-	-	-	++	+++
		Shinoda	-	-	-	-	+	-
Flavonoids		Brontrager's	+	++	-	-	+	-

'+' presence in traces; '++' presence in moderates; '+++' presence in abundance; '-' absence. Seed extract in H: hexane; C: Chloroform; A: Acetone; M: Methanol and DW.: Distil water

**Table 2:** Physico-chemical properties of different extract of *Lepidium sativum* seed

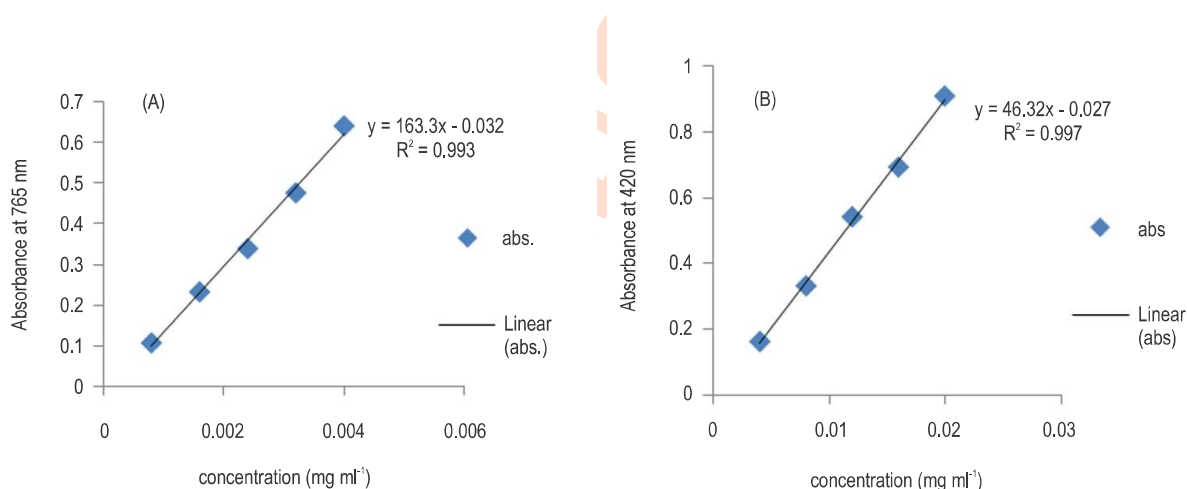
Physicochemical Parameters	Hexane	Chloroform	Acetone	Ethanol	Methanol	Water
Refractive Index (ns)	1.3787±0.013	1.3631±0.012	1.3571±0.015	1.3409±0.019,	1.3592±0.018	1.3315±0.016
Conductivity at 2 (mS)	0.00	0.04±0.01	0.00	0.54±0.09	0.00	1.043±0.02
pH	6.82±0.29	6.62±0.014	5.44±0.08	6.23±0.10	6.86±0.10	7.92±0.13
Colour Value	0	0	0	0	0	4±0.07
Density (kg m <sup>-3</sup> )	8.492±0.07	10.398±0.05	9.951±0.04	9.983±0.07	9.897±0.04	12.576±0.07

Values are mean ± SD

**Table 3:** Estimation of fatty acid composition in *Lepidium sativum* seed oil

Fatty acid	Carbon number	Retention time (min.)	Proportion (% of Total, on peak area basis)
Palmitic acid	16:0	35.645	12.278±0.06
Stearic acid	18:1	44.056	3.828±0.04
Oleic acid	18:0	44.645	24.413±0.09
Linoleic acid	18:2	45.966	11.085±0.11
Linolenic acid	18:3	47.870	24.447±0.04
Arachidic acid	20:0	51.658	4.137±0.05
Eicosanoic acid	20:1	52.207	11.789±0.07
Erucic acid	22:1	59.201	5.464±0.05

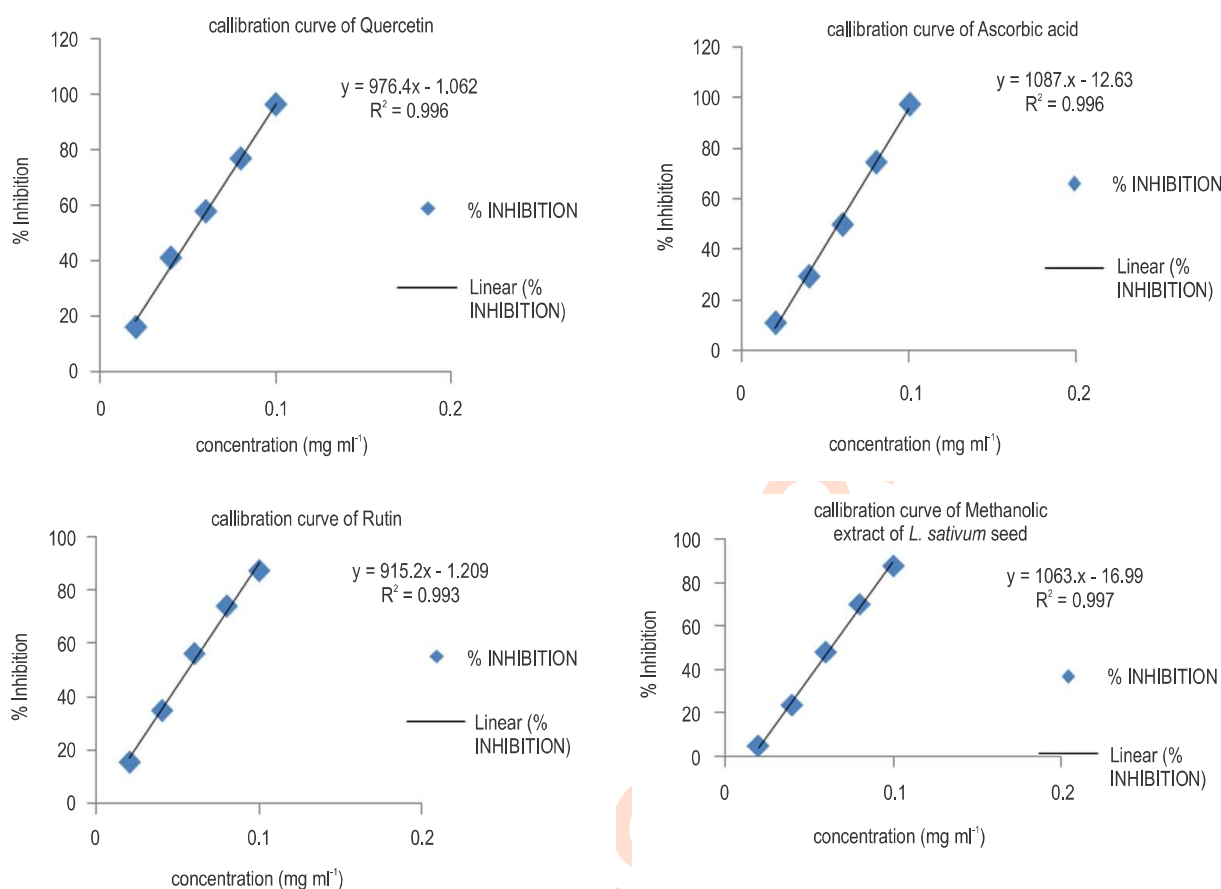
Values are mean ± SD

**Fig. 1:** Quantitative evaluation of (A) phenolics content using Gallic acid as standard and (B) Flavanoids using Quercetin as standard.

as standard was detected as 0.5% and 0.375%, respectively (Fig. 1). The results revealed that phytochemicals, phenolics and flavanoids are important components of *L. sativum* seeds for their antioxidant activity. The presence of phytochemicals depicts the potential of plants for enormous value in nutrition and health.

The physico-chemical parameters support important information about storage, structural stability and quality of

different extractives. The physico-chemical properties of different extracts of *lepidium* seeds (Table 2) showed some variations as compared to earlier report *i.e.* refractive index of different extracts ranged from 1.3315nD to 1.3787nD, while Ghada *et al.* (2014) reported refractive index of 1.47nD in *L. sativum* seeds oil maximum conductivity 1.043 in the water extract at 2 mS. Different extracts possessed pH ranging from 5.44 in acetone to 7.92 in water. The maximum colour value of extract



**Fig. 2:** Minimum inhibition conc DPPH free radical scavenging activity in standard (Quercetin, Ascorbic, Rutin) and methanolic extract of *Lepidium sativum* seed.

was found in water, likewise 3.20 colour value (4) was reported by Ghada *et al.* (2014). The colour of extracts is an important feature which often determines the consumer's acceptance. The extract of *L. sativum* extracts are yellow in color generally due to the occurrence of some pigments like chlorophyll and carotenoids (Diwakar *et al.*, 2010). The minimum and maximum density was recorded 8.492 in hexane and 12.576 in water extract. Further, these characteristics indicate the better shelf life of seed. Diwakar *et al.* (2010) reported that the oil of *Lepidium* seed is stable at refrigerated condition due to the presence of tocopherol and carotenoids, which are natural antioxidants.

DPPH is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule. It is usually used as a substrate to evaluate the antioxidative activity of compounds that are reported to be antioxidants (Duh *et al.*, 1999; Srivastava *et al.*, 2015). The extract at a concentration of 0.02 mg ml<sup>-1</sup> showed inhibition of 4.73% and 0.10 mg ml<sup>-1</sup> it was 87.78. The concentration of Ascorbic acid, Quercetin and Rutin varied from 0.02 to 0.10 mg ml<sup>-1</sup>. Ascorbic acid, Quercetin and

Rutin at a concentration of 0.02 mg ml<sup>-1</sup> exhibited inhibition of 11.29, 16.18 and 15.57% whereas at 0.10 mg ml<sup>-1</sup> concentration, 97.55, 96.03 and 87.48% inhibition was observed. A gradual increase in inhibition percent was observed with increase in the concentration of ascorbic acid, quercetin and rutin at same concentration in methanol extract of *L. sativum* seed. The IC<sub>50</sub> value of methanol extract of *L. sativum* seed (MLSS) was calculated by the concentration/effect regression lines (Fig.2). A lower value of IC<sub>50</sub> indicated a higher antioxidant activity. The IC<sub>50</sub> value of DPPH scavenging activity by MLSS (0.063 mg ml<sup>-1</sup>) were found to be extremely significant as compared to standards Ascorbic acid (0.057 mg ml<sup>-1</sup>), Quercetin (0.052 mg ml<sup>-1</sup>) and Rutin (0.055 mg ml<sup>-1</sup>). Nayan *et al.*, (2011) also showed similar IC<sub>50</sub> value for DPPH scavenging activity in seed extract i.e. 0.0395 mg ml<sup>-1</sup>.

In this study, the oil yield was found to be 21.567% using modified cold percolation method, which was reported to be significantly higher 14.73% as compared to earlier report (Yenge *et al.*, 2017). In case of nondestructive analysis to detect oil

content through TD-NMR, total oil content was significantly higher, (24.38%) in the seed. Niu *et al.* (2014) also determined oil content through TD-NMR in seed as compared to standard soxhlet extraction method for more accuracy. Thus, a standardized extraction protocol and non-destructive detection method established to obtain highly yielding seed oil from *L. sativum* seed. *L. sativum* seed is characterized by 20.243% saturated and 77.198% unsaturated fatty acids. Fatty acid analysis indicated palmitic (12.278%), arachidic (4.137%) and stearic (3.828%) as most abundant saturated fatty acids whereas the major unsaturated fatty acids detected were linolenic (24.447%), oleic (24.413%), eicosanoic (11.789%), linoleic (11.085%) and erucic (5.464%), respectively, (Table 3) which showed variable fatty acid composition as reported earlier (Ghada *et al.*, 2014).

The oil has 29.877% monounsaturated fatty acids (oleic and erucic) and 47.321% polyunsaturated fatty acids (linolenic, linoleic and eicosanoic). The unsaturated fatty acids are of significant importance for the nutritional quality of oil. The high content of linoleic acid and oleic acid up to 35.498 % in oil makes seed suitable for edible use. High linolenic content in oil (24.447%) prospects for fortification, preparing biscuits enriched with  $\alpha$ -linoleic acids for human consumption and use as a surfactant and softening agents (Umesha *et al.*, 2015). In addition, high collective content of Linoleic and Linolenic acid upto 35.532% maybe beneficial for clinical use. Similarly, Phang *et al.* (2019) reported that Linolenic and linoleic acid, these two fatty acids are essential for human health. Thus, *L. sativum* seeds are a good source of medicinal and economically important phytochemicals. Studies ascertain utilization of plant for domestic, industrial purpose and explore the real potential of seeds as/for markers, functional nutraceutical foods rich in antioxidant and healthy fats. Further studies to standardize fatty acid of value within permissible limits through breeding mechanism and functional modification will scope for health care and nutraceutical products.

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