Research Article



Lepidium sativum L. (Garden Cress) seed mucilage: Quantitative Assessment (Total Phenols, Flavonoids and Tannins) and FT-IR Analysis

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ABSTRACT

Objectives: *Lepidium sativum* L. (Garden Cress) is an herb of Brassicaceae family growing in warm climates. The seeds contain mucilaginous compounds that are known to have bioactive properties. In the present investigation, extraction of *Lepidiumsativum* L. seed mucilage was followed by preliminary screening for carbohydrates, protein, mucilage, and qualitative and quantitative assessment of phytochemicals viz. total phenols, total flavonoids and tannin content. The extracted mucilage was also subjected to FT-IR analysis.

Methods: Extraction of seeds of *L.sativum* was performed and it is dried at 60°C for 6-8 hrs, followed by qualitative tests for carbohydrates, protein, mucilage and phytochemicals. The total phenols were estimated using Folin Ciocalteu method, total flavonoids using Aluminium chloride method and tannin content using Folin Denis method. The FT-IR spectra was obtained using the KBr-pellet technique.

Results: The screening tests showed the presence of carbohydrates, amino acids and phytochemicals. The total phenolic, flavonoid and tannin content estimated was 109 ± 1.8 mgGAE/100 g, 96.2 ± 2.4 mgQE/100 g and 87.4 ± 3.2 mgTAE/100 g respectively. The FT-IR spectra of mucilage showed the presence of hydroxyl groups, carbohyd groups and confirm the region for carbohydrates and protein.

Conclusion: The phytochemical composition of garden cress seed mucilage can be a vital element in food functions which could strengthen the gum structure, improve food and gum interactions and enhance the shelf- life stability. In conclusion, garden cress mucilage appears to have distinct health benefits with added advantages as efficient additive in food products.

Keywords: Lepidium sativum, Hydrocolloids, Phenolic compounds, Antioxidants, Flavonoids, Tannins, FT-IR

Introduction

The Brassicaceae family, recognized as one of the largest plant families, includes over 300 genera and approximately 1,500 species, encompassing both medicinal plants and vegetable crops. Among these genera, *Anstatica, Arabis, Diplotaxis, Zilla and Lepidium.* are the most widespread. ^[1] Predominantly found in warm climates, members of this family are distributed globally, with the highest diversity located in West and Central Asia, as well as certain regions of North America.^[2,3] *Lepidium sativum*, commonly known as garden pepper cress or pepperwort, is an edible, fast-growing plant closely related to mustard and watercress, characterized by its tangy and peppery flavor. ^[4] This upright, glabrous, annual herb can reach heights of 15 to 45 cm and belongs to the mustard family. It is also referred to by several other names, including Halim, Chandrashura, Aaliv, and Asaliyo. Recent studies have highlighted the traditional uses of Lepidium sativum seed extract in addressing various clinical conditions, noting its properties as an aperient, diuretic, galactagogue, stimulant, anti-asthmatic, and antiscorbutic.^[5] The leaves of this plant also exhibit stimulant, diuretic, and antiscorbutic characteristics. Furthermore, oral intake of aqueous extract from *Lepidium sativum* has been shown to significantly reduce blood pressure.^[6] The seeds of *Lepidium sativum* contain

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mucilage present in the outermost layer^[7] comprising approximately 6-15% of the total seed composition.^[8,9] The present study aims to analyze the phytochemical composition this mucilage, focusing on total phenolic, total flavonoid, and tannin content, while also identifying functional groups using FT-IR analysis.

Materials and Methods

Garden cress seeds were purchased from the local market of Gwalior. All the chemicals used in the present investigation were of analytical reagent quality.

Extraction of Seed mucilage

Extraction of seed mucilage was carried out following the method adopted by Cui, 2001.^[10] The cress seeds (100 g) were washed and then mixed with 900 mL of distilled water. The seeds and water were stirred for 5 hours at 300 rpm at 60°C. Finally, the precipitated mucilage was dried in hot air oven for 24 hours at 60°C and the dried mucilage was stored in airtight containers.

Screening Tests for Carbohydrates, Proteins (Amino Acids), Mucilage, Alkaloids, Phenols, Flavonoids, Tannins and Glycosides present in *Lepidium sativum* seed mucilage were carried out according to protocols described in practical pharmacognosy by Khandelwal, 2008, p. 149-156.^[11]

Determination of Total Phenolic Content

Total phenolics were quantified by the FolinCiocalteu (FC) method as outlined by Siddhu et al., 2017.^[12] A gallic acid standard was prepared in concentrations ranging from 10 to 100 µg/ml. The sample was dissolved in distilled water to achieve a concentration of 100 µg/ml. To this, 500 µl of gallic acid and the test sample were combined with 2 ml of Folin-Ciocalteu reagent and a Na₂CO₃ solution. The mixture was then incubated at room temperature for 30 minutes, after which absorbance was measured at 765 nm. A standard curve for gallic acid was established for quantification purposes. The resulting data is expressed in milligrams of gallic acid per 100 grams of extract.

Estimation of Total Flavonoid Content

Aluminum chloride was utilized to assess the total flavonoid content, following the method outlined by Zou et al., 2004.^[13] A standard curve was established using Quercetin concentrations ranging from 10 to 100 μ g/ml. A sample concentration of 100 μ g/ml was prepared in distilled water. After a 6-minute incubation period, 150 μ l of AlCl3 solution was added and allowed to incubate for another 6 minutes. Subsequently, 2 ml of NaOH solution was introduced and incubated for an additional 15 minutes. Absorbance at 510 nm was measured. The results are presented in milligrams of QE per 100 grams of the extract.

Estimation of Tannins

The extraction of tannins was performed following method outlined by Rana et al., 2023^[14], utilizing the Folin-Denis reagent. In this procedure, 0.5 g of sample is dissolved in 75 mL of distilled and boiled for 30 minutes. It is then centrifuged at 2000 g rpm for 20 minutes. Subsequently, 1 mL of the extracted sample is again diluted with 75 mL of water. 10 mL Na2CO3 is added to 5 mL of Folin-Denis reagent, then diluted to 100 mL. Absorbance was measured at 700 nm. To establish a standard curve, tannic acid concentrations ranging from 0 to 100 mg were utilized.

FTIR analysis

The FTIR spectra of the mucilage sample was acquired using the KBr-pellet technique in which the samples were pulverized with potassium bromide to form pellets under 1 ton/cm² pressure. This analysis was conducted on a Perkin-Elmer spectrometer (MODEL: Spectrum One, USA) within the range of 4000–450 cm–1. For the infrared measurements, the transmission mode was employed, allowing the infrared beam to pass directly through the samples.

Results and Discussion

The results of the screening tests indicated the presence of carbohydrates, proteins (amino acids), mucilage, flavonoids, phenols, and tannins while demonstrating the absence of reducing sugars, ketohexose sugars, alkaloids, glycosides, and other compounds. These findings are presented in Table 1.

Table 1 shows preliminary confirmatory tests results ofGarden cress seed mucilage

S.N.	Test	Observation	Inference	
1	Molisch's	+	Carbohydrate is Present	
2	Benedict's	-	Reducing Sugar is Absent	
3	Barfoed's	-	Monosaccharide is Ab- sent	
4	Bials	+	Pentose sugar is Present	
5	Selwinoff's	-	Ketohexose (Fructose) is Absent	
6	Ruthenium Red	+	Mucilage is Present	

7	Wagner's	-	Alkaloid is Absent
8	Hager's	-	Alkaloid is Absent
9	Borntrag- er's	-	Glycosides are Absent
10	Alkaline Reagent	+	Flavonoids Present
11	Ferric Chlo- ride	+	Phenols and Tannins
12	Ninhydrin	+	Amino Acid is Present

Total Phenolic Content

In the present investigation the total phenolic content estimated using Folin–Ciocalteu (FC) method was 109 ± 1.8 mgGAE/100 g as shown in the Table 2. Findings of Tadele $(2019)^{[15]}$ showed higher values for TPC in 80% methanolic extracts of different garden cress seed varieties which were higher in black seeds than in red seeds, ranging from 10.55 ± 0.80 to 12.66 ± 0.70 mg GAE/g. Yet another study by Chatoui et al., $(2016)^{[16]}$ reported almost similar values as Tadale $(2019)^{[15]}$ ranging from 9.1 to 11.3 mg GAE/g. Zia-Ul-Haq et al., $(2012)^{[17]}$ reported TPC values ranging from 8.5 to 10.2 mg GAE/g, which is marginally lower than Ethiopian variants which could be due of climatic, geographical and soil variations. In contrast, TPC in methanolic extract was found to be 46.0 mg GAE/100 g in a study conducted by Rizwan Ahamad et al., $2015.^{[18]}$

Alternatively, variations in total phenol concentration were seen in the ethanolic and methanolic extracts of garden cress which ranged between 19.34 and 25.13 µg/ml in gallic equivalents. With 25.13 µg/ml of gallic acid, the ethanolic extract had the highest concentration. While methanolic extract yielded the lowest level, 19.34 µg/ml of gallic acid (Mohamed et al., 2023).^[19] According to Türkoğlu et al. (2018)^[20], the phenolic compounds act as antioxidants because of polyphenols and organosulphur compounds. These compounds as active antioxidant agents are secondary metabolites that have hydroxyl substituted and benzene rings with metal chelating abilities to aid in the protection against free radical effects caused by iron and copper.^[21,22,23]

Total Flavonoid Content

In this study, the estimated total flavonoids content (TFC) was 96.2 ± 2.4 mg QE/100 g as presented in Table 2. Tadele $(2019)^{[15]}$ reported that TFC values for black seeds from Fogera was notably higher, ranging from 5.93 ± 0.39 mg

CE/g to 8.47 ± 1.29 mg CE/g, exceeding the results of this study. Similarly, Deshmukh et al., $(2017)^{[24]}$ observed TFC values between 6.0 and 8.1 mg CE per gram of sample. Additionally, Rizwan Ahamad et al. $(2015)^{[18]}$ documented a TFC of 4.2 mg QE/100 g in their methanolic extract analysis.

Flavonoids represent a diverse class of phenolic compounds found in plant-based foods, comprising over six thousand distinct varieties. These low molecular weight compounds are characterized by a 15-carbon skeleton that includes two aromatic rings connected by a three-carbon bridge, forming a heterocyclic ring structure (Lafay et al., 2008; Vuolo et al., 2019).^[25,26] In culinary applications, flavonoids contribute to the taste, exhibit enzyme inhibition properties, serve as components of vitamin C, and possess the ability to protect against lipid peroxidation. Additionally, they are responsible for imparting color to various foods (Ferrer et al., 2008).^[27]

Tannin Content

The estimated tannin content in the current investigation was found to be 87.4 \pm 3.2 mg of tannic acid equivalent per 100g as depicted in the Table 2. Phytochemical screening conducted by Chatoui et al. (2016), Ahmad et al. (2015), Al-Snafi (2019), and Ghante et al. (2011) confirmed the presence of tannins in the methanolic extract of garden cress seeds.)^[16,18,28,29].

Tannins are complex polyphenols that are water-soluble and have molecular masses ranging from 500 to 3000 Da. They play a significant role in plant ecosystem interactions and act as antimicrobial agents by forming complexes with water, proteins, or alkaloids (Ferrer et al., 2008; Vuolo et al., 2019).^[27,26]

The properties of phenolic compounds, flavonoids, and tannins have been detailed in numerous studies. These compounds are found in significant quantities in seed mucilage, with results consistent with previous research and literature. Their presence positively impacts rheological properties and favourably alters sensory characteristics. Specifically, they can enhance the viscosity, elasticity, and stability of gums through non-covalent interactions with the gel network.^[28] (Ferrer et al., 2008). Additionally, these compounds contribute to increased firmness and water-holding capacity, which positively influence the development of stable product textures. Furthermore, phenolic compounds are known for their antioxidant properties, which improve the oxidative stability of food gums, thereby extending their shelf life.^[30] (Tsao, 2010).

In addition to enhancing the structural interactions of food

gums, phenolic compounds possess anti-inflammatory, anti-diabetic, and cardioprotective properties, which further enrich the functionality of food gums. This aligns with the growing consumer demand for 'nutritionally modified' food products (Williamson, 2017).^[31] The influence of phenolic compounds on the sensory attributes of food gums has also been recognized. As noted by Chung et al. (1998) ^[32], these compounds can either enhance or diminish the sensory characteristics of food products, depending on the formulation. Furthermore, phenolics exhibit antibacterial properties and have been shown to extend the shelf life of various processed food items (Chikezie et al., 2015).^[33]

Flavonoids, in particular, play a crucial role in the functionality of food gums, contributing to their structural and functional roles, as well as providing antioxidant benefits through free radical neutralization. These compounds enhance the functional performance of food gums regarding rheological properties, stability, and shelf-life, which can also be associated with sensory qualities and health benefits.

 Table 2 shows Total Phenolic content, Total Flavonoid content and Tannin content of Garden cress seed mucilage

FT-IR Analysis

The FT-IR spectra of Garden cress seed mucilage are illustrated in Fig. 1, with the peaks identified at specific wavenumbers. Table 3 summarizes the absorption peaks and the corresponding functional groups present in each region. The band in the 3500 to 3000 cm-1 range indicates free hydroxyl groups, characterized by stretching vibrations (Razavi et al., 2014; S. Razmkhah et al., 2016).^[34,35] The region between 1600 and 1500 cm-1 reveals Amide II, associated with bending vibrations, confirming the presence of proteins (Naji-Tabasi et al., 2016).^[36] Additionally, the band from 1600 to 1400 cm-1 signifies a carbonyl group with symmetrical stretching vibrations (Jindal et al., 2013; S. Razmkhah et al., 2016).^[37,35] The region between 800 and 1200 cm-1 is recognized as a fingerprint region for carbohydrates (Kang et al., 2011; S. Razmkhah et al., 2016). ^[38,35] Moreover, Guo et al. (2024)^[39] noted that the presence of free hydroxyl groups contributes to the reinforcement of the gel structure.

 Table 3 shows FT-IR Spectra of Garden cress seed mucilage

	Total flavonoids (mgQE/100g)	Tannin content (mgTAE/100g)	S.N.	Wavenumbers (cm ¹)	Functional Groups
Total Phenols			1	3279.98	(O-H)
(mgGAE/100g)			2	1598.47	N-H
109 ± 1.8	96.2± 2.4	87.4 ± 3.2	3	1411.34	COO
			4	1034.81	С-О-Н

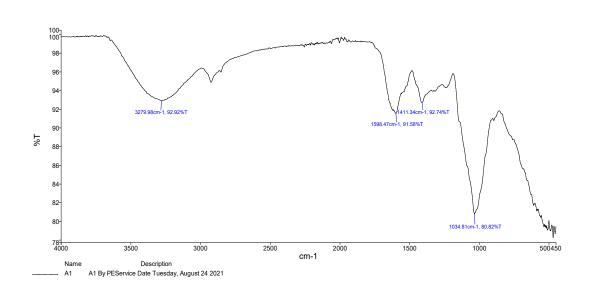


Fig 1 shows FT-IR spectra of Garden cress seed mucilage

Conclusion

Phenolic compounds serve a structural role and possess radical scavenging abilities, contributing to the overall biological activity in the interactions between food gums. Additionally, these compounds can influence the sensory and rheological properties, stability, and shelf life of food gums, as well as their health benefits. The pres-

Singh& Charu

Lepidium sativum L. (Garden Cress) seed mucilage: Quantitative Assessment

ence of functional groups such as hydroxyl and carboxyl can be advantageous in various food applications, as they facilitate nutrient absorption and digestion. Furthermore, these compounds demonstrate good degradability. Future efforts should focus on optimizing these attributes for a diverse array of applications within food matrices

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Conflicts of Interest

The authors declare no conflict of interest.

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