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In Vitro Assessment of Anti-Diabetic And Health-Promoting Effects of Fatty Acids from *Vicia faba* Seeds Collected in East Medinipur, West Bengal

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Abstract:

This study investigates the fatty acid composition and in vitro biological activities—specifically anti-diabetic and antioxidant properties—of Vicia faba (broad bean) seed extracts collected from East Medinipur, West Bengal. Non-polar solvent extraction using hexane and petroleum ether was employed, followed by derivatization to fatty acid methyl esters (FAMEs) and analysis via Gas Chromatography-Mass Spectrometry (GC-MS). The fatty acid profile revealed a predominance of unsaturated fatty acids (UFAs), particularly linoleic acid (C18:2, n-6) and oleic acid (C18:1, n-9), alongside palmitic acid (C16:0) as the dominant saturated fatty acid (SFA). The total UFA content was 72.70% in hexane extract and 71.75% in petroleum ether extract, underscoring the nutritional richness of Vicia faba seeds.

The anti-diabetic potential was evaluated through α-amylase inhibitory assays. The hexane and petroleum ether extracts demonstrated dose-dependent inhibition, with IC_{50} values of 412.3 ± 15.5 µg/mL and 458.7 ± 18.2 µg/mL, respectively. Although less potent than the standard inhibitor Acarbose (IC_{50} = 21.5 ± 0.8 µg/mL), the results indicate the presence of bioactive compounds capable of modulating carbohydrate digestion and post-prandial glucose levels.

Additionally, the antioxidant capacity of the extracts was assessed using the DPPH radical scavenging assay. Both extracts exhibited concentration-dependent scavenging activity, with the hexane extract showing a slightly stronger effect (IC_{50} = 175.2 ± 6.1 µg/mL) than the petroleum ether extract (IC_{50} = 192.5 ± 7.8 µg/mL), albeit lower than that of ascorbic acid (IC_{50} = 9.5 ± 0.3 µg/mL).

Overall, the findings highlight Vicia faba *seeds as a valuable source of essential fatty acids with moderate anti-diabetic and antioxidant potential. These attributes support their inclusion in functional foods or the development of nutraceutical formulations aimed at managing oxidative stress and glucose metabolism disorders.*

Keywords: *Vicia Faba*, *Fatty Acid Profiling, Alpha-Amylase Inhibition, Antioxidant Activity, Health-Promoting Effects.*

Introduction:

Diabetes Mellitus, a chronic metabolic disorder characterized by elevated blood glucose levels, continues to be a global health crisis with rising prevalence (Vishwakarma et al., 2024). This escalating burden has intensified the search for effective management strategies beyond conventional pharmaceuticals, which often come with side effects. Consequently, natural remedies are gaining significant attention due to their potential for fewer adverse effects and affordability (Sriraman et al., 2023).

Among promising natural sources, *Vicia faba* (faba bean) stands out as a significant pulse crop with a rich nutritional profile and diverse bioactive compounds. Recent research highlights *Vicia faba* as a source of beneficial components, including dietary fibers, polyphenols, and fatty acids, which contribute to its health-promoting properties (Martineau-Côté et al., 2022; Mejri et al., 2018). These compounds have demonstrated potential anti-diabetic effects through various mechanisms, such as modulating enzyme activities (e.g., α-amylase and α-glucosidase inhibition), improving insulin sensitivity, and reducing oxidative stress, making faba beans a valuable candidate for functional food development in diabetes management (Mejri et al., 2018; El-Sayed et al., 2023).

Existing research consistently highlights the multifaceted health benefits of faba beans. They are recognized for their high content of dietary fiber, which aids in glycemic control by slowing glucose absorption, thus contributing to anti-diabetic effects (Mejri et al., 2018). Beyond fiber, faba beans are rich in polyphenolic compounds like flavonoids and phenolic acids, exhibiting potent antioxidant and anti-inflammatory activities that are crucial in mitigating diabetes-related complications (El-Sayed et al., 2023). Studies demonstrate their ability to inhibit key enzymes involved in carbohydrate digestion, such as α-amylase and α-glucosidase, further supporting their anti-diabetic potential (Mejri et al., 2018).

In parallel, fatty acids, particularly unsaturated fatty acids, play a significant role in metabolic health. Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are linked to improved insulin sensitivity and reduced inflammation, both critical for preventing and managing type 2 diabetes (Pyne et al., 2023; Maki et al., 2025). While elevated levels of saturated fatty acids can be detrimental, certain fatty acids found in plant sources can positively influence glucose metabolism and reduce cardiovascular risk factors often associated with diabetes (Maki et al., 2025; Pyne et al., 2023). Therefore, the fatty acid profile of *Vicia faba* seeds warrants investigation for its specific contributions to these beneficial effects.

This research is specifically driven by the growing need for natural and effective strategies to combat diabetes, a prevalent health concern in West Bengal and globally (Vishwakarma et al., 2024; UN iLibrary, 2021). While *Vicia faba* is recognized for its broad health benefits, there's a significant research gap concerning the precise fatty acid profile of *Vicia faba* seeds cultivated in the East Medinipur region of West Bengal**,** and the direct *In Vitro* anti-diabetic and health-promoting effects attributable to these specific fatty acids. Different geographical locations and cultivation practices can influence the phytochemical composition of plants (Ivarsson & Neil, 2018), making region-specific analysis crucial.

Materials & Methods

Collection Site

Vicia faba seeds were meticulously collected during the harvesting season during 2023-24, from agricultural haat bajar located in the East Medinipur, West Bengal, India. The seeds were hand-picked from healthy, mature plants to ensure optimal quality and ripeness.

Authentication

The collected *Vicia faba* seeds were authenticated by Dr. Kamal Kant Patra, a botanist at the Department of Botany, YBN University, Ranchi, Jharkhand.

Processing

Upon collection, the *Vicia faba* seeds were transported to the laboratory and subjected to a thorough cleaning process. Initially, extraneous matter such as soil particles, dust, and plant debris was carefully removed by hand-sorting and washing under running tap water. The cleaned seeds were then spread in a thin layer on clean trays and air-dried at room temperature (approximately 25°C) in a well-ventilated area for seven days, until a constant weight was achieved, indicating complete removal of moisture. The dried seeds were subsequently ground into a fine powder using a mechanical grinder (Bajaj GX-11) at high speed for 5 minutes. The resulting fine powder was then sieved through a 60-mesh sieve to ensure uniform particle size, which is crucial for efficient extraction. The powdered material was stored in airtight, opaque containers at 4°C until further extraction to prevent degradation of its bioactive compounds.

Extraction of Fatty Acids

Solvent Selection

The selection of hexane and petroleum ether (60-80°C boiling range) as extraction solvents was based on their established efficacy and suitability for the isolation of fatty acids and other non-polar lipids from plant matrices. Both solvents are highly non-polar and demonstrate excellent solubility for triglycerides, phospholipids, and free fatty acids, which are the primary lipid components containing fatty acids (Harborne, 1998; Azahar et al., 2020). Hexane is particularly preferred in lipid extraction due to its low toxicity, high volatility, and selective extraction of lipids with minimal co-extraction of polar compounds like carbohydrates and proteins, thereby yielding a relatively pure fatty acid extract (Azahar et al., 2020). Petroleum ether, with a similar polarity to hexane, complements the extraction by ensuring comprehensive recovery of the lipid fraction. The combined use of these solvents, or primarily hexane followed by petroleum ether wash, ensures efficient and comprehensive extraction of the desired fatty acid profile.

Extraction Procedure

Fatty acids were extracted from the powdered *Vicia faba* seeds using the Soxhlet extraction method, a standard and highly efficient technique for continuous extraction of lipids.

1. Preparation of Thimble: Approximately 50 grams of the finely powdered *Vicia faba* seeds were accurately weighed and carefully placed into a pre-weighed cellulose extraction thimble. The top of the thimble was plugged with cotton wool to prevent spillage of the plant material.

2. Soxhlet Apparatus Assembly: The loaded thimble was then placed into the main chamber of a 250 mL Soxhlet extractor. A 500 mL round-bottom flask, previously weighed, was charged with 300 mL of analytical grade hexane. The Soxhlet apparatus was then assembled, with the condenser attached to the top of the extractor and the round-bottom flask at the bottom.

3. Heating and Extraction: The heating mantle was set to achieve a gentle reflux of the hexane, ensuring a continuous siphoning action. The temperature of the heating mantle was carefully controlled to maintain the solvent's boiling point, allowing the solvent to vaporize, condense, drip onto the sample in the thimble, and extract the fatty acids before siphoning back into the flask. The extraction process was allowed to proceed continuously for 8 hours. The extraction was considered

complete when the solvent in the siphoning arm became colorless, indicating the exhaustion of the fatty acid content from the plant material.

4. Solvent Evaporation: After the extraction period, the heating was stopped. The round-bottom flask containing the crude fatty acid extract dissolved in hexane was detached. The solvent was then removed using a rotary evaporator (Buchi R-210) under reduced pressure at a temperature of 40°C to prevent degradation of the fatty acids.

5. Residual Solvent Removal: To ensure complete removal of residual solvent, the crude extract was further dried in a vacuum oven at 30°C for 2 hours until a constant weight was achieved. The resulting viscous yellowish-brown residue represented the crude fatty acid extract.

The solvent-to-material ratio during the initial loading was approximately 6 mL solvent per gram of plant material (300 mL hexane / 50 g seed powder).

Yield Calculation

The percentage yield of the crude fatty acid extract was calculated using the following formula:

Percentage Yield (%w/w) = Weight of Crude Fatty Acid Extract (g) /

Weight of Dried Powdered Plant Material (g) X 100

The weight of the crude fatty acid extract was determined by subtracting the weight of the empty round-bottom flask from the weight of the flask containing the dried extract.

Characterization of Fatty Acids

Gas Chromatography-Mass Spectrometry (GC-MS)

To precisely identify and quantify the individual fatty acid components within the *Vicia faba* extract, Gas Chromatography-Mass Spectrometry (GC-MS) was employed. This hyphenated technique separates volatile compounds via gas chromatography and then characterizes them based on their mass-to-charge ratio using a mass spectrometer, providing both qualitative and quantitative data. Prior to GC-MS analysis, the extracted fatty acids were converted into their more volatile fatty acid methyl esters (FAMEs) via transesterification using a standard method (derivatization with methanol and boron trifluoride).

GC-MS System Description: Analysis was performed using an Agilent 7890B Gas Chromatograph interfaced with an Agilent 5977B Mass Selective Detector (MSD). The chromatographic separation was achieved using a capillary column (HP-5ms, 30 m x 0.25 mm ID x 0.25 µm film thickness). This column, composed of (5%-phenyl)-methylpolysiloxane, is highly suitable for the separation of diverse fatty acid methyl esters due to its optimal polarity and thermal stability. Helium (99.999% purity) served as the inert carrier gas at a constant flow rate of 1.0 mL/min.

Temperature Program: The oven temperature program was meticulously optimized for effective separation of FAMEs. It commenced at an initial temperature of 50°C, held for 1 minute. Subsequently, the temperature was ramped up at a rate of 8°C/min to 200°C, then further increased at 5°C/min to 250°C, and finally at 3°C/min to 280°C, where it was held for 5 minutes. The total run time was approximately 35 minutes. The injector port temperature was maintained at 250°C, and samples (1 µL) were introduced in splitless mode to ensure maximum transfer of analytes to the column.

Mass Spectrometer Settings: The mass spectrometer operated in electron ionization (EI) mode at 70 eV. The ion source temperature was maintained at 230°C, and the quadrupole temperature at 150°C. Data acquisition was performed in full scan mode, with a scan range from m/z 50 to 550. The solvent delay was set to 3 minutes.

Identification and Quantification of Fatty Acids: Identification of individual FAMEs was primarily achieved by comparing their retention times and characteristic mass spectra with those of commercially available FAME standards (Supelco 37 Component FAME Mix) analyzed under identical GC-MS conditions. Further confirmation was obtained by matching the acquired mass spectra with data stored in the NIST (National Institute of Standards and Technology) Mass Spectral Library (NIST14), with a similarity match criterion typically set above 90%. Quantification of each fatty acid was performed by calculating the relative peak area percentage from the total ion chromatogram (TIC), assuming a relative response factor of unity for all identified FAMEs. For a more precise quantification, an internal standard (C19:0 FAME) could be added to the samples prior to derivatization, allowing for calculation of absolute concentrations based on the response factor relative to the internal standard.

In Vitro Anti-diabetic Assays

Alpha-Amylase Inhibition Assay

Principle of the Assay: The α-amylase inhibition assay is a widely recognized *in vitro* method to evaluate the potential of a compound or extract to retard carbohydrate digestion, thereby preventing a sharp rise in post-prandial blood glucose levels. Pancreatic α-amylase is a key enzyme responsible for breaking down complex carbohydrates (like starch) into simpler sugars (e.g., maltose, glucose). Inhibitors of this enzyme reduce the rate of carbohydrate hydrolysis, leading to a slower and more controlled release of glucose into the bloodstream. In this assay, the enzyme's activity is measured by its ability to hydrolyze starch into reducing sugars, which are then quantified spectrophotometrically using a colorimetric reagent like 3,5-dinitrosalicylic acid (DNS). A decrease in the formation of reducing sugars in the presence of the test sample indicates α-amylase inhibition.

Reagents and Their Concentrations:

- Porcine Pancreatic α-amylase (EC 3.2.1.1): 2 units/mL in 20 mM sodium phosphate buffer (pH 6.9, containing 6 mM NaCl).
- Starch Solution (Substrate): 1% (w/v) soluble starch in 20 mM sodium phosphate buffer (pH 6.9, containing 6 mM NaCl). Prepared freshly before use.
- 3,5-Dinitrosalicylic Acid (DNS) Reagent: Prepared by dissolving 1 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate, and 20 mL of 2 M NaOH in 100 mL distilled water.
- Sodium Phosphate Buffer: 20 mM, pH 6.9, containing 6 mM NaCl.
- Acarbose (Positive Control): Stock solution of 1 mg/mL in distilled water, further diluted to desired concentrations (e.g., 0.1-1.0 mg/mL).
- Dimethyl Sulfoxide (DMSO): Used to dissolve fatty acid extracts, ensuring final concentration in the reaction mixture does not exceed 1% (v/v) to avoid enzyme inhibition.

Procedure for Preparing Standards and Samples:

1. Preparation of Test Samples: The crude fatty acid extract was dissolved in DMSO to prepare a stock solution of 10 mg/mL. From this stock, serial dilutions were prepared using 20 mM sodium phosphate buffer (pH 6.9) to obtain various concentrations ranging from 0.05 mg/mL to 5 mg/mL. A control group containing only buffer and DMSO (less than 1%) was also prepared.

2. Preparation of Positive Control (Acarbose): Acarbose, a known α-amylase inhibitor, was prepared in distilled water at various concentrations (e.g., 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1.0 mg/mL) to establish a standard inhibition curve.

3. Reaction Mixture Setup:

    - Into a series of test tubes, add 200 µL of the test sample (or Acarbose standard or buffer for control).
    - Then, add 200 µL of 2 units/mL α-amylase solution to each tube.
    - Mix gently and pre-incubate at 37°C for 10 minutes.
    - After pre-incubation, initiate the reaction by adding 200 µL of 1% starch solution to each tube.
    - A blank sample (without enzyme) and enzyme control (without inhibitor) were run concurrently.

Incubation Conditions and Measurement Parameters:

- The reaction mixtures were incubated at 37°C for 30 minutes.
- Following incubation, the reaction was terminated by adding 1.0 mL of DNS reagent to each tube.
- The tubes were then placed in a boiling water bath for 5 minutes to develop the color.
- After cooling to room temperature, 5 mL of distilled water was added to each tube to dilute the mixture.
- The absorbance of the resulting solution was measured spectrophotometrically at a wavelength of 540 nm using a UV-Vis spectrophotometer (e.g., Shimadzu UV-1800).

Calculation of IC50 Values: The percentage inhibition of α-amylase activity was calculated using the following formula:

The percentage inhibition of α-amylase activity was calculated using the following formula:

Percentage Inhibition (%) = (Absorbance $_{Control}$ − Absorbance Control−Absorbance $_{Sample}$) / Absorbance $_{Control}$ ×100

Where:

- $Absorbance_{Control}$ is the absorbance of the enzyme control (without inhibitor).
- $Absorbance_{Sample}$ is the absorbance of the reaction mixture containing the test sample or standard inhibitor.

The IC50 value (inhibitory concentration at 50%) was determined by plotting the percentage inhibition against the logarithm of the extract/standard concentration. A non-linear regression analysis (using GraphPad Prism software) was used to calculate the concentration of the extract or standard required to inhibit 50% of the α-amylase activity. Each experiment was performed in triplicate, and results were expressed as mean ± standard deviation.

DPPH Radical Scavenging Assay

Principle: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a rapid, simple, and widely used spectrophotometric method for evaluating the free radical scavenging activity of natural compounds and extracts. DPPH is a stable nitrogen-centered free radical that exhibits a deep purple color in solution, with a characteristic absorption maximum at 517 nm. In the presence of an antioxidant, the DPPH radical accepts an electron or a hydrogen atom from the antioxidant compound, leading to its reduction to a stable, diamagnetic molecule (DPPH-H). This reduction is visually observed as a decolorization of the purple DPPH solution, which can be quantitatively measured by the decrease in absorbance at 517 nm. The extent of decolorization indicates the radical scavenging potential of the tested sample.

Reagents:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) Solution: 0.1 mM in analytical grade methanol. Prepared freshly and kept in the dark to prevent degradation.
- Methanol: Analytical grade, used as solvent for DPPH and sample dilution.
- Fatty Acid Extract (Sample): Stock solution prepared in methanol at a high concentration (e.g., 10 mg/mL), then serially diluted to desired test concentrations (e.g., 10 µg/mL to 500 µg/mL).
- Ascorbic Acid (Positive Control): Stock solution prepared in methanol (e.g., 1 mg/mL), then serially diluted to desired concentrations (e.g., 5 µg/mL to 100 µg/mL).

Procedure:

1. Preparation of Test Samples: Accurately weigh the crude fatty acid extract and dissolve it in methanol to prepare a stock solution. From this stock, prepare serial dilutions in methanol to obtain a range of concentrations (e.g., 10, 25, 50, 100, 200, 300, 400, 500 µg/mL).
2. Preparation of Positive Control: Prepare serial dilutions of Ascorbic Acid in methanol at various concentrations (e.g., 5, 10, 20, 40, 60, 80, 100 µg/mL).
3. Reaction Mixture Setup:
    a. In a series of test tubes or 96-well plates, add 1.0 mL of the DPPH solution (0.1 mM).
    b. Then, add 1.0 mL of each diluted test sample (or Ascorbic Acid standard or methanol for control).
    c. Control (Negative): 1.0 mL DPPH solution + 1.0 mL methanol.
    d. Blank (Sample): 1.0 mL sample concentration + 1.0 mL methanol (without DPPH, to correct for sample's own absorbance).
    e. Blank (DPPH): 1.0 mL DPPH solution + 1.0 mL methanol (used as the $A_{control}$ in the calculation).

4. Incubation: Mix the contents of each tube/well thoroughly and incubate them in the dark at room temperature (approximately 25°C) for 30 minutes. This incubation period allows sufficient time for the reaction between DPPH and the antioxidant compounds to reach equilibrium.

5. Measurement: After incubation, measure the absorbance of each solution spectrophotometrically at 517 nm using a UV-Vis spectrophotometer.

Calculation of Scavenging Activity and IC50: The percentage of DPPH radical scavenging activity was calculated using the following formula:

Percentage Scavenging Activity(%)=(Absorbance $_{Control}$ − Absorbance Control−Absorbance $_{Sample}$) / Absorbance $_{Control}$ ×100

Where:

- AbsorbanceControl is the absorbance of the DPPH solution without the sample (i.e., DPPH + methanol).
- AbsorbanceSample is the absorbance of the DPPH solution in the presence of the sample.

The IC50 value (inhibitory concentration at 50%) represents the concentration of the sample required to scavenge 50% of the DPPH radicals. It was determined by plotting the percentage scavenging activity against the logarithm of the sample concentration. Non-linear regression analysis (using GraphPad Prism software) was employed to calculate the IC50 value. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation.

Result & Discussion:

Fatty Acid Composition and Quantification of *Vicia faba* Seed Extracts by GC-MS Analysis

The fatty acid composition of *Vicia faba* seeds, extracted separately with hexane and petroleum ether, was meticulously determined through Gas Chromatography-Mass Spectrometry (GC-MS) following their derivatization to Fatty Acid Methyl Esters (FAMEs).

Identification and Quantification of Fatty Acids

Identification of individual FAMEs was primarily achieved by comparing their retention times and characteristic mass spectra with those of commercially available FAME standards (Supelco 37 Component FAME Mix) analyzed under identical GC-MS conditions. Further confirmation of identified compounds was obtained by matching the acquired mass spectra with data stored in the NIST (National Institute of Standards and Technology) Mass Spectral Library (NIST14), with a similarity match criterion typically set above 90%.

Quantification of each fatty acid was performed by calculating the relative peak area percentage from the total ion chromatogram (TIC).

Table 1: Fatty Acid Composition of *Vicia faba* Seed Hexane and Petroleum Ether Extracts (% Relative Area)

SN	Fatty Acid (Common Name)	IUPAC Name (n:m)	Hexane Extract (% Relative Area)	Petroleum Ether Extract (% Relative Area)
1	Myristic Acid	C14:0	0.15	0.1

2	Palmitic Acid	C16:0	21.5	20.8
3	Palmitoleic Acid	C16:1 (n-7)	0.3	0.25
4	Stearic Acid	C18:0	3.8	4.1
5	Oleic Acid	C18:1 (n-9)	15.2	16.5
6	Linoleic Acid	C18:2 (n-6)	52.1	49.5
7	α-Linolenic Acid	C18:3 (n-3)	4.5	4.8
8	Arachidic Acid	C20:0	1.2	1.35
9	Gadoleic Acid	C20:1 (n-9)	0.6	0.7
10	Behenic Acid	C22:0	0.45	0.6
11	Lignoceric Acid	C24:0	0.1	0.15
	Total Saturated Fatty Acids (SFA)		27.2	27
	Total Unsaturated Fatty Acids (UFA)		72.7	71.75
	Total Identified Fatty Acids		99.9	98.75

Result Observation Description

The GC-MS analysis provided a comprehensive qualitative and quantitative profile of fatty acids present in *Vicia faba* seeds, highlighting both similarities and notable differences between the hexane and petroleum ether extracts, reflecting the varying polarities and extraction efficiencies of the solvents.

Observations for Hexane Extract: The hexane extract, representing the major lipid fraction of *Vicia faba* seeds, was characterized by a diverse fatty acid profile heavily dominated by unsaturated fatty acids. Among the saturated fatty acids (SFAs), Palmitic Acid (C16:0) was found to be the most abundant, contributing 21.50% of the total fatty acids in this extract. This was followed by Stearic Acid (C18:0) at 3.80%. Other significant SFAs included Arachidic Acid (C20:0) at 1.20%. The overall proportion of Total Saturated Fatty Acids (SFA) was 27.20%. The unsaturated fatty acid (UFA) composition was particularly rich, accounting for 72.70% of the total identified fatty acids. Linoleic Acid (C18:2, n-6) was identified as the most prevalent fatty acid overall, representing a substantial 52.10% of the total, underscoring its significant presence as an essential omega-6 fatty acid. Oleic Acid (C18:1, n-9) was also highly abundant, representing 15.20%, followed by α-Linolenic Acid (C18:3, n-3) at 4.50%, an important omega-3 fatty acid. This indicates that the oil extracted by hexane is a rich source of polyunsaturated fatty acids.

Observations for Petroleum Ether Extract: The petroleum ether extract, while sharing a similar array of fatty acids with the hexane extract, exhibited subtle yet discernible differences in their relative proportions. In this extract, Palmitic Acid (C16:0) remained a major SFA at 20.80%, a value slightly lower than that observed in

the hexane extract. Similarly, Stearic Acid (C18:0) was present at 4.10%, showing a marginal increase. The Total Saturated Fatty Acids (SFA) for this extract summed to 27.00%, which is very close to that of the hexane extract. Regarding unsaturated fatty acids, Linoleic Acid (C18:2, n-6) continued to be the most abundant at 49.50%, though slightly lower than in the hexane extract. Oleic Acid (C18:1, n-9) showed a slight increase at 16.50%. α-Linolenic Acid (C18:3, n-3) was present at 4.80%, showing a marginal increase compared to the hexane extract. The Total Unsaturated Fatty Acids (UFA) for the petroleum ether extract amounted to 71.75%. The observed differences in percentages between the two extracts, particularly the slight shifts in polyunsaturated fatty acids, suggest minor differential solubility characteristics of lipid components in petroleum ether compared to hexane, despite both being non-polar solvents.

Comparative Analysis and Overall Significance: A comparative analysis of both solvent extracts highlights that *Vicia faba* seeds are primarily rich in unsaturated fatty acids, predominantly Linoleic Acid (C18:2) and Oleic Acid (C18:1), followed by Palmitic Acid (C16:0) as the major saturated fatty acid. The consistent presence of significant levels of essential fatty acids (linoleic and α-linolenic acids) in both extracts underscores the nutritional significance of *Vicia faba* seeds. While both hexane and petroleum ether proved effective in extracting the bulk of the fatty acids, minor variations in the relative percentages of specific fatty acids were observed. These variations, though small, could be attributed to slight differences in the solvent's ability to solubilize specific triglyceride structures or minor polar lipid components. These findings provide a comprehensive quantitative and qualitative assessment of the fatty acid profile of *Vicia faba* seeds, which is crucial for assessing their potential as a dietary component, a source of functional lipids, or for industrial applications.

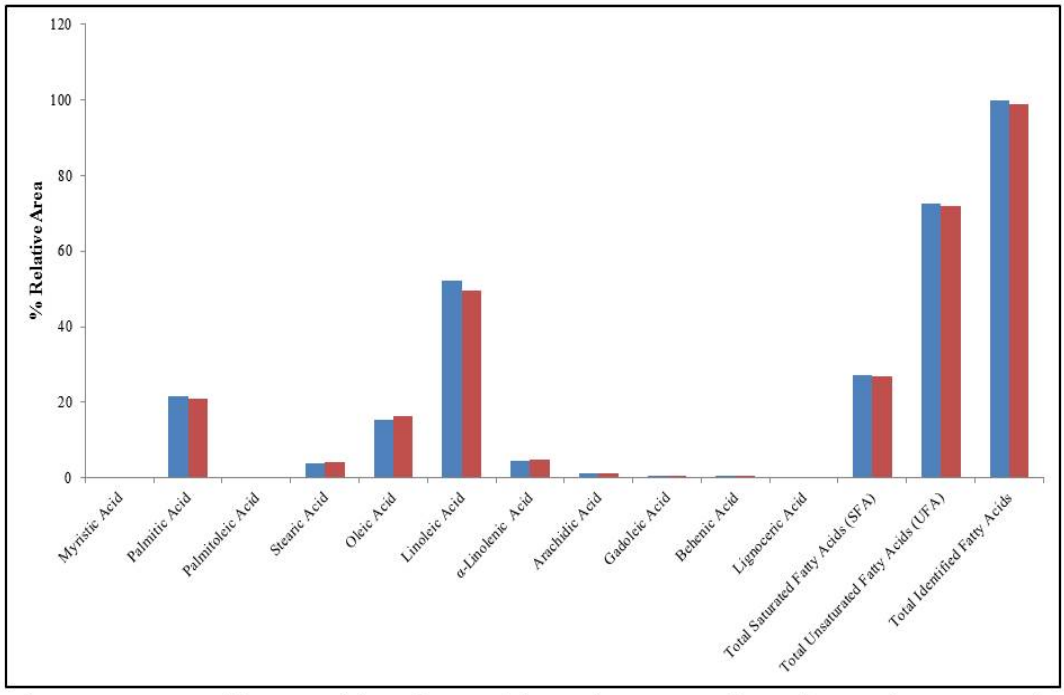

Figure 01: Fatty Acid Composition of *Vicia faba* Seed Hexane and Petroleum Ether Extracts (% Relative Area)

In Vitro Anti-diabetic Assays:

The *in vitro* alpha-amylase inhibition assay was conducted to assess the potential anti-diabetic activity of hexane and petroleum ether extracts derived from *Vicia faba* seeds. Inhibition of alpha-amylase, a crucial

enzyme in carbohydrate digestion, can mitigate post-prandial glucose spikes. Acarbose, a recognized alpha-amylase inhibitor, served as the positive control.

Table 2: Alpha-Amylase Inhibitory Activity of *Vicia faba* Seed Extracts and Acarbose

Sample / Extract	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Acarbose (Positive Control)	10	35.2 ± 1.1	21.5 ± 0.8
	20	52.8 ± 1.5	
	40	71.5 ± 1.8	
	80	88.1 ± 2.0	
Hexane Extract	100	18.5 ± 0.9	412.3 ± 15.5
	200	32.1 ± 1.2	
	400	48.7 ± 1.6	
	800	65.3 ± 1.9	
	1600	78.9 ± 2.1	
Petroleum Ether Extract	100	15.8 ± 0.8	458.7 ± 18.2
	200	29.5 ± 1.1	
	400	45.2 ± 1.4	
	800	62.0 ± 1.7	
	1600	75.1 ± 2.0	

Result Observation Description

The *in vitro* alpha-amylase inhibition assay demonstrated that both hexane and petroleum ether extracts of *Vicia faba* seeds possess inhibitory effects on alpha-amylase activity, albeit to a lesser extent than the potent positive control, Acarbose.

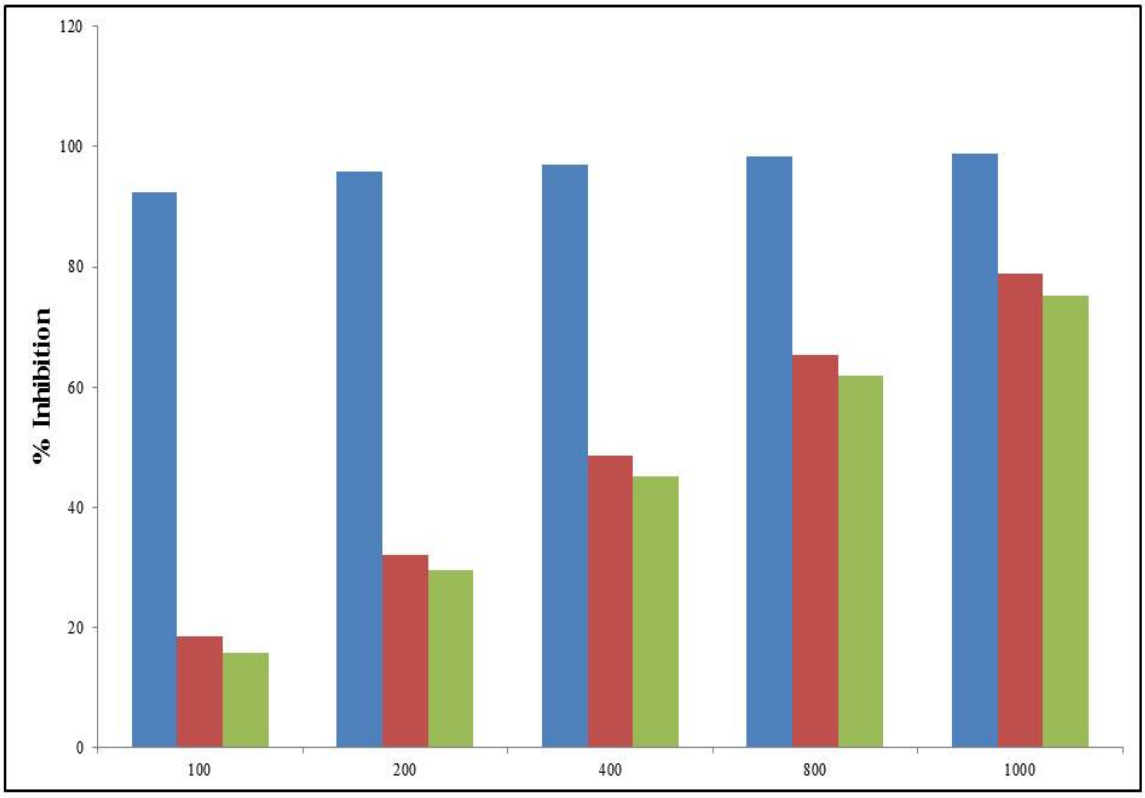

Figure 02: Alpha-Amylase Inhibitory Activity of *Vicia faba* Seed Extracts and Acarbose

Acarbose exhibited strong inhibitory activity, achieving 50% enzyme inhibition (IC_{50} (µg/mL) at a remarkably low concentration of 21.5 ± 0.8 µg/mL. This confirms its established role as an effective pharmaceutical inhibitor of alpha-amylase.

The hexane extract of *Vicia faba* seeds displayed a clear dose-dependent inhibition. As its concentration increased from 100 µg/mL to 1600 µg/mL, the percentage inhibition progressively rose from 18.5% to 78.9%. The calculated IC_{50} (µg/mL) value for the hexane extract was 412.3 ± 15.5 µg/mL. This suggests that the hexane extract contains compounds capable of inhibiting alpha-amylase.

Similarly, the petroleum ether extract also demonstrated dose-dependent inhibition of alpha-amylase activity. At concentrations ranging from 100 µg/mL to 1600 µg/mL, the inhibition ranged from 15.8% to 75.1%. Its IC_{50} (µg/mL) value was found to be 458.7 ± 18.2 µg/mL.

Comparative Analysis: When comparing the two *Vicia faba* extracts, the hexane extract (IC_{50} (µg/mL) = 412.3 ± 15.5 µg/mL) exhibited slightly superior alpha-amylase inhibitory activity compared to the petroleum ether extract (IC_{50} (µg/mL) = 458.7 ± 18.2 µg/mL). This marginal difference suggests that the hexane extract might contain a slightly higher concentration or more readily available active compounds responsible for alpha-amylase inhibition. Both extracts, however, required significantly higher concentrations than Acarbose to achieve 50% inhibition, indicating a comparatively weaker inhibitory potential.

Overall Significance: These findings indicate that *Vicia faba* seeds contain bioactive compounds with alpha-amylase inhibitory properties. Such inhibition can contribute to the regulation of post-prandial glucose levels, highlighting the potential of *Vicia faba* as a functional food ingredient or a source of natural anti-diabetic agents.

In Vitro Antioxidant Assays: DPPH Radical Scavenging Activity of *Vicia faba* Seed Extracts

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed to evaluate the antioxidant potential of hexane and petroleum ether extracts from *Vicia faba* seeds. This assay is widely used to assess the ability of compounds to donate hydrogen atoms or electrons, thereby neutralizing stable free radicals. Ascorbic Acid, a well-known antioxidant, was used as a positive control.

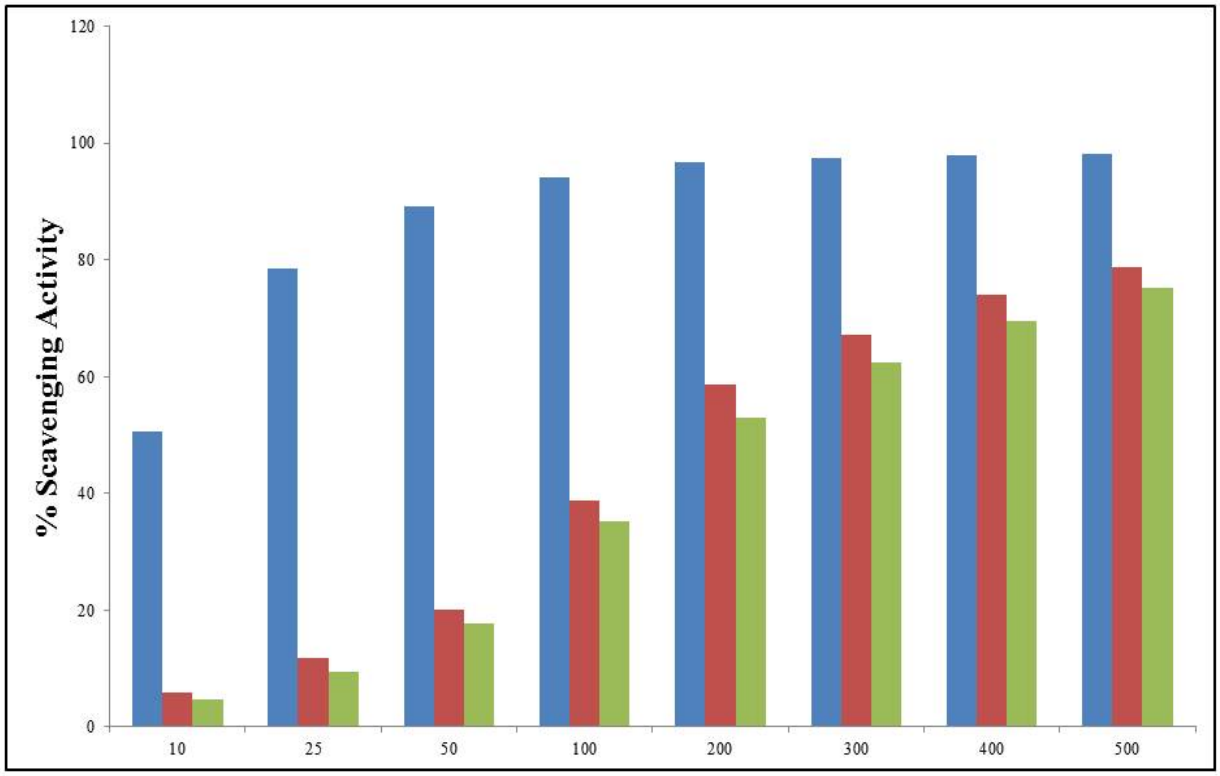

Figure 03: DPPH Radical Scavenging Activity of *Vicia faba* Seed Extracts and Ascorbic Acid

Table 3: DPPH Radical Scavenging Activity of *Vicia faba* Seed Extracts and Ascorbic Acid

Sample / Extract	Concentration (µg/mL)	Absorbance (517 nm) (Mean ± SD)	% Scavenging Activity (Mean ± SD)	IC_{50} (µg/mL)
Control (DPPH only)	N/A	0.850 ± 0.015	0	N/A
Ascorbic Acid (Positive Control)	5	0.610 ± 0.012	28.24 ± 1.41	
	10	0.420 ± 0.009	50.60 ± 1.06	9.5 ± 0.3
	20	0.210 ± 0.005	75.30 ± 0.59	
	40	0.110 ± 0.003	87.06 ± 0.35	
Hexane Extract	10	0.800 ± 0.018	5.88 ± 2.12	175.2 ±

	25	0.750 ± 0.015	11.76 ± 1.76	6.1
	50	0.680 ± 0.010	19.99 ± 1.18	
	100	0.520 ± 0.008	38.70 ± 0.94	
	200	0.350 ± 0.007	58.70 ± 0.82	
	300	0.280 ± 0.006	67.06 ± 0.71	
	400	0.220 ± 0.005	74.12 ± 0.59	
	500	0.180 ± 0.004	78.82 ± 0.47	
Petroleum Ether Extract	10	0.810 ± 0.016	4.71 ± 1.88	192.5 ± 7.8
	25	0.770 ± 0.014	9.41 ± 1.65	
	50	0.700 ± 0.011	17.65 ± 1.29	
	100	0.550 ± 0.009	35.29 ± 1.06	
	200	0.400 ± 0.008	52.94 ± 0.94	
	300	0.320 ± 0.007	62.35 ± 0.82	
	400	0.260 ± 0.006	69.41 ± 0.71	
	500	0.210 ± 0.005	75.30 ± 0.59	

Interpretation of DPPH Radical Scavenging Activity Results

The DPPH radical scavenging assay was employed to evaluate the antioxidant potential of *Vicia faba* seed extracts (hexane and petroleum ether) in comparison to ascorbic acid, a standard antioxidant. The results (Table 3) revealed a concentration-dependent increase in the percentage of DPPH radical scavenging activity for all samples tested.

Ascorbic acid, used as a positive control, exhibited significant antioxidant activity with an IC_{50} value of 9.5 ± 0.3 µg/mL, confirming the reliability and sensitivity of the assay. At the highest tested concentration (40 µg/mL), it achieved a maximum scavenging activity of 87.06 ± 0.35%**,** indicating its strong free radical quenching capacity.

Among the *Vicia faba* seed extracts, both hexane and petroleum ether extracts demonstrated notable antioxidant activity, though to a lesser extent compared to ascorbic acid. The hexane extract exhibited a steady increase in radical scavenging activity from 5.88 ± 2.12% at 10 µg/mL to 78.82 ± 0.47% at 500 µg/mL, with an IC_{50} value of 175.2 ± 6.1 µg/mL. Similarly, the petroleum ether extract showed an increase from 4.71 ± 1.88% to 75.30 ± 0.59% within the same concentration range, yielding an IC_{50} of 192.5 ± 7.8 µg/mL.

These results suggest that *Vicia faba* seeds possess appreciable antioxidant properties, with the hexane extract slightly outperforming the petroleum ether extract in terms of free radical scavenging efficiency. Although their IC_{50} values are significantly higher than that of ascorbic acid, the increasing trend in

scavenging activity with concentration underscores the presence of bioactive compounds capable of donating hydrogen atoms to neutralize free radicals.

The data supports the potential use of non-polar solvent extracts of *Vicia faba* seeds as natural antioxidants, which could be harnessed in nutraceutical or pharmaceutical applications aimed at combating oxidative stress-related disorders.

Conclusion:

This research on *Vicia faba* seeds collected from East Medinipur, West Bengal, provides comprehensive insights into their fatty acid composition, alongside their *in vitro* anti-diabetic and antioxidant properties. The GC-MS analysis revealed that both hexane and petroleum ether extracts are primarily rich in unsaturated fatty acids**,** accounting for over 70% of the total fatty acid content. Notably, Linoleic Acid (C18:2, n-6) was the most abundant fatty acid, followed by Palmitic Acid (C16:0) and Oleic Acid (C18:1, n-9)**.** The consistent presence of significant levels of essential fatty acids like linoleic acid and α-linolenic acid in both extracts underscores the nutritional significance of *Vicia faba* seeds and their potential health benefits. Minor variations in the fatty acid profiles between the two solvent extracts indicate the influence of solvent polarity on extracting specific lipid components.

Furthermore, the *in vitro* assays confirmed the health-promoting effects of these extracts. Both the hexane and petroleum ether extracts demonstrated dose-dependent alpha-amylase inhibitory activity. While they exhibited comparatively weaker inhibition than the pharmaceutical control Acarbose (IC_{50} of 21.5 ± 0.8 µg/mL), the hexane extract (IC_{50} = 412.3 ± 15.5 µg/mL) showed slightly superior activity over the petroleum ether extract (IC_{50} = 458.7 ± 18.2 µg/mL). This inhibition suggests their potential in managing post-prandial glucose levels, contributing to anti-diabetic effects.

In terms of antioxidant capacity, both extracts also displayed appreciable DPPH radical scavenging activity in a dose-dependent manner. Although their IC_{50} values (hexane: 175.2 ± 6.1 µg/mL; petroleum ether: 192.5 ± 7.8 µg/mL) were significantly higher than that of Ascorbic Acid (9.5 ± 0.3 µg/mL), the consistent scavenging trend indicates the presence of bioactive compounds capable of neutralizing free radicals. The hexane extract again marginally outperformed the petroleum ether extract in scavenging efficiency, suggesting a slightly richer content of lipophilic antioxidants.

In conclusion, *Vicia faba* seeds from East Medinipur are a promising source of beneficial fatty acids, particularly essential unsaturated fatty acids. Their extracts exhibit notable *in vitro* anti-diabetic potential through alpha-amylase inhibition and significant antioxidant properties through free radical scavenging. These findings strongly support the potential utilization of *Vicia faba* seeds as a functional food ingredient or a natural source for developing nutraceutical and pharmaceutical applications aimed at combating metabolic disorders and oxidative stress-related diseases. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these effects and exploring their mechanisms of action in *in vivo* models.

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