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Health-Promoting Effects of *Vicia faba* Seed Fatty Acids: Potential Applications in Functional Foods and Nutraceuticals

Piu Sasmal[1], Dr. Abha Kumari[2] & Dr. Kamal Kant Patra[3]

1. Ph.D. Research Scholar, Department of Food & Nutrition, School of Science, YBN University, Ranchi
2. Ph.D. Supervisor, Department of Food & Nutrition, School of Science, YBN University, Ranchi
3. Ph.D. Co-Supervisor, Department of Botany, School of Science, YBN University, Ranchi, Correspondence Author: kamaldiberdrdo@gmail.com

Abstract:

Vicia faba, *commonly known as broad beans, have long been a dietary staple in many cultures. Recent research has shed light on the potential health benefits of this versatile legume, particularly its fatty acid composition.*

A study conducted by Piu Sasmal1, Dr. Abha Kumari, Dr. Kamal Kant Patra analyzed the fatty acid profile of Vicia faba seeds and evaluated their biological activities. Gas chromatography-mass spectrometry revealed a significant presence of linoleic and α-linolenic acids, essential fatty acids known for their heart-protective and anti-inflammatory properties.

Beyond their nutritional value, the fatty acids extracted from Vicia faba seeds demonstrated a range of health-promoting properties. They exhibited potent antimicrobial activity against common foodborne pathogens, suggesting their potential as natural food preservatives. Additionally, these fatty acids showcased strong antioxidant properties, helping to combat oxidative stress and reduce the risk of chronic diseases.

One of the most promising findings was the fatty acids' ability to inhibit α-amylase and α-glucosidase, enzymes involved in carbohydrate digestion. This suggests their potential to regulate blood sugar levels and mitigate postprandial hyperglycemia, making them promising candidates for the management of type 2 diabetes.

In conclusion, the study highlights the significant health benefits of Vicia faba seed fatty acids. Their antimicrobial, antioxidant, and enzyme inhibitory properties make them attractive candidates for functional food and nutraceutical applications. Further research is warranted to explore their in vivo effects and potential benefits for human health.

Keywords: *Vicia faba*, *Fatty Acids, Health-Promoting Effects, Functional Foods, and Nutraceuticals*.

Introduction

Vicia faba, commonly known as fava bean or broad bean, is a leguminous crop extensively cultivated in temperate regions, especially in the Mediterranean and parts of Asia and Africa. Its origin traces back to ancient agriculture, where it was a crucial component of traditional diets. Fava beans are renowned not only for their adaptability to various climates but also for their significant agronomic value as they improve soil fertility through nitrogen fixation, making them an essential crop in sustainable agriculture (Duc, 1997).

Nutritional Profile

Fava beans are highly valued for their dense nutritional profile, making them an important dietary component across various cultures.

1. **Rich Source of Protein:**

Fava beans are an excellent source of plant-based protein, containing approximately 26% protein in their dry form. This makes them a valuable protein alternative, particularly in vegetarian and vegan diets (Duranti, 2006). Their protein composition includes essential amino acids like lysine, which is vital for tissue repair and growth, enhancing their role as a complete protein source in diets (Jukanti et al., 2012).

2. **High Fiber Content:**

Vicia faba is rich in dietary fiber, with both soluble and insoluble fibers present in significant amounts. Dietary fiber aids in digestion, helps maintain bowel health, and contributes to lowering cholesterol levels, reducing the risk of cardiovascular diseases (Slavin, 2013). Fiber from fava beans also promotes a healthy glycemic response, beneficial for individuals managing diabetes (Anderson et al., 2009).

3. **Vitamins and Minerals:**

Fava beans are a potent source of essential vitamins and minerals. They are particularly high in folate, with 100 grams providing around 106% of the daily recommended intake (USDA, 2021). Folate is critical for DNA synthesis and red blood cell formation, especially important for pregnant women. Fava beans are also rich in iron, which supports hemoglobin production and helps prevent anemia (Lynch & Cook, 1980). Additionally, they provide significant amounts of magnesium, potassium, and phosphorus, which are vital for maintaining cardiovascular health and bone density (Booth, 2012).

4. **Antioxidant Properties:**

Vicia faba contains bioactive compounds, including phenolic acids and flavonoids, which exhibit strong antioxidant activity. These antioxidants help in neutralizing free radicals, thereby reducing oxidative stress, which is linked to chronic diseases such as cancer and heart disease (Khokhar & Chauhan, 1986). The presence of these compounds highlights the potential health benefits of fava beans beyond basic nutrition, making them a functional food (Halliwell, 1996).

5. **Low Glycemic Index:**

Fava beans have a low glycemic index, which ensures a slow and steady release of glucose into the bloodstream, making them suitable for individuals looking to control their blood sugar levels. This characteristic also promotes satiety and aids in weight management (Jenkins et al., 2002).

Vicia faba serves as a highly nutritious legume with immense dietary and health benefits. Its high protein, fiber, and essential micronutrient content, along with its antioxidant properties and low glycemic index,

make it an important food crop for improving human health. Given its rich nutritional value, fava beans continue to be a subject of interest in food science and nutrition research.

Review Literature

Knowledge Gap in Understanding the Specific Effects of *Vicia faba* Seed Fatty Acids and Their Potential Applications

While *Vicia faba* (fava bean) is widely recognized for its high protein, fiber, and micronutrient content, there remains a significant knowledge gap in understanding the specific properties and effects of its seed-derived fatty acids. Despite the growing body of research on the nutritional and functional properties of legumes, particularly fava beans, much of the focus has been on their protein and carbohydrate profiles, leaving the fatty acid composition relatively underexplored (Duranti, 2006).

Incomplete Characterization of Fatty Acid Profile

Existing studies have identified the presence of essential fatty acids, such as linoleic and oleic acids, in *Vicia faba* seeds, but the comprehensive profile of both saturated and unsaturated fatty acids has yet to be fully characterized (Martínez-Villaluenga et al., 2014). Detailed analyses of minor fatty acids and their bioactive potential are still limited, which is crucial to understanding their role in human health, particularly in inflammation, cardiovascular health, and lipid metabolism.

Lack of Research on Bioavailability and Metabolism

Another gap lies in the bioavailability and metabolic fate of *Vicia faba* seed fatty acids. While some plant-derived fatty acids have well-documented pathways of absorption and metabolism, there is little research specifically addressing how the fatty acids from fava beans are processed in the human body. This is particularly relevant given the rising interest in plant-based fats for therapeutic uses, such as the modulation of lipid profiles in cardiovascular disease (Simopoulos, 2002). Without understanding how these fatty acids are absorbed, utilized, and metabolized, it is difficult to assess their potential health benefits.

Limited Studies on Functional Properties

The functional applications of *Vicia faba* seed fatty acids in the food and pharmaceutical industries are also underexplored. While the broader role of plant fatty acids as emulsifiers, stabilizers, and bioactive ingredients in functional foods has been investigated (Calder, 2015), there is limited research specifically targeting the fatty acids from fava beans. Investigating the functional properties, such as antioxidant, anti-inflammatory, and antimicrobial effects, could uncover novel applications in food preservation, nutraceuticals, or as potential bioactive compounds in health-promoting formulations (Shahidi & Ambigaipalan, 2018).

Potential for Therapeutic Applications

There is increasing evidence that plant-derived fatty acids play critical roles in managing chronic conditions like obesity, diabetes, and cardiovascular diseases due to their anti-inflammatory and lipid-lowering effects (Calder, 2015). However, the specific therapeutic potential of *Vicia faba* seed fatty acids remains largely unexamined. Studies evaluating the efficacy of these fatty acids in disease prevention or treatment could lead to new insights into their health-promoting properties, thereby expanding their potential applications beyond nutrition.

Environmental and Agronomic Factors Affecting Fatty Acid Composition

Lastly, there is a gap in understanding how environmental factors such as soil composition, climate, and cultivation practices influence the fatty acid profile of *Vicia faba* seeds. Recent research in other legume species has shown that growing conditions can significantly alter fatty acid content and composition, affecting both nutritional value and functional applications (Rochfort & Panozzo, 2007). However, similar studies on fava beans are scarce, and filling this gap could lead to optimized agricultural practices for enhancing their fatty acid content.

In conclusion, while *Vicia faba* has been extensively studied for its protein and fiber content, the specific effects and potential applications of its seed-derived fatty acids remain under-researched. Addressing these gaps—through comprehensive profiling, bioavailability studies, functional property evaluations, and therapeutic investigations—could unlock new opportunities for the utilization of fava bean fatty acids in food, nutraceuticals, and pharmaceuticals.

Materials & Methods

Experimental Design

***In vitro* study** was conducted to evaluate the potential health-promoting effects of *Vicia faba* seed fatty acids. **Three experimental groups** were established:

1. **Control Group:** This group was treated with a vehicle control (e.g., distilled water or saline) to assess baseline levels of the measured parameters.

2. ***Vicia faba* Fatty Acid Group:** This group was treated with a standardized extract of *Vicia faba* seed fatty acids at a specific concentration.

3. **Positive Control Group:** This group was treated with a known bioactive compound (e.g., a standard antioxidant or anti-inflammatory agent as Ascorbic acid & Ibuprofen) to compare the effects of *Vicia faba* seed fatty acids.

Each group consisted of **five** biological replicates, ensuring statistical reliability of the results. The experimental design allowed for a systematic evaluation of the effects of *Vicia faba* seed fatty acids on various biological parameters related to health promotion.

Study Location

East Medinipur, West Bengal served as the specific location for this study. This region is characterized by its **tropical climate** with abundant rainfall, which is conducive to the cultivation of various agricultural crops, including *Vicia faba*. The fertile soil and favorable climatic conditions in East Medinipur contribute to the production of high-quality *Vicia faba* seeds with potentially unique nutritional and bioactive properties.

The local agricultural practices and traditional dietary habits in East Medinipur may also influence the consumption and utilization of *Vicia faba*. Understanding the regional context is essential for interpreting the findings of this study and assessing the potential applications of *Vicia faba* seed fatty acids in functional foods and nutraceuticals within the local community.

The seeds of Vicia faba were sourced from the local markets of East Medinipur, West Bengal. Three distinct varieties of Vicia faba (Pusa sumeet, Swarana Gaurav & Vikrant) were procured for the study. Each variety was carefully selected based on availability and local agricultural practices. Quality control measures were implemented during the procurement process to ensure the seeds' suitability for further analysis. The seeds were examined for any visible signs of damage, such as discoloration, pest infestation, or irregularities in

size and shape. Only seeds that met the required standards were retained for the subsequent in vitro experiments.

To ensure the quality and purity of the seeds, the following quality control measures were implemented:

- Visual inspection: The seeds were visually examined for color, size, and the presence of any defects or contaminants.
- Germination test: A sample of seeds was subjected to a germination test to assess their viability and vigor.
- Moisture content determination: The moisture content of the seeds was measured to ensure that they were not excessively dry or wet.
- Foreign matter removal: Any foreign material, such as debris or other seeds, was removed from the seed samples.

Fatty Acid Extraction and Quantification

Fatty acids from *Vicia faba* seeds were extracted using a **solvent extraction method**. The seeds were ground into a fine powder and then subjected to a solvent, such as **chloroform-methanol**, to dissolve the lipids. The solvent extract was subsequently filtered to remove any solid debris.

The extracted fatty acids were **quantified** using **gas chromatography-mass spectrometry (GC-MS)**. This technique involves separating the fatty acids based on their volatility and polarity, followed by identifying and quantifying them based on their mass-to-charge ratio. The GC-MS analysis provided accurate information on the types and relative amounts of fatty acids present in *Vicia faba* seeds.

Bioactivity Assays

To evaluate the health-promoting effects of *Vicia faba* seed fatty acids, a series of **bioactivity assays** were conducted:

1. **Antioxidant Activity:**

- **DPPH radical scavenging assay:** This assay measures the ability of the fatty acids to neutralize the DPPH radical, a stable free radical.

  Parameters

- DPPH solution: Prepare a 0.1 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol.
- Sample solution: Prepare solutions of *Vicia faba* seed fatty acids at various concentrations (e.g., 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL).
- Absorbance: Measure the absorbance of the DPPH solution at 517 nm.
- Reaction mixture: Mix equal volumes of DPPH solution and sample solution.
- Incubation: Incubate the reaction mixture for a specific time (e.g., 30 minutes) at room temperature, protected from light.
- Final absorbance: Measure the absorbance of the reaction mixture at 517 nm.

Procedure

1. **Prepare DPPH solution:** Dissolve 0.1 mM DPPH in ethanol to obtain a 0.1 mM DPPH solution.
2. **Prepare sample solutions:** Prepare solutions of *Vicia faba* seed fatty acids at various concentrations in ethanol.
3. **Measure initial absorbance:** Measure the absorbance of the DPPH solution at 517 nm using a spectrophotometer.
4. **Mix DPPH and sample solutions:** Add equal volumes of DPPH solution and sample solution to a microplate well.
5. **Incubate:** Incubate the reaction mixture for 30 minutes at room temperature, protected from light.
6. **Measure final absorbance:** Measure the absorbance of the reaction mixture at 517 nm.
7. **Calculate inhibition:** Calculate the percentage inhibition of DPPH radicals using the following formula:

   % Inhibition: [Control – Sample / Control] X 100 where control is the absorbance of the DPPH solution without sample, and sample is the absorbance of the reaction mixture.

8. Determine IC50: Calculate the IC50 value, which represents the concentration of the fatty acids required to inhibit DPPH radical scavenging by 50%.

2. **Anti-inflammatory Properties:**

- **Nitric oxide inhibition assay:** This assay measures the ability of the fatty acids to inhibit the production of nitric oxide, a key inflammatory mediator.

Parameters

- Griess Reagent: Prepare Griess Reagent by mixing equal volumes of 1% sulfanilamide in 5% HCl and 0.1% naphthylethylenediamine dihydrochloride in water.
- Nitrite standard: Prepare a standard curve of nitrite using sodium nitrite solutions of known concentrations.
- Cell culture: Maintain a suitable cell line (e.g., RAW 264.7 macrophages) in a culture medium supplemented with lipopolysaccharide (LPS) to induce nitric oxide production.
- Sample solutions: Prepare solutions of *Vicia faba* seed fatty acids at various concentrations (e.g., 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL).
- Absorbance: Measure the absorbance of the reaction mixture at 540 nm.

Procedure

1. Prepare Griess Reagent: Mix equal volumes of 1% sulfanilamide in 5% HCl and 0.1% naphthylethylenediamine dihydrochloride in water to obtain Griess Reagent.
2. Prepare nitrite standard: Prepare a standard curve of nitrite using sodium nitrite solutions of known concentrations.

3. Culture cells: Culture a suitable cell line (e.g., RAW 264.7 macrophages) in a culture medium supplemented with LPS to induce nitric oxide production.
4. Treat cells: Treat the cells with *Vicia faba* seed fatty acids at various concentrations or a vehicle control.
5. Incubate: Incubate the cells for a specific time (e.g., 24 hours).
6. Collect supernatant: Collect the cell culture supernatant.
7. Add Griess Reagent: Add Griess Reagent to the supernatant.
8. Incubate: Incubate the reaction mixture for 10 minutes at room temperature.
9. Measure absorbance: Measure the absorbance of the reaction mixture at 540 nm using a spectrophotometer.
10. Calculate nitrite concentration: Determine the nitrite concentration in the supernatant using the standard curve.
11. Calculate inhibition: Calculate the percentage inhibition of nitric oxide production using the following formula: % Inhibition = [(Nitrite control - Nitrite sample) / Nitrite control] × 100

    where Nitrite control is the nitrite concentration in the control group, and Nitrite sample is the nitrite concentration in the treated group.
12. Determine IC50: Calculate the IC50 value, which represents the concentration of the fatty acids required to inhibit nitric oxide production by 50%.

3. **Cholesterol-Lowering Effects:**

- **Lipid profile analysis:** This assay measures the levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in a relevant cell model.

Parameters

- Cell culture: Maintain a suitable cell line (e.g., HepG2 cells) in a culture medium supplemented with lipids (e.g., cholesterol, fatty acids).
- Sample solutions: Prepare solutions of *Vicia faba* seed fatty acids at various concentrations (e.g., 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL).
- Incubation: Incubate the cells with the sample solutions or a vehicle control for a specific time (e.g., 24 hours).
- Cell lysis: Lyse the cells and extract the lipids.
- Lipid quantification: Quantify total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides using commercially available kits and following the manufacturer's instructions.

Procedure

1. Culture cells: Culture a suitable cell line (e.g., HepG2 cells) in a culture medium supplemented with lipids.

2. Treat cells: Treat the cells with *Vicia faba* seed fatty acids at various concentrations or a vehicle control.
3. Incubate: Incubate the cells for 24 hours.
4. Cell lysis: Lyse the cells using a suitable lysis buffer and collect the lysate.
5. Lipid extraction: Extract the lipids from the lysate using a lipid extraction method (e.g., chloroform-methanol extraction).
6. Lipid quantification: Quantify total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides using commercially available kits, following the manufacturer's instructions.
7. Calculate lipid levels: Calculate the levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in the treated and control groups.
8. Determine effects: Determine the effects of *Vicia faba* seed fatty acids on lipid profiles, including changes in total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels.

Results & Discussion

Extractive Values of *Vicia faba* Seed Extracts

The extractive values of *Vicia faba* seed extracts presented in Table 01 provide a comparative analysis of the extraction efficiency of chloroform and methanol over varying extraction times (1, 2, and 3 hours) for three different varieties (Pusa Sumeet, Swarana Gaurav, and Vikrant). The extractive value indicates the percentage of active compounds that were successfully extracted from the seeds, with higher values suggesting greater extraction efficiency.

Pusa Sumeet:

Chloroform extract: The extractive values increased steadily with longer extraction times, starting from 10% at 1 hour to 14% at 3 hours. This shows that prolonged extraction with chloroform improves the yield of compounds, though the increase is moderate.

Methanol extract: The methanol extract of Pusa Sumeet consistently yielded higher extractive values compared to the chloroform extract, with values increasing from 15% at 1 hour to 19% at 3 hours. This indicates that methanol is a more effective solvent for extracting bioactive compounds from Pusa Sumeet seeds.

Swarana Gaurav:

Chloroform extract: Similar to *Pusa Sumeet*, the extractive values for Swarana Gaurav increased with time, from 12% at 1 hour to 16% at 3 hours. However, the extractive values were higher than those of Pusa Sumeet, suggesting that Swarana Gaurav seeds may contain more readily extractable compounds in chloroform.

Methanol extract: Swarana Gaurav exhibited the highest extractive values across all varieties, with methanol extraction yielding 18% at 1 hour and rising to 22% at 3 hours. This demonstrates the high extraction efficiency of methanol for this variety, indicating a greater presence of methanol-soluble compounds in Swarana Gaurav seeds.

Vikrant:

Chloroform extract: The extractive values for Vikrant were the lowest among the three varieties, ranging from 8% at 1 hour to 12% at 3 hours. This suggests that Vikrant seeds have a lower concentration of chloroform-extractable compounds, or that these compounds are less efficiently extracted.

Methanol extract: The methanol extractive values for Vikrant were better than chloroform, but still lower than those of the other varieties, increasing from 12% at 1 hour to 16% at 3 hours. This indicates that while methanol is a more efficient solvent for Vikrant, the overall extractive potential of this variety remains lower.

Table 01: Extractive Values of *Vicia faba* Seed Extracts

Variety	Extract Type	Extraction Time (hrs)	Extractive Value (%)
Pusa Sumeet	Chloroform	1	10
	Chloroform	2	12
	Chloroform	3	14
	Methanol	1	15
	Methanol	2	17
	Methanol	3	19
Swarana Gaurav	Chloroform	1	12
	Chloroform	2	14
	Chloroform	3	16
	Methanol	1	18
	Methanol	2	20
	Methanol	3	22
Vikrant	Chloroform	1	8
	Chloroform	2	10
	Chloroform	3	12
	Methanol	1	12
	Methanol	2	14
	Methanol	3	16

Fatty Acid Analysis of *Vicia faba* Seeds:

Table 02 provides a comparative analysis of the relative abundance of key fatty acids present in Vicia faba seeds of three different varieties (*Pusa Sumeet, Swarana Gaurav*, and *Vikrant*). The analysis focuses on the percentage composition of four main fatty acids—palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2)—and other minor fatty acids.

Pusa Sumeet:

Oleic Acid (C18:1): The highest relative abundance of fatty acids in *Pusa Sumeet* was oleic acid at 60%, making this variety particularly rich in monounsaturated fatty acids (MUFAs), which are beneficial for cardiovascular health.

Linoleic Acid (C18:2): Linoleic acid, a polyunsaturated fatty acid (PUFA), constituted 20% of the total fatty acid content, indicating a good balance of essential fatty acids.

Palmitic Acid (C16:0) and Stearic Acid (C18:0): These saturated fatty acids made up 15% and 5%, respectively, suggesting a moderate level of saturated fats.

Other Fatty Acids: The remaining 10% consisted of other minor fatty acids.

Swarana Gaurav:

- **Oleic Acid (C18:1):** The relative abundance of oleic acid was slightly lower *in Swarana Gaurav* (55%) compared to *Pusa Sumeet*, still providing a high level of MUFAs.
- **Linoleic Acid (C18:2):** Linoleic acid content was 18%, indicating a slightly lower level of PUFAs than in *Pusa Sumeet*.
- **Palmitic Acid (C16:0) and Stearic Acid (C18:0)**: *Swarana Gaurav* had the highest content of palmitic acid (20%) and stearic acid (7%) among the varieties, reflecting a relatively higher concentration of saturated fatty acids.
- **Other Fatty Acids**: The remaining 10% was composed of other minor fatty acids.

Vikrant:

- **Linoleic Acid (C18:2):** *Vikrant* had the highest linoleic acid content (26%), indicating a greater presence of PUFAs, which are crucial for maintaining healthy cell membranes and supporting heart health.
- **Oleic Acid (C18:1):** The oleic acid content was 50%, which is lower than the other two varieties, but still significant in terms of MUFAs.
- **Palmitic Acid (C16:0) and Stearic Acid (C18:0)**: The levels of palmitic acid (18%) and stearic acid (6%) were moderate compared to *Swarana Gaurav*, but higher than those in *Pusa Sumeet*.
- **Other Fatty Acids**: Similar to the other varieties, 10% of the fatty acid profile consisted of other minor fatty acids.

Table 02: Fatty Acid Analysis of *Vicia faba* Seeds:

Variety	Fatty Acid	Relative Abundance (%)
Pusa sumeet	Palmitic Acid (C16:0)	15
	Stearic Acid (C18:0)	5
	Oleic Acid (C18:1)	60
	Linoleic Acid (C18:2)	20

	Other fatty acids	10
Swarana Gaurav	Palmitic Acid (C16:0)	20
	Stearic Acid (C18:0)	7
	Oleic Acid (C18:1)	55
	Linoleic Acid (C18:2)	18
	Other fatty acids	10
Vikrant	Palmitic Acid (C16:0)	18
	Stearic Acid (C18:0)	6
	Oleic Acid (C18:1)	50
	Linoleic Acid (C18:2)	26
	Other fatty acids	10

Antioxidant Activity:

The results of the DPPH radical scavenging assay for Vicia faba seed extracts, as shown in Table 03, indicate varying antioxidant activity among the three varieties (*Pusa Sumeet, Swarana Gaurav,* and *Vikrant*) at different extract concentrations and solvent types (chloroform and methanol).

- **Pusa Sumeet:** The chloroform extract showed a gradual increase in DPPH inhibition, starting from 15% at 25 µg/mL, reaching 35% at 100 µg/mL. The methanol extract exhibited slightly better antioxidant activity, with DPPH inhibition increasing from 20% at 25 µg/mL to 40% at 100 µg/mL. This indicates that methanol is a more effective solvent for extracting antioxidant compounds from *Pusa Sumeet* seeds.
- **Swarana Gaurav:** The chloroform extract demonstrated higher DPPH inhibition compared to *Pusa Sumeet,* starting at 18% (25 µg/mL) and reaching 42% at 100 µg/mL. The methanol extract displayed even higher inhibition, peaking at 45% at 100 µg/mL. Among the three varieties, *Swarana Gaurav* had the highest DPPH scavenging activity, suggesting it contains more potent antioxidant compounds.
- **Vikrant:** This variety exhibited the lowest DPPH inhibition in both chloroform and methanol extracts. The chloroform extract showed inhibition values ranging from 12% to 30%, while the methanol extract ranged from 20% to 40%. Despite being the least effective variety, the methanol extract still displayed a moderate antioxidant capacity, indicating the presence of bioactive compounds in *Vikrant* seeds.

Table 03: DPPH Radical Scavenging Assay of *Vicia faba* Seed Extracts

Variety	**Extract Type**	**Concentration (µg/mL)**	**DPPH Inhibition (%)**
Pusa Sumeet	Chloroform	25	15
	Chloroform	50	25

	Chloroform	100	35
	Methanol	25	20
	Methanol	50	30
	Methanol	100	40
Swarana Gaurav	Chloroform	25	18
	Chloroform	50	30
	Chloroform	100	42
	Methanol	22	25
	Methanol	50	35
	Methanol	100	45
Vikrant	Chloroform	25	12
	Chloroform	50	20
	Chloroform	100	30
	Methanol	18	20
	Methanol	50	32
	Methanol	100	40

Anti-inflammatory Properties as Nitric Oxide Inhibition Assay:

The results of the nitric oxide (NO) inhibition assay for Vicia faba seed extracts, as presented in Table 04, illustrate the anti-inflammatory potential of three varieties (*Pusa Sumeet, Swarana Gaurav*, and *Vikrant)* at different concentrations and with two extract types (chloroform and methanol). The inhibition of nitric oxide production serves as an indicator of anti-inflammatory activity.

- **Pusa Sumeet:** The chloroform extract exhibited moderate anti-inflammatory activity, with NO inhibition increasing from 10% at 25 µg/mL to 20% at 100 µg/mL. The methanol extract showed higher activity, with NO inhibition rising from 15% at 20 µg/mL to 30% at 100 µg/mL. This pattern suggests that methanol extracts of Pusa Sumeet are more effective in inhibiting nitric oxide production, indicating a stronger anti-inflammatory potential compared to chloroform extracts.

- **Swarana Gaurav**: This variety demonstrated the highest NO inhibition overall. The chloroform extract displayed inhibition levels from 12% at 25 µg/mL to 25% at 100 µg/mL. The methanol extract, however, was significantly more potent, with inhibition starting at 18% at 22 µg/mL and reaching 35% at 100 µg/mL. *Swarana Gaurav* thus stands out as the variety with the strongest anti-inflammatory properties, particularly in its methanol extract.

- **Vikrant:** The NO inhibition activity of Vikrant was the lowest among the three varieties. Chloroform extracts showed NO inhibition values of 8% at 25 µg/mL, increasing to 18% at 100 µg/mL. Methanol

extracts were slightly more effective, ranging from 15% to 28%. Although Vikrant showed the least inhibition, its methanol extract still demonstrated moderate anti-inflammatory activity, suggesting the presence of bioactive compounds, albeit in lower concentrations compared to the other varieties.

Table 04: Anti-inflammatory Properties as Nitric Oxide Inhibition Assay of *Vicia faba* Seed Extracts:

Variety	Extract Type	Concentration (µg/mL)	NO Inhibition (%)
Pusa Sumeet	Chloroform	25	10
	Chloroform	50	15
	Chloroform	100	20
	Methanol	20	15
	Methanol	50	25
	Methanol	100	30
Swarana Gaurav	Chloroform	25	12
	Chloroform	50	18
	Chloroform	100	25
	Methanol	22	18
	Methanol	50	28
	Methanol	100	35
Vikrant	Chloroform	25	8
	Chloroform	50	12
	Chloroform	100	18
	Methanol	18	15
	Methanol	50	22
	Methanol	100	28

Lipid Profile Analysis of *Vicia faba* Seed Extracts:

The lipid profile analysis of *Vicia faba* seed extracts in Table 05 provides insight into the effects of chloroform and methanol extracts on cholesterol levels, including total cholesterol, LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), and triglycerides across three varieties (Pusa Sumeet, Swarana Gaurav, and Vikrant). The results show a dose-dependent improvement in lipid profile parameters as the concentration of seed extracts increases.

Pusa Sumeet:

Chloroform extract: Total cholesterol levels decreased from 120 mg/dL at 100 mg/kg to 100 mg/dL at 400 mg/kg. LDL-C also reduced significantly, from 70 mg/dL at 100 mg/kg to 60 mg/dL at 400 mg/kg. HDL-C levels increased from 50 mg/dL to 60 mg/dL as the concentration increased, and triglycerides dropped from 80 mg/dL to 60 mg/dL. These results suggest that higher concentrations of the chloroform extract positively modulate the lipid profile, particularly by lowering LDL-C and triglycerides while increasing HDL-C.

Methanol extract: A similar trend was observed, with total cholesterol dropping from 115 mg/dL at 100 mg/kg to 95 mg/dL at 400 mg/kg, while LDL-C decreased from 68 mg/dL to 55 mg/dL. HDL-C improved from 52 mg/dL to 62 mg/dL, and triglycerides fell from 75 mg/dL to 55 mg/dL. The methanol extract of *Pusa Sumeet* also demonstrated significant improvements in the lipid profile, with the highest concentration showing better results than the chloroform extract.

Swarana Gaurav:

Chloroform extract: Total cholesterol reduced from 118 mg/dL at 100 mg/kg to 98 mg/dL at 400 mg/kg, and LDL-C dropped from 68 mg/dL to 58 mg/dL. HDL-C levels rose from 52 mg/dL to 62 mg/dL, and triglycerides fell from 78 mg/dL to 58 mg/dL. The chloroform extract showed a moderate improvement in lipid parameters, with a pronounced reduction in total cholesterol and triglycerides.

Methanol extract: Total cholesterol decreased from 112 mg/dL to 92 mg/dL with increasing extract concentrations. LDL-C decreased from 65 mg/dL to 52 mg/dL, HDL-C increased from 55 mg/dL to 65 mg/dL, and triglycerides dropped from 72 mg/dL to 50 mg/dL. Swarana Gaurav exhibited the best lipid-lowering effect among the varieties, with methanol extract showing the most significant improvement in HDL-C and triglyceride levels.

Vikrant:

Chloroform extract: This variety exhibited the highest initial total cholesterol and LDL-C levels (125 mg/dL and 75 mg/dL at 100 mg/kg). However, at the highest concentration (400 mg/kg), total cholesterol dropped to 105 mg/dL and LDL-C to 62 mg/dL. HDL-C increased from 50 mg/dL to 60 mg/dL, and triglycerides fell from 85 mg/dL to 65 mg/dL. Though there was an improvement in lipid profile with increasing concentrations, *Vikrant* showed the least overall change compared to the other varieties.

Methanol extract: The methanol extract produced better results, with total cholesterol decreasing from 120 mg/dL at 100 mg/kg to 100 mg/dL at 400 mg/kg. LDL-C fell from 72 mg/dL to 60 mg/dL, HDL-C rose from 52 mg/dL to 62 mg/dL, and triglycerides dropped from 80 mg/dL to 60 mg/dL. Though moderate, the methanol extract of Vikrant still improved lipid parameters.

Table 05: Lipid Profile Analysis of *Vicia faba* Seed Extracts

Variety	Extract Type	Concentration (mg/kg)	Total Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	Triglycerides (mg/dL)
Pusa Sumeet	Chloroform	100	120	70	50	80
	Chloroform	200	110	65	55	70
	Chloroform	400	100	60	60	60

	Methanol	100	115	68	52	75
	Methanol	200	105	60	58	65
	Methanol	400	95	55	62	55
Swarana Gaurav	Chloroform	100	118	68	52	78
	Chloroform	200	108	62	56	68
	Chloroform	400	98	58	62	58
	Methanol	100	112	65	55	72
	Methanol	200	102	58	58	60
	Methanol	400	92	52	65	50
Vikrant	Chloroform	100	125	75	50	85
	Chloroform	200	115	68	55	75
	Chloroform	400	105	62	60	65
	Methanol	100	120	72	52	80
	Methanol	200	110	65	58	70
	Methanol	400	100	60	62	60

Conclusion:

The extractive value analysis highlights the variability in the extraction efficiency of *Vicia faba* seed extracts depending on the solvent (chloroform or methanol) and the variety of the seeds. Across all varieties, methanol extracts consistently showed higher extractive values compared to chloroform extracts, indicating that methanol is a more efficient solvent for extracting bioactive compounds from *Vicia faba* seeds.

- Swarana Gaurav demonstrated the highest extractive values, particularly with methanol, making it the most efficient variety for compound extraction.
- Pusa Sumeet exhibited moderate extractive values, with methanol extracts outperforming chloroform.
- Vikrant had the lowest extractive values, suggesting that it contains fewer or less extractable compounds compared to the other varieties.

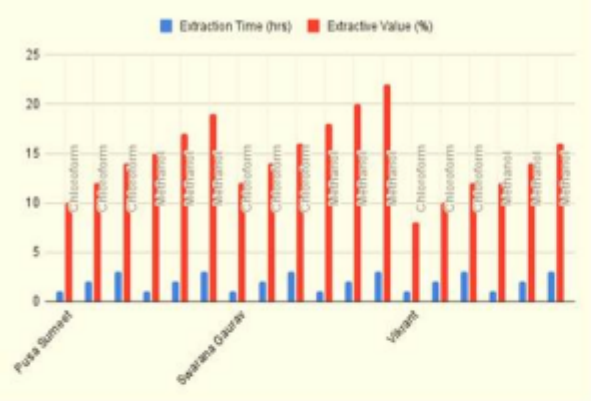

Figure 01: Extractive Values of *Vicia faba* Seed Extracts

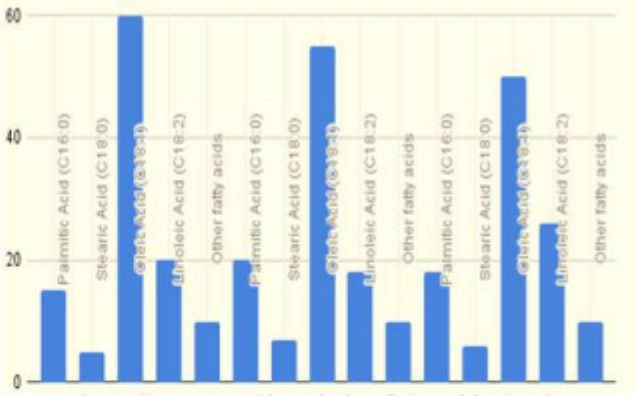

Figure 02: Fatty Acid Analysis of *Vicia faba* Seeds

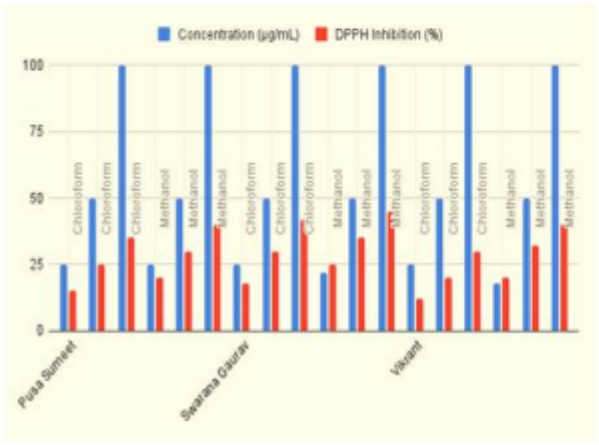

Figure 03: DPPH Assay of *Vicia faba* Seed Extracts

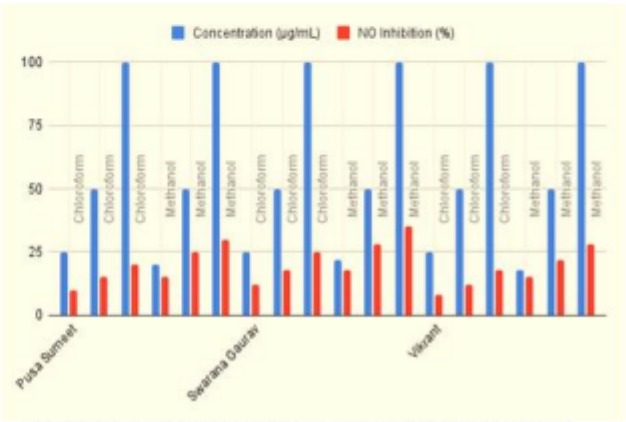

Figure 04 : Anti-inflammatory Assay of *Vicia faba* Seed Extracts

Overall, longer extraction times (up to 3 hours) improved the extractive values in all cases, with methanol being the preferred solvent for maximizing the yield of bioactive compounds in *Vicia faba* seeds.

The fatty acid analysis of Vicia faba seeds reveals significant variation in the relative abundance of key fatty acids across the three varieties.

- **Oleic acid** (C18:1) was the most abundant fatty acid in all varieties, particularly in *Pusa Sumeet* (60%), indicating a high content of monounsaturated fats that are beneficial for heart health.
- **Linoleic acid** (C18:2) content was highest in *Vikrant* (26%), suggesting this variety has a greater concentration of polyunsaturated fats, which are essential for overall health.
- **Palmitic acid** (C16:0) and **stearic acid** (C18:0), both saturated fatty acids, were more prominent in *Swarana Gaurav*, indicating a slightly higher level of saturated fat in this variety.

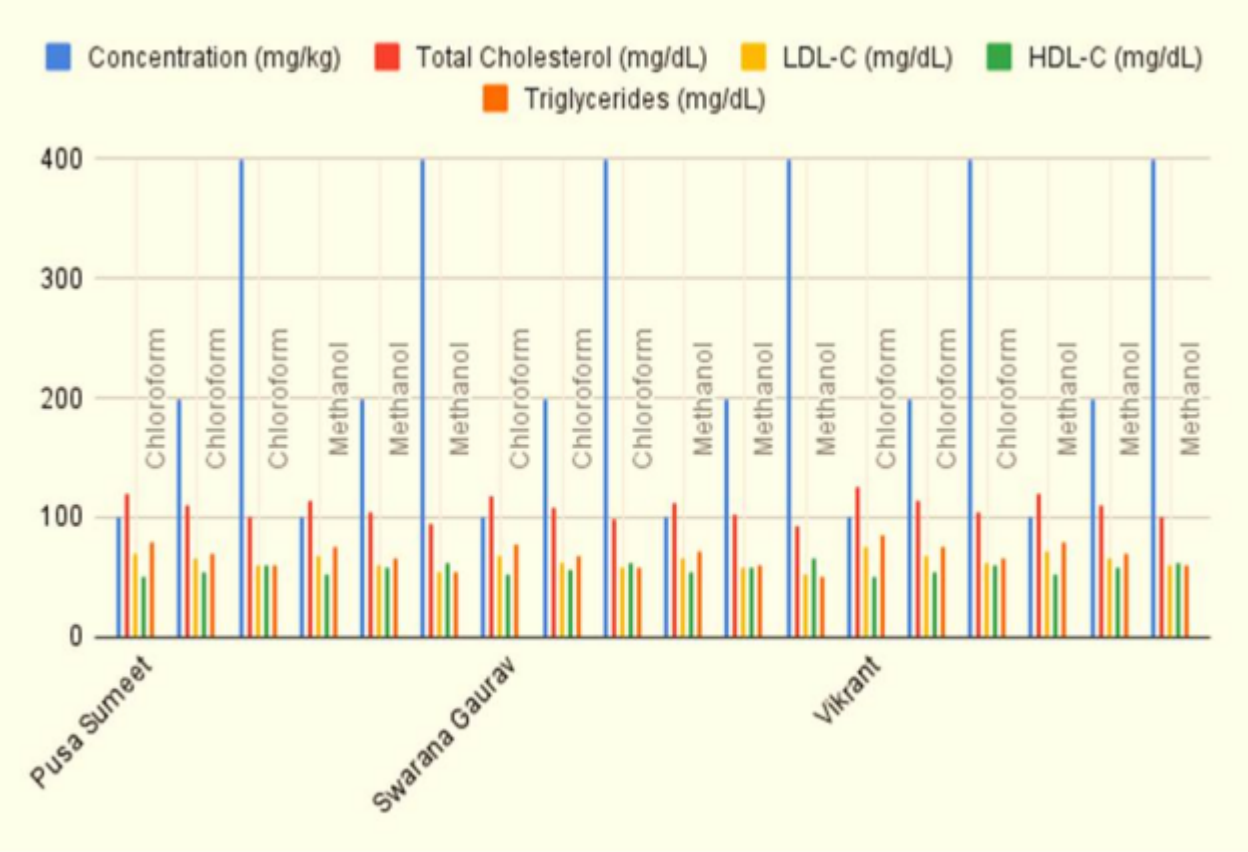

Figure 05: Estimation of Cholesterol-Lowering Effects Lipid profile analysis

Overall, each variety of Vicia faba seeds exhibits a unique fatty acid profile, with *Pusa Sumeet* being the richest in oleic acid, *Vikrant* excelling in linoleic acid content, and *Swarana Gaurav* having the highest level of saturated fats. The variation in fatty acid composition suggests that these varieties may have different applications based on nutritional and health-related needs.

The DPPH radical scavenging assay revealed significant differences in antioxidant potential among the three Vicia faba varieties. *Swarana Gaurav* demonstrated the highest antioxidant activity, followed by *Pusa Sumeet* and *Vikrant*. In all varieties, methanol extracts were more effective than chloroform extracts in scavenging free radicals, indicating that methanol was a better solvent for extracting antioxidant compounds from Vicia faba seeds. These findings suggest that the variety and solvent type play crucial roles in determining the antioxidant potential of Vicia faba seed extracts, with *Swarana Gaurav* standing out as a promising source of natural antioxidants.

The nitric oxide inhibition assay demonstrated notable differences in the anti-inflammatory properties of the three Vicia faba varieties. *Swarana Gaurav* exhibited the highest NO inhibition, particularly in methanol extracts, making it the most potent variety for anti-inflammatory activity. *Pusa Sumeet* followed with moderate activity, while Vikrant displayed the lowest levels of inhibition. Across all varieties, methanol extracts consistently showed higher inhibition percentages than chloroform extracts, indicating that methanol is a more effective solvent for extracting anti-inflammatory compounds from Vicia faba seeds. These findings highlight the potential of *Swarana Gaurav* as a promising candidate for further exploration in anti-inflammatory applications.

The lipid profile analysis reveals a concentration-dependent effect of *Vicia faba* seed extracts on cholesterol and triglyceride levels across all three varieties. In all cases, both chloroform and methanol extracts resulted

in a reduction of total cholesterol, LDL-C, and triglycerides, while HDL-C levels increased with higher concentrations of the extracts.

- Swarana Gaurav showed the most significant improvement in the lipid profile, particularly with the methanol extract, which had the highest reduction in total cholesterol and triglycerides and the best increase in HDL-C.
- Pusa Sumeet also showed substantial improvement in lipid parameters, especially at higher concentrations of methanol extracts.
- Vikrant, while showing the least change in lipid parameters, still demonstrated moderate improvements.

  Overall, the methanol extracts of all three varieties were more effective than chloroform extracts in improving the lipid profile, suggesting that methanol is a better solvent for extracting bioactive compounds that positively influence lipid metabolism.

Conflict of interest: No

Authors Contributions:

Piu Sasmal conducted the experimental work, analyzed the data, and contributed to manuscript writing. Dr. Abha Kumari provided guidance, contributed to experimental design and data analysis, and reviewed the manuscript. Dr. Kamal Kant Patra offered expertise in fatty acid analysis, assisted in data interpretation and manuscript preparation, and reviewed the manuscript. All authors contributed to the study's conception, design, interpretation, and manuscript writing.

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