

## From Berry to Powder: Unveiling the Proximate Composition, Techno-functional Traits, and Characterization of *Piper nigrum* Linn

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### Abstract

Black pepper, a widely popular spice and medicinal plant, has attracted considerable attention for its versatile applications across diverse industries. This abstract presents an exhaustive study on black pepper, encompassing proximate analysis, techno-functional properties, antioxidant analysis, *in vitro* anti-obesity assay, and characterization using scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectroscopy, high performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) techniques. Proximate analysis uncovered essential nutritional components within black pepper powder, including moisture content (4.67%), ash content (4.32%), fiber content (6.9%), fat content (4.32%), and protein estimation (14.69%). Techno-functional properties like tap density, bulk density, Hausner's ratio, angle of repose, and Carr's ratio were assessed to understand its physical characteristics, particularly for applications in food processing. Phytochemical analysis revealed a rich content of phenolic compounds (6.34% GAE) and flavonoids (3.49%), arousing interest within the pharmaceutical and nutraceutical sectors due to their potential health benefits. Antioxidant potential was evaluated using the DPPH assay (4.23% inhibition), highlighting black pepper's natural antioxidant properties. Anti-obesity assays showcased promising results, including  $\alpha$ -amylase inhibition at different concentrations and glucose inhibition over time, suggesting a potential role in combating obesity-related health issues. Characterization studies using FTIR spectroscopy unveiled several prominent peaks, indicating the presence of functional groups like hydroxyl, methylene, phenyl ring, aromatic ring, organic acid, and nucleic acids. These findings provide valuable insights into black pepper's chemical composition and functional properties, opening new avenues for its applications. In conclusion, this comprehensive study sheds light on the proximate composition, techno-functional properties, phytochemical content, antioxidant potential, and anti-obesity properties of black pepper. The characterization data using FTIR spectroscopy further enhances our understanding of its chemical profile, contributing significantly to black pepper's utility in culinary, medicinal, and functional food applications across various industries.

### Keywords

$\alpha$ -amylase inhibition, Antioxidant analysis, Health benefits, *In vitro* anti-obesity assay, Nutraceutical applications, Obesity-related health issues, Scanning electron microscope

### Introduction

Black pepper, scientifically known as *Piper nigrum* L., is a member of the Piperaceae family and originates from the southwestern Ghats of India. This plant, characterized by its perennial climbing nature and elliptical leathery leaves (sized 10-15 cm in length and 5-9 cm in width), gives rise to small round berries, re-

ferred to as peppercorns, measuring up to 8 mm in diameter [1]. These vines exhibit optimal growth in shaded areas, often supported by trees or poles, reaching heights of up to 10 meters [2]. Globally recognized for its extensive use, black pepper holds a significant place as one of the most employed spices, even earning prestigious titles like the "King of Spices" and "Black Gold" due to its remarkable value in international trade [3]. The cultivation of black pepper is largely concentrated in nations such as India, Malaysia, Indonesia, China, Thailand, Sri Lanka, Vietnam, Brazil, and Madagascar [2]. Throughout history, black pepper has played roles in food preparation, preservation, flavor enhancement, and traditional remedies for various ailments [4].

In India, the annual production amounts to roughly 62,000 metric tons, with approximately 10-12% of this quantity earmarked for export [3]. The chemical makeup of black pepper includes alkaloids, flavonoids, lignans, tannins, anthraquinones, as well as alkaloids, piperidine, wisanine, dipiperamide, and other essential oils [5, 6]. In the realm of essential oils derived from *Piper* plants, a variety of volatile chemical compounds exist, contributing to rich diversity. However, these compositions exhibit notable variability due to factors such as plant polymorphism, geographical distinctions, environmental conditions, and chemotypes. The essential oil constituents span a range, including compounds like  $\alpha$ -pinene, myrcene, limonene,  $\alpha$ -terpinene, p-cymene,  $\beta$ -pinene,  $\alpha$ -phellandrene, and (Z)- $\beta$ -ocimene among others [7]. Among the array of bioactive components found in black pepper, significant substances include piperine, piperonylamine, pipericide, sarmenosine, sarmentine, and chavicine. Notably, piperine stands out for its pronounced pharmacological effects, emerging as a principal bioactive element [8]. Traditionally, black pepper has found application in ayurveda for addressing fevers, gastrointestinal concerns, neurological disorders, and broncho-pulmonary conditions like asthma.

Similarly, traditional Chinese medicine employs it to alleviate pain, treat rheumatism, combat infections, and enhance blood circulation [9]. Its potential as an antioxidant, anti-inflammatory, antimicrobial, neuroprotective agent, and its capacity to aid gastrointestinal issues have also been recognized [10]. Beyond its role in culinary arts, black pepper boasts a rich historical backdrop, a diverse spectrum of chemical components, and a global influence that shapes our appreciation and enjoyment of food.

## Materials and Methods

### Materials

The *P. nigrum* were obtained from the market and all the analytical grade chemicals utilized in the experiments were obtained from the Department of Food Science and Technology at Lovely Professional University, located in Phagwara, Punjab.

### Methods

#### Powder preparation

The examination of the *P. nigrum* was meticulous to eliminate any external elements. Subsequently, they underwent

drying within a tray drier (manufactured by B. S. exports) at a precisely regulated temperature of  $50^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$  for a duration of 24 h. Once the berries were thoroughly dried, they were finely ground using a grinder and then stored in a Ziplock bag under normal room conditions.

#### Determination of total yield of black pepper powder

For yield determination the procedure involved depositing the sample onto a sieve shaker (Harrison) and subjecting it to vibrations with an amplitude of 60 for a duration of 20 min. A test sieve with a mesh size of 250  $\mu\text{m}$  was utilized. The procedure was outlined in the work of Manuwa et al. [11]. The weight of the sieved sample was gauged, and the yield was computed using the subsequent formula:

$$\text{Total yield (\%)} = \frac{\text{Weight of sieved powder (g)}}{\text{Initial weight of sample (g)}} \times 100$$

#### Moisture content (%)

The moisture content of the desiccated powder was determined through the application of the oven drying method using a hot air oven (Vidya Udyog) set at a temperature of  $105^{\circ}\text{C}$ . The consecutive weight was recorded once a consistent weight was attained [12]. The moisture content of the powdered black pepper was computed using the subsequent equation:

$$\text{Moisture content (\%)} = \frac{\text{Weight of the crucible before drying (g)} - \text{Weight of the crucible after drying (g)}}{\text{Weight of the crucible before drying (g)}} \times 100$$

#### Ash content (%)

To initiate the procedure, a crucible was used to measure 2.0 g of black pepper powder. This powder was then exposed to incineration within a muffle furnace (Narang scientific work) at a temperature of  $560^{\circ}\text{C}$  for a duration of 16 h, until it was completely transformed into ash. Following this, the crucible was allowed to cool within a desiccator, and the ultimate weight of the crucible was determined [13]. The content of ash was determined using the subsequent equation:

$$\text{Ash content (\%)} = \frac{\text{Weight of the crucible before drying (g)} - \text{Weight of the crucible after drying (g)}}{\text{Weight of the crucible before drying (g)}} \times 100$$

#### Fat content (%)

5.0 g of *P. nigrum* powder into a thimble to initiate the process with hexane as the chosen solvent for extraction. The procedure is conducted over a duration of 6 h, following the protocol delineated by Khassetia and Affrina [14], ensuring thorough lipid extraction. After the extraction phase, the solvent is removed, and the resulting lipid extract is concentrated through a rotary evaporator or a similar apparatus. The concentrated lipid extract then undergoes drying within either a desiccator or a drying apparatus to eliminate any residual solvent traces. The quantification of the lipid content is achieved through the application of the provided mathematical equation.

$$\text{Fat content (\%)} = \frac{\text{Weight of the round bottom flask after extraction (g)}}{\text{Weight of the sample (g)}} \times 100$$

### Protein estimation

An accurately measured quantity of 0.5 to 1 g of black pepper powder was carefully placed into a digestion vessel. This sample was then combined with concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and a digestion catalyst, such as copper sulfate (CuSO<sub>4</sub>), for the digestion process. The digestion vessel was gradually heated, either by using a Bunsen burner or a heating mantle, until the contents became clear or exhibited a pale-yellow hue, indicating the digestion's completion. Following the cooling phase, the contents were diluted using distilled water and transferred into a distillation setup. The ammonia released during the distillation procedure was collected within an acidic solution, while the resulting distillate underwent titration with standardized sulfuric acid like Sharma and Dhuria [15]. Subsequently, the protein content was evaluated and computed using the provided mathematical formula.

$$\text{Protein estimation (\%)} = \frac{N \times F \times 6.25}{\text{Weight of the sample (g)}}$$

In this context, the parameters are denoted as follows: 'N' stands for the quantity of ammonia nitrogen acquired through titration, measured in milligrams (mg). The symbol 'F' signifies the coefficient that reflects the concentration of the sulfuric acid solution utilized during the titration process. Furthermore, the numerical value 6.25 is utilized as a transformation coefficient to convert nitrogen content into protein content. This conversion coefficient assumes an average protein nitrogen content of 16%.

### Fiber estimation

Approximately 2.5 g of the defatted dry sample were utilized for fiber analysis employing fibraplus FES06E. The sample underwent boiling with a 1.25% diluted H<sub>2</sub>SO<sub>4</sub> solution, followed by a water rinse. Afterward, it was subjected to boiling once more, this time with a 1.25% diluted NaOH solution. The remaining residue post-digestion was collected as the raw fibrate residue, which was subsequently placed in a furnace and digested at 600 °C. The procedure was outlined by Akter et al. [16]. The quantification of the crude fiber content was determined using the subsequent mathematical expression.

$$\text{Crude fiber (\%)} = \left[ (\text{Loss in weight on ignition} \div \text{Weight of the sample}) \times 100 \right]$$

### Tapped density and bulk density

The measurement of bulk density (ρ<sub>bulk</sub>) entails assessing the combined volume occupied by solid particles and the empty spaces within a subdivided powder. In this procedure, the powder was placed into a 50 ml measuring cylinder up to the 50 ml mark. Subsequently, the cylinder was dropped from a height of 1 inch onto a hard wooden surface at 2-second intervals, and the resultant powder volume was meticulously measured.

On a different note, tap density (ρ<sub>tab</sub>) presents an alternative version of bulk density, achieved by tapping or vibrating the container in a specific manner. This tapping or vibration facilitates a more organized arrangement of particles, resulting in heightened particle packing density. The determination of

tap density necessitates noting the initial volume or mass of the powder. Then, the measuring cylinder or vessel containing the powder is subjected to mechanical tapping for 500 repetitions. Periodic measurements of volume or mass are recorded until minimal or no further changes are observed. The calculation of bulk density for black pepper powder was executed in accordance with the approach elucidated by Mulani et al. [17] and Etti et al. [18]. Tapped density serves as an indicator of the powder's compactness after mechanical tapping. Consequently, tap density generally surpasses bulk density.

$$\tilde{n}_{\text{bulk}} = \frac{W_t}{V_{\text{bulk}}}$$

To ascertain the tapped density (ρ<sub>tab</sub>) of powders, a formula is employed that integrates the powder's weight (W<sub>t</sub>) and the volume of the powder acquired from a calibrated graduated cylinder without tapping (V<sub>bulk</sub>).

$$\tilde{n}_{\text{tab}} = \frac{W_t}{V_{\text{tab}}}$$

The given formula employs the powder's weight (W<sub>t</sub>) and the volume of the powder bed following 500 tapping actions (V<sub>tab</sub>).

### Hausner's ratio and Carrs index

The Carr index is calculated by dividing the discrepancy between the tapped density and bulk density by the tapped density. Following Carr's flowability index, powders with a Carr index between 5% and 15% are categorized as having exceptional flowability, while a Carr index surpassing 25% typically signifies inadequate flowability.

$$\text{Carrs Index} = \frac{\tilde{n}_{\text{tab}} - \tilde{n}_{\text{bulk}}}{\tilde{n}_{\text{tab}}}$$

Hausner ratio serves as a metric used to gauge the flow properties of a powder. It is determined by dividing the tapped density by the bulk density. Hausner ratio values within the range of 1.0 to 1.1 signify a powder that moves easily, while Hausner ratio values falling between 1.1 and 1.25 indicate a powder with moderate flowability. When the Hausner ratio surpasses 1.25 and extends up to 1.4, it indicates a powder that presents difficulty in flowing, and a Hausner ratio exceeding 1.4 points to a powder with exceedingly low flowability. The protocol was outlined by Mochahary et al. [19]

$$\text{Hausner Ratio} = \frac{\tilde{n}_{\text{tab}}}{\tilde{n}_{\text{bulk}}}$$

### Angle of repose

The procedure to ascertain the angle of repose (θ) for *P. nigrum* powder followed the methodology detailed in the study by Kundan et al. [20]. The specific quantity of the dried powder is accurately placed within a funnel situated 6 cm above a flat surface. The powder is then permitted to flow naturally, creating a mound over a sheet of paper on the horizontal plane. In this process, the height and radius of the powder heap are precisely measured and documented. The angle of repose is computed using the provided formula.

$$\text{Angle of response (}^\circ\text{)} = \tan^{-1}\left(\frac{h}{r}\right)$$



The height of the mound is symbolized by 'h', and its radius is designated as 'r'.

The angle of repose plays a significant role as an indicator for assessing the powder's flow properties. Typically, an angle of repose at approximately 30 degrees signals a substance that moves effortlessly, indicating favorable flowability. Conversely, angles of repose approaching 40 degrees generally point to a substance that exhibits sluggish flow, highlighting diminished flowability.

### Plant extract preparation

For the preparation of these extracts from the herbal materials, 1 g of powdered herb was dissolved in 10 ml of various extraction solutions, including methanol, chloroform, ethanol, hexane, and acetone, at a temperature of 35 °C. This mixture was then introduced into an orbital shaker and subjected to incubation at 150 rpm for 48 h. Following the incubation period, the tubes underwent centrifugation at 4000 rpm for 5 min. The resultant extracts were allowed to evaporate within a refrigerated temperature range of 4 - 7 °C. The protocol was adopted by Kumar et al. [21]. The weight of each extract was measured to determine the yield as a percentage. Among the extracts, the one with the highest yield was chosen and preserved in sealed glass vials at a temperature of -18 °C for further analysis.

### Total phenolic content determination

For the determination of total phenolic content a solution containing 300 µl of the ethanol extract was mixed with 1 ml of methanol and 200 µl of Folin-Ciocalteu reagent. After an incubation period of 5 min, 600 µl of a 0.02 mM sodium carbonate solution was added, followed by a 2-hour incubation at 40 °C. For calibration, a blank solution was created using methanol instead of the extract, and a gallic acid standard curve was established. The absorbance of the resultant solutions was measured at 765 nm using a UV-spectrophotometer (visible spectrophotometer 168). Total phenol content was calculated using the equation  $y = 4.61x + 0.0848$  ( $R^2 = 0.9703$ ), where y represents absorbance and x represents gallic acid concentration in micrograms per milliliter (µg/ml) [22]. Subsequently, the total phenolic content in the was determined using the subsequent equation, and the outcome was expressed as milligram gallic acid equivalents per gram of dried weight sample (mg GAE/g d.w.).

Total phenolic content =

$$\frac{\text{Concentration} \left( \frac{\text{mg}}{\text{ml}} \right) \times \text{Volume of solvent (ml)}}{\text{Weight of dried sample used (g)}}$$

### Total flavonoid content estimation

The black pepper powder was dissolved in methanol at a concentration of 500 µg/ml. In a test tube, 300 µl of the extract was mixed with 3.4 ml of an aqueous methanol solution (30% v/v). Then, solutions of sodium nitrite (0.5 M) and aluminum chloride (0.3 M) were added. After incubation period of 5 min, sodium hydroxide solution (1 M) was introduced, and

the mixture was thoroughly mixed before measuring the absorbance at 506 nm against a blank using visible spectrophotometer 168. A calibration curve using a quercetin standard was established for accurate determination. The total flavonoid content was calculated using the equation  $y = 0.005312x + 0.01153$  ( $R^2 = 0.9861$ ), where y represents the absorbance at 506 nm and x represents the amount of quercetin in micrograms of quercetin equivalents (RE) per milliliter of extract. The procedure is based on the method developed by Alara et al. [22]. The results were reported as micrograms of quercetin equivalents (RE) per milligram of extract.

### DPPH estimation

DPPH radicals were generated by dissolving about 0.004 g of DPPH reagent in 100 ml of methanol. For the assay, 20 µl of the extract was combined with 180 µl of DPPH. The mixture was left in darkness for a duration of 15 min, and the absorbance at 517 nm was measured using a Visible Spectrophotometer 168. The analysis encompassed blank samples containing only methanol and standard samples containing ascorbic acid in methanol. The extracts were evaluated at various concentrations to ascertain the  $IC_{50}$ , which signifies the concentration causing a 50% reduction in DPPH absorbance. This approach closely aligns with the methods employed by previous researchers [22, 23]. The extent of scavenging was determined through the subsequent formula:

DPPH estimation =

$$\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

### Amylase inhibition assay

A substrate solution was prepared by dissolving 500 mg of soluble starch in 25 ml of 0.4 M NaOH and subjecting it to a temperature of 100 °C for 5 min. The resulting solution's pH was adjusted to 7.0 using HCl, and the final volume was made up to 100 ml with distilled water. Various concentrations of plant extract solutions were created using acetate buffer with a pH of 6.5 as the medium. In a microplate well, a mixture of 20 µl of the sample and 40 µl of the substrate solution was combined. Then, 20 µl of α-amylase solution (50 µg/ml) was added to the mixture (Figure 1). This was followed by a short incubation of 15 min at 25 °C. To halt the enzymatic reaction, 80 µl of 0.1 M HCl was added, followed by the addition of 200 µl of 1 mM iodine solution. Subsequently, the optical density was measured at 650 nm using a visible spectrophotometer (Visible Spectrophotometer 168) [21]. The quantification of amylase inhibitory activity was carried out using the following formula:

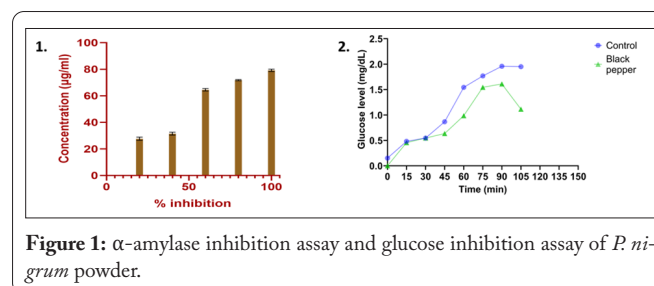


Figure 1: α-amylase inhibition assay and glucose inhibition assay of *P. nigrum* powder.

$$\text{Amylase inhibition assay (\%)} = 1 - \frac{(OD2 - OD1)}{(OD4 - OD3)} \times 100$$

The assessment of amylase inhibition activity involved the comparison of optical densities across various solutions. OD1 indicated the optical density of the solution containing plant extract, starch, and amylase. OD2 represented the optical density of the solution containing plant extract and starch. OD3 denoted the optical density of the solution containing starch and amylase. Lastly, OD4 indicated the optical density of the solution containing solely starch.

#### Glucose uptake assays using dialysis bag

The dialysis membrane was soaked in distilled water to facilitate expansion. Subsequently, one end of the dialysis tube was sealed, and 1 mg of the herbal extract was introduced into 10 ml of the dialysis membrane. Following this, 15 ml of a 0.22 mM glucose solution was added and thoroughly mixed. The system was then subjected to an incubation period at 37 °C for 4 h. Upon completion of the incubation, centrifugation was conducted at 4800 rpm for 20 min. A centrifugal tube containing 45 ml of 0.15 M NaCl was prepared. The movement of glucose from the dialysis membrane to the external solution was assessed at different time intervals using a glucometer (control d glucometer) [21].

#### FTIR analysis

The FTIR analysis was carried out. Around 5 mg of each specimen was mixed with 5 mg of KBr to enable qualitative evaluation. Subsequently, the dehydrated and powdered state of each sample underwent assessment using a PERKIN ELMER FTIR spectrometer equipped with a KBr beam splitter. The FTIR spectrophotometer functioned at a resolution of 0.5 cm<sup>-1</sup>, utilizing Spectrum 10 software. The analyzed spectral range encompassed values spanning from 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup>. The procedure detailed in the available literature by Asema et al. [23].

#### HPLC analysis

HPLC system equipped with CBM 20 alite controller, DGU-20 A5 prominence online degasser, LC-20AD binary pumps, SIL-20A prominence auto sampler, CTO-20 prominence column oven and SPD-20A prominence UV-Vis detector (Shimadzu Co., Kyoto, Japan). The chromatographic separation was performed in the reversed phase C18 analytical column of dimension 4.6 x 150 mm and 5 µm particle size. Isocratic elution containing 48% acetonitrile and 52% of 1% acetic acid in water was used as mobile phase for the analysis. The constant flow rate of 1 ml/min was maintained throughout the run. Column was kept in the column oven maintained at a constant temperature of 40 °C. 10 µl of both standards and samples were injected into the system and piperine peak was monitored at 343 nm. The run time was set to 25 min. This methodology draws inspiration from prior research Shrestha et al. [24].

#### GC-MS analysis

A quantity of two grams of the sample was placed in a 20

ml amber vial with PTFE/silicone septa. It was then subjected to equilibration at 80 °C for 30 min using a headspace sampler, with agitation set at 300 rpm. For GC-MS analysis, a 6890 GC-MS system (SHIMADZU) with an HP-INNOWax column (60m x 0.25 mm x 0.25 m; Agilent Co.) was utilized. A syringe injected 1 ml of the sample in split injection mode (20:1) at 230 °C, employing helium (> 99.9% purity) as the carrier gas at a flow rate of 1 ml/min. The oven temperature was initially held at 40 °C for 2 min, then increased at a rate of 5 °C/min until it reached 230 °C, maintaining this temperature for 10 min. Flavor compounds were identified by comparing their spectra to a library (APAWLY-9781119376743 NIST Mass Spectral Library 2017). The relative percentage composition of each component was determined by comparing its average peak area against the total areas. The software used to manage mass spectra and chromatograms was the GCMS-solution™ software. The procedure was outlined by Liu et al. [25].

#### SEM analysis

The dried ethanolic extract of black pepper underwent morphological characterization through SEM employing a JEOL microscope. The samples were observed at magnifications of 100, 200, 500, 1000, and 2000x, following the methodology outlined in the work by Fernandes et al. [26].

#### Statistical analysis

The research carried out a series of experiments in triplicate and reported the outcomes as the mean ± standard deviation. To evaluate significant variations among the averages, they utilized a statistical technique called one-way analysis of variance, followed by the application of the Duncan post hoc test at a significance threshold of p < 0.05.

## Results and Discussion

#### Proximate analysis

The proximate analysis of black pepper has been depicted in table 1. Moisture content was decreased to 4.67% through tray drying. The ash content of 4.32% aligned with the findings of Abukawsar et al. [27], who reported a range of ash content from 4.08% to 5.55% for Indigenous cultivars and Kerala cultivars of *P. nigrum* seeds. The fiber content was determined to be 6.9%, a value in line with work by Pavithra and Anuradha [28]. The fat content was measured at 4.47%, which was notably lower than the estimation by Otunola et al. [29] who reported a fat content of 12.70%. Regarding protein estimation, it was found to be 14.69%, a value closely resembling the findings recorded by Pavithra and Anuradha [28] and Liu

**Table 1:** Results for the proximate analysis for black pepper powder.

Proximate analysis of black pepper powder	
Moisture content (%)	4.67 ± 0.32
Ash content (%)	4.32 ± 0.44
Fiber content (%)	6.9 ± 0.24
Fat content (%)	4.32 ± 0.84
Protein estimation (%)	14.69 ± 0.04

et al. [30]. Their studies indicated protein content ranging from 12.50% to 12.59% based on hammer mill and cryogenic grinding of black pepper.

### Techno-functional properties

The micrometric properties analysis (Table 2) of the ground pepper revealed a tap density of 0.743 gm/cm<sup>3</sup>, a value consistent with the findings of Barnwal et al. [31], where the range was reported as 625–671.5 kg/m<sup>3</sup>. Another study noted a range of 603.79–760.10 kg/m<sup>3</sup> for cryogenic and ambient grinding of green pepper with moisture content under 10%. The bulk density was measured at 0.66 gm/cm<sup>3</sup>, a result akin to Barnwal et al. [31], who studied ambient temperature grinding of black pepper powder with a 4% moisture content like our experiment. Similarly, Meghwal and Goswami [32], reported a range of 432.03–563.85 kg/m<sup>3</sup> for cryogenic and ambient grinding of green pepper with moisture content below 10%. The Hausner's ratio was determined as 1.22, comparable to the range of 1.35 to 1.38 for ambient ground powder and 1.30 to 1.34 for cryogenically ground powder found by Meghwal and Goswami [32]. The angle of repose was measured at 24.40°, closely resembling the result of 28.6° obtained by Barnwal et al. [31]. Carr's ratio was found to be 15.90, a value consistent with the cryogenically ground black pepper studied by Meghwal and Goswami [32].

**Table 2:** Results for the techno-functional properties for *P. nigrum* powder.

Techno-functional properties	
Tap density (gm/cm <sup>-3</sup> )	0.743 ± 0.01
Bulk density (gm/cm <sup>-3</sup> )	0.66 ± 0.24
Hausner's ratio	1.22 ± 0.42
Angle of repose (°)	24.40 ± 0.30
Carr's ratio	15.90 ± 0.75

### Phytochemical analysis

In table 3 the total phenolic content was assessed at 6.34 µg/ml, a value consistent with the 5.46 µg GAE/ml documented by Feng et al. [33] and slightly higher than the 4.12 µg GAE/ml recorded by Uyoh et al. [34] and in the case of *P. longum* by Krishna et al. [35]. The total flavonoid content was determined to be 3.49 µg QUE/ml, which closely resembles the 3.97 µg QUE/ml reported by Feng et al. [33] and Krishna et al. [35]. Regarding DPPH (% inhibition), the recorded value was 4.93%, which is comparable to the 4.23% DPPH activity reported by Uyoh et al. [34] and slightly higher than the 1.19% mentioned by Feng et al. [33].

### In vitro lipid digestion activity

The inhibitory effect of solvent fractions derived from

**Table 3:** Results for the phytochemical analysis for *P. nigrum* powder.

Phytochemical analysis for <i>P. nigrum</i> powder	
Total phenolic content ((as percent gallic acid equivalents (GAE))	6.34 ± 0.72
Total flavonoid content (as percent as percentage of quercetin)	3.49 ± 0.24
DPPH (% inhibition)	4.23 ± 0.42

the ethanol berry extract of *P. nigrum* on α-amylase activity is summarized in table 4. As per the findings, the ethanol fraction displayed a significant (p < 0.05) inhibition of α-amylase activity, with an IC<sub>50</sub> value of 49.56 µg/ml, in contrast to acarbose, which exhibited an inhibitory activity of 66.66 ± 0.19 µg/ml. The other fractions showed significantly lower activity compared to acarbose. These results are consistent with those reported by Sulaimon et al. [36], who studied the antidiabetic potential of *Piper guineense*. In the glucose uptake assay, the ethanol fraction demonstrated maximum inhibition at a concentration of 100 µg/ml. The glucose uptake assay for the ethanolic extract revealed an IC<sub>50</sub> value of 1.74, with the highest uptake observed at 90 min.

**Table 4:** Results for the *in vitro* lipid digestion assay for black pepper powder.

In vitro lipid digestion assay			
Inhibition assay	Concentration (µg/ml)	% of inhibition	IC <sub>50</sub> (mg/ml)
α-amylase inhibition assay	20	27.48 ± 0.39	49.56 ± 0.11
	40	31.3 ± 0.26	
	60	64.65 ± 0.33	
	80	71.89 ± 0.01	
	100	79.10 ± 0.34	
Glucose inhibition assay	Time (min)	Glucose (mg/ml)	IC <sub>50</sub>
	15	0.456 ± 0.45	1.74 ± 0.42
	30	0.546 ± 0.13	
	45	0.634 ± 0.11	
	60	0.986 ± 0.15	
	75	1.544 ± 0.04	
	90	1.611 ± 0.1	
105	1.113 ± 0.08		

### FTIR analysis

Infrared spectroscopy, often referred to as "infrared fingerprints," is a highly informative and specific analytical technique used for identifying the primary chemical constituents within medicinal materials. This method relies on the fact that different chemical components exhibit their distinctive infrared characteristic peaks. Notable peak values and their corresponding references underscore the utility of this technique: At 3284.45 cm<sup>-1</sup>, H-bonded–OH stretching in hydroxyl groups was documented by Verma et al. [37] during their study of onion skin (Figure 2). Additionally, they reported a peak at 2924.57 cm<sup>-1</sup>, associated with C–H<sub>2</sub> stretching in methylene groups (Table 5). Zhang et al. [38], while investigating *Lonicera japonica*, revealed several peaks, including 1585.59 cm<sup>-1</sup> (representing phenyl ring stretching), 1491.81 cm<sup>-1</sup> (indicative of C=C stretching in aromatic rings), 1441.76 cm<sup>-1</sup> (related to O–H stretching in organic acids), and 1076.59 cm<sup>-1</sup> (signifying PO<sub>2</sub><sup>-</sup> symmetric stretching, associated with nucleic acids, phospholipids, glycogen, polysaccharides, and glycolipids).

Furthermore, Kaur et al. [39] explored the antidiabetic activity of *Ficus semicordata*, discovering a distinctive peak at



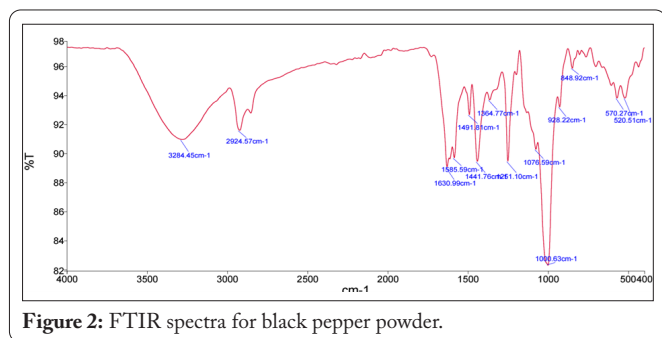


Figure 2: FTIR spectra for black pepper powder.

928.22  $\text{cm}^{-1}$ , corresponding to  $\text{-C-H}$  out-of-plane bending in aromatic hydrocarbons. In summary, infrared spectroscopy is a valuable tool for characterizing diverse chemical components based on their unique infrared signatures, facilitating comprehensive analysis in the study of medicinal materials and various other substances.

### HPLC analysis

The HPLC data provided in table 6 and figure 3 offers a comprehensive analysis of the compounds detected through chromatography. There was a total of 13 peaks observed, and among them, 3 peaks were identified and investigated. Peak 2, which had a retention time of 4.38 min, was determined to be eugenol, as documented by Rehman et al. [40]. Peak 2 at 6.29 min retention time indicated the presence of piperine, which was corroborated by Shingate et al. [41] at 6.43 min. Additionally, peaks at 7.05 min by Rehman et al. [40], and 6.4 min by Namjoyan et al. [42] also document the presence of piperine. Peak 3, observed at 22.36 min, showed the presence of piperine, consistent with the findings of Friedman et al. [43].

### GC-MS analysis

The analysis of black pepper's active compounds involved examining the GC-MS chromatogram, where the ethanol extract of black pepper powder revealed 41 distinct compounds shown in table 7 and figure 4. Among these compounds, piperine, the primary component of black pepper, constituted 65.40% of the total composition, as reported by Vadivel et al. [44]. Additionally, piperlongumine accounted for 0.67% of the composition, and (2E, 4E)-N-Isobutyloctadeca-2,4-dienamide made up 5.40%, as observed by Dawid et al. [45] while studying black pepper powder's key pungent and tingling

Table 6: HPLC data for *P. nigrum* at 364 nm.

HPLC (364 nm)			
Peak	Retention time (min)	Compound	Ref.
1	4.384	Eugenol	[40]
2	6.293	Piperine	[40-42]
3	22.368	Piperyline	[43]

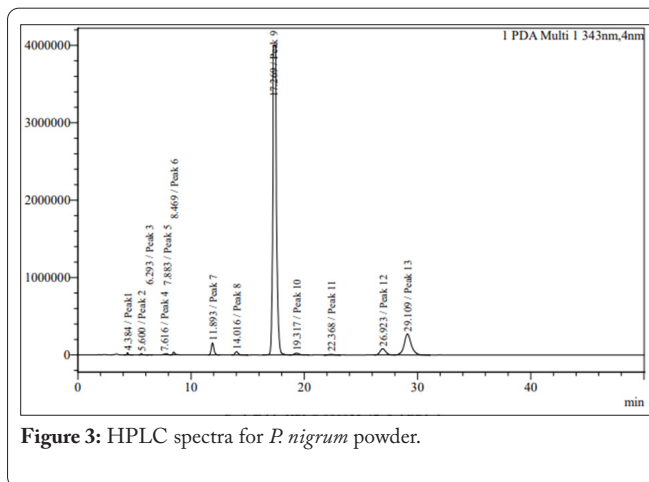


Figure 3: HPLC spectra for *P. nigrum* powder.

compounds. Mercer et al. [46] research on the volatile organic compounds profile of Malaysian and Indian black peppers also identified (2E,4E,14E)-N-Isobutylicos-2,4,14-trienamide as a unique compound in Indian black pepper, constituting 5.40% of the composition. Finally, (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one was discovered to be present at a level of 5.24% in the current study, a compound previously identified by Pedersen et al. [47] during their research on amides from *Piper capense* with central nervous system activity.

### SEM analysis

The investigation into the structure of ground black pepper was conducted through SEM, with the corresponding images displayed in figure 5. These visual findings bear a resemblance to the outcomes obtained by Ghodki and Goswami [48] during their research on the impact of temperature variations on pepper powder.

Table 5: FTIR data for *P. nigrum* powder.

FTIR					
Peak	Peak ( $\text{cm}^{-1}$ )	Bond	Bond vibration	Functional group	Ref.
1	3284.45	H-bonded-OH	Stretching	Hydroxyl group	[37]
2	2924.57	C-H <sub>2</sub>	Stretching	Methylene group	[37]
3	1585.59		Stretching	Phenyl ring	[38]
4	1491.81	C=C	Stretching	Aromatic ring	[38]
5	1441.76	O-H	Stretching	Organic acid	[38]
6	1076.59	PO <sub>2</sub> <sup>-</sup>	Symmetric stretching	Nucleic acids, phospholipids, glycogen, polysaccharides, glycolipids	[38]
7	928.22	-C-H	Out of bending	Aromatic hydrocarbon	[39]

**Table 7:** GC-MS data.

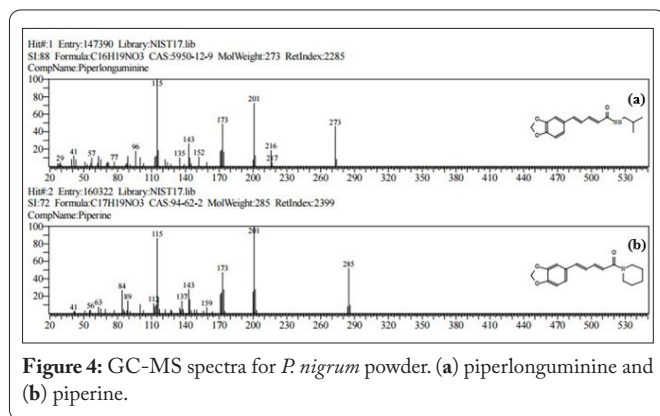
Peak	Compound found	Common name	Retention time	Area %
1	2,2'-Bis-trimethylsilylbenzhydryl methyl ether	Specific chemical structure	13.554	0.08
2	2,4-Di-tert-butylphenol	Bis(2,4-di-tert-butylphenyl) ether	17.724	0.21
3	1-(1-Butoxypropan-2-yloxy) propan-2-yl 4-chlorobenzoate	Dibutylated perylene	20.825	0.04
4	Butane,1,4-bis(9,10-dihydro-9-methylantracen-10-yl)	Diisobutyl phthalate	20.865	0.12
5	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Specific chemical structure	21.763	0.08
6	Morpholine	Specific chemical structure	22.1	0.11
7	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	Specific chemical structure	22.265	0.36
8	Hexadecanoic acid	Palmitic acid	22.392	0.6
9	2,4-Decadienamide, N-isobutyl-, (E,E)-	N-Isobutyl-2,4-decadienamide	22.541	2.02
10	2,3,8-Trimethyl-1H,9H-pyrrolo[3,2-H] quinolin-6-one	Specific chemical structure	22.915	0.06
11	Methyl stearate	Specific chemical structure	24.339	0.53
12	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo [3.3.3] undecane	Specific chemical structure	24.42	2.16
13	(2E,4E)-N-Isobutyldodeca-2,4-dienamide	Specific chemical structure	24.585	0.32
14	(2E,4E)-1-(Piperidin-1-yl) deca-2,4-dien-1-one	Specific chemical structure	24.617	0.47
15	(E)-1-(Piperidin-1-yl) octadec-5-en-1-one	Specific chemical structure	25.08	0.06
16	1-Hexanoyl-3-pivaloyl-5-[3,4-dimethoxybenzal] hydantoin	Specific chemical structure	25.212	0.07
17	Glutarimide, N-(2-octyl)-	Specific chemical structure	25.279	0.25
18	Methyl 6,6,8,8-tetramethyl-3-oxo-2,5,7,9-tetraoxa-6, 8-disilaundecan-11-oate	Specific chemical structure	25.594	0.21
19	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	Specific chemical structure	26.221	0.35
20	Tetrakis (dimethylsilyloxy)silane	Specific chemical structure	26.775	0.25
21	(2E,4E)-N-Isobutylhexadeca-2,4-dienamide	Specific chemical structure	28.108	0.23
22	(2E,4E,6E)-7-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,4,6-trien-1-one	Specific chemical structure	28.5	0.27
23	Benzo[b]benzofuran-2-carboxaldehyde	Specific chemical structure	28.613	0.42
24	2-Phenyl-4,5 methylenedioxybenzaldehyde	Specific chemical structure	28.685	0.59
25	(E)-5-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidin-1-yl) pent-2-en-1-one	Specific chemical structure	28.732	5.24
26	Piperlonguminine	Specific chemical structure	28.846	0.67
27	Carbonic acid, monoamide, N-octadecyl-, (1S)-(+)-menthyl ester	Specific chemical structure	29.079	0.19
28	(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	Specific chemical structure	29.506	0.43
29	(2E,4E,14E)-N-Isobutylicos-2,4,14-trienamide	Specific chemical structure	29.617	4.16
30	(2E,4E)-N-Isobutyloctadeca-2,4-dienamide	Specific chemical structure	29.658	5.4
31	N-Isobutyl-11-(3,4-methylenedioxyphenyl)-2E,4E,10E-undecatrienoic amid	Specific chemical structure	29.965	0.08
32	2H-Benzo[f]oxireno[2,3-E] benzofuran-8(9H)-one, 9-[[1,3-benzodioxol-5-y	Specific chemical structure	30.141	0.8
33	2-Furanol, 3,4-bis(1,3-benzodioxol-5-ylmethyl) tetrahydro-	Cubebin	30.251	0.14
34	Benzoic acid, 3-methoxy-, butyl ester	Butyl 3-methoxybenzoate	30.371	0.27
35	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	Specific chemical structure	30.532	0.97
36	(E)-1-(Piperidin-1-yl) hexadec-2-en-1-one	Specific chemical structure	30.636	0.06
37	Piperine	Specific chemical structure	30.87	65.4
38	(2E,4E,14E)-N-Isobutylicos-2,4,14-trienamide	Specific chemical structure	31.1	1.38
39	(2E,6E)-7-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,6-dien-1-one	Specific chemical structure	31.242	1.64
40	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl) dihydro-, (3R-trans)-	Specific chemical structure	32.205	0.07
41	(E)-9-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidin-1-yl) non-8-en-1-one	Specific chemical structure	32.928	3.21

## Conclusion

This study offers a comprehensive insight into dried black pepper powder, uncovering its chemical composition, func-

tional properties, and phytochemical attributes. Proximate analysis revealed key components, including moisture content, ash content, fiber content, fat content, and protein estimation, providing valuable nutritional information and potential ap-





**Figure 4:** GC-MS spectra for *P. nigrum* powder. (a) piperlonguminine and (b) piperine.

plications in various food products. Techno-functional properties such as tap density, bulk density, Hausner's ratio, angle of repose, and Carr's ratio were explored, contributing to our understanding of how black pepper powder influences product characteristics, flowability, and stability, essential for food processing and development. Phytochemical analysis demonstrated significant phenolic and flavonoid content, indicating potential antioxidant properties.

The DPPH assay highlighted its ability to scavenge free radicals, suggesting health-related benefits. Utilizing advanced techniques like FTIR, HPLC, GC-MS, and SEM can further enhance the characterization and quality control of black pepper powder, paving the way for diverse applications across industries, including pharmaceuticals and cosmetics. Looking forward, black pepper powder holds promising prospects. Its versatile properties align with the growing demand for natural and functional ingredients in the food, health, and wellness sectors. As consumers seek healthier and more flavorful options, black pepper powder is poised to satisfy these preferences. Its potential in non-food sectors, such as pharmaceuticals and cosmetics, presents exciting opportunities for exploration and innovation. In conclusion, black pepper powder stands as a versatile and promising ingredient, with a bright future in both traditional and contemporary industries.

## Acknowledgements

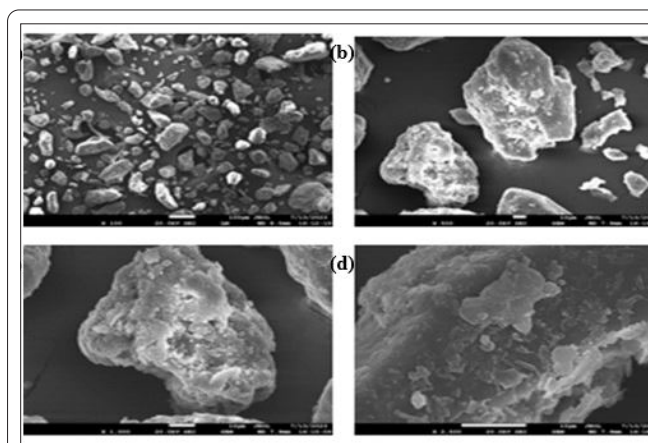
None.

## Conflict of Interest

None.

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**Figure 5:** Scanning electron microscopy (SEM) for *P. nigrum* powder at different magnification. (a) ×100 magnification (b) × 500 magnification (c) × 1000 magnification and (d) × 2500 magnification.

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