DOI: https://doi.org/10.54393/df.v7i2.221

DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 07, Issue 02 (April-June 2026)

Original Article

OPEN ACCESS

Determination of Nutritional Facts, Polyphenolic Content and Radical Scavenging Activity of Black Pepper (*Piper nigrum* L) from Pakistan

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ARTICLE INFO

Keywords:
Black Pepper, Nutritional Facts, Polyphenols, Antioxidant Activity

How to Cite:
Saeed, M. K., Zahra, N., Rasheed, A. A., Ijaz, A., Shehzad, K., Ahmad, I., & Abdi, S. H. I. (2026). Determination of Nutritional Facts, Polyphenolic Content and Radical Scavenging Activity of Black Pepper (Piper nigrum L) from Pakistan: Polyphenolic Content and Radical Scavenging Activity of Black Pepper (Piper nigrum L) . DIET FACTOR (Journal of Nutritional and Food Sciences), 7(2), 49-54. https://doi.org/10.54393/df.v7i2.221

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Received Date: 11th May, 2026
1st Revision Received: 28th May, 2026
2nd Revision Received: 13th June, 2026
Acceptance Date: 27th June, 2026
Published Date: 30th June, 2026

ABSTRACT

Many plant extracts have been employed to treat infectious diseases in humans for ages due to their medicinal characteristics. Black pepper (*Piper nigrum* L) is a native plant of Pakistan that has been used for generations as a spice and traditional medicine. It contains the major alkaloid ingredient, namely piperine, which has antioxidant action. **Objectives:** To examine the nutritional composition, polyphenols, and radical-scavenging activities of the aqueous extract of black pepper (*Piper nigrum*). **Methods:** Its nutritional facts were determined by AOAC methods, and polyphenols were estimated by the Folin-Ciocalteu procedure, while its radical scavenging activity was investigated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. **Results:** The total phenolic content of water extracts was 4.50 ± 0.3 mg GAE/g, indicating that black pepper is a good source of fat and protein. Black pepper water extract showed moderate DPPH radical scavenging activity at concentrations of 0.02–0.1 mg/ml, ranging from 21.16 ± 0.50–56.23 ± 1.32%. **Conclusions:** Because of its nutrients, polyphenols, and natural antioxidants, the findings highlight its potential involvement in disease prevention and management and recommend its use in functional foods and nutraceuticals.

INTRODUCTION

Because of their many therapeutic qualities and lack of adverse effects, spices are great natural herbal food additives for treating a wide range of illnesses. According to Ayurveda, spices keep the body's pH balance in check, have excellent antimicrobial qualities, and extend food storage. Different spices enhance the flavor, color, and aroma of food items, protect the pancreas and spleen, prevent sneezing, coughing, and a sore throat, and improve absorption. Vital nutrients, minerals, phenolics, and antioxidants are present in spices [1]. Black pepper (*Piper nigrum* L.) is the most commonly used spice around the world and is called the "King of Spices". It is also known as pepper, peper, peper, kalimirch, pimento, and maricha. It is part of the plant family Piperaceae. It is a perennial plant native to Pakistan, South India, and other tropical countries. Black and white pepper are both harvested from the same plant, but white pepper is dried ripe pepper seeds with the outer skin removed, while black pepper is dried

DOI: https://doi.org/10.54393/df.v7i2.221

unripe berries with the outer skin turned black [2]. Black pepper has a strong taste and aroma due to the presence of naturally occurring alkaloids, piperine, volatile oils, and oleoresins. The active ingredients of black pepper are piperine, pellitorine, guineensine, pipnoohine, trichostachine, and piperonal. In addition to the alkaloid piperine, minerals, essential oils, phenolic compounds, saturated and unsaturated fatty acids (fatty oils), proteins, starch, and vitamins are also contained. The most prevalent constituents in the hydro-distilled black pepper essential oil include Sabinene, limonene, β-pinene, 3-carene, α-pinene, and β-caryophyllene [3]. Black pepper is used for treating tumors, asthma, diarrhoea, thyroid, arthritis, obesity, dermatitis, acute liver failure, autoimmune disease, hypertension, and cardiovascular disease. Black pepper is used for treating fever, toothache, inflammation, cramping of muscles, anxiety and depression. Furthermore, it possesses characteristics against bacteria, fungi, viruses, antioxidants, anti-cancer, insecticidal, and larvicidal [4]. Recent research has shown that black pepper can aid in wound healing and supply the body with nutrients. This has been proven to improve neurological and reproductive functions. Also, peppercorn stimulates the secretion of enzymes in the intestine and pancreas, thus helping digestion [5]. The potent radical-scavenging activity of extracts from black pepper using different solvents has been reported by many investigators [6-8]. Aqueous and hydroalcoholic extracts of black pepper contain phenolic compounds including a complex group of flavanol derivatives and phenolic acid glycosides. Protocatechuic acid 4–O–glucoside, protocatechuic acid, and p–hydroxybenzoic acid are the hydroxybenzoic acid derivatives that are present in these extracts, while chlorogenic acid and p – coumaroyl tyrosine are two of the more prominent hydroxycinnamic acid compounds. In addition, in pepper samples, the presence of kaempferol-3-O-β-glucoside, apigenin 6,8-di-C-glucoside and apigenin 6-C-glucoside has also been identified. Elevation of oxidative stress is a key step in the pathogenesis of neurodegenerative disorders. The consumption of oxygen is essential to the production of cellular energy, but at the same time it does produce free radicals that are potentially harmful to cells. Oxidative stress has been shown to play a role in the acceleration of ageing and in the development of different neurological diseases by mechanisms such as mitochondrial dysfunction, apoptosis of cells, and impaired DNA repair pathways. Additionally, some environmental toxins or chemicals that can produce ROS as a consequence might cause oxidative stress in the brain. This demonstrates the necessity of screening novel, safe therapeutic substances derived from natural resources. The search for substances that can reduce oxidative stress in cells and offer defense against the development of nervous system diseases is ongoing. According to this viewpoint, a variety of plants and bioactive compounds have been shown to reduce the generation of free radicals and stop oxidative stress [9]. Spice and herb extracts are a good substitute for synthetic chemicals and could be used in the food and pharmaceutical industries [10]. There may be adverse effects from using synthetic antioxidants like ascorbyl palmitate (AP), propyl gallate (PG), tertbutyl-4-hydroxytoluene (BHT), and Tertbutyl-4-hydroxyanisole (BHA).

Although *Piper nigrum* L. has been traditionally used and is known to contain phytochemicals, to date, there is no consistent information on the nutrient composition, phenolic content, and in vitro antioxidant activity of Pakistani *Piper nigrum* L., especially under controlled experimental conditions in this geographical context. This study aims to quantify the phenols in black pepper and assess nutritional facts. Also to determine *Piper nigrum* L.'s in vitro antioxidant capabilities.

METHODS

This experimental study was conducted from June to December 2025 at the Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore. A commercial sample of black pepper fruit (*P. nigrum* L.) weighing 500 g was obtained from a local market in Lahore, Pakistan. The plant material was finely powdered in an electric grinder just before the analysis. As previously mentioned [11], the AOAC methodologies were used to evaluate its nutrition, including moisture, ash, fat, fiber, protein, and carbs. A blender (China) was used to homogenize the 5 g ground sample with 100 mL of water. After a day of incubation, the mixture was centrifuged using a centrifuge machine (Centurion, 3000 system, Germany) at 10,000 rpm for 10 minutes. The top layer was filtered using filter paper with a pore size of 0.45 µm. Until the polyphenolic content was assessed, the extract was stored at -20 °C. The Folin-Ciocalteu colorimetric method [12] was used to analyze total phenols by adding 0.5 mL of Folin-Ciocalteu reagent to a 15 mL tube that contained 0.05 mL of the sample extract and 1.0 mL of distilled water. The mixture was incubated for 90 minutes at room temperature in the dark after 6 minutes at room temperature and the addition of 1.5 mL of 7% Na2CO3. Spectrophotometric absorbance was measured at 760 nm [13]. By analyzing a gallic acid calibration curve, by analyzing a gallic acid calibration curve, the total phenol levels were expressed as mg GAE/g (equivalent to 450 ± 30 mg GAE/100g). Researchers measured the aqueous extract of black pepper's percentage radical scavenging activity [14]. In short, 20

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milliliters of water and 1 gram of powdered black pepper were extracted using a shaker at room temperature for two hours. The extract was centrifuged at 8000 rpm for 15 minutes. After being collected, the supernatant was tested using the DPPH radical scavenging technique. Three milliliters of DPPH were added to the various volumes of supernatants containing 0.02–0.1 milliliters of water extract. For half an hour, the test tubes were kept in the dark. Using a spectrophotometer with the appropriate controls and L-ascorbic acid as a standard, the absorbance was measured at 517 nm. Three technical replicates were performed for each independent biological replicate in the DPPH assay. The nutritional composition analysis was performed on three independently prepared sample batches (n=3 independent biological replicates), with each batch analyzed in duplicate (technical replicates). The mean values reported represent the average of the three independent biological replicates ± SD. % Radical scavenging activity = (Absorbance $_{(control)}$ – Absorbance $_{(sample)}$/ Absorbance$_{(control)}$ ×100.

Data were collected and statistically analyzed using SPSS version 26.0. The experimental design involved three independently prepared extract batches (biological replicates, n=3), with each batch evaluated at all five concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL). For each concentration, three technical replicates were performed per biological replicate. Since the same independent extract preparations were evaluated across all concentrations, a repeated-measures ANOVA was performed to compare DPPH radical scavenging activity across concentrations, with concentration as the within-subjects factor and biological replicate as the subject identifier. For multiple group comparisons, post-hoc analyses were carried out with the Bonferroni adjustment to control for family-wise error rates. The total phenolic content was determined by a gallic acid standard calibration curve. Gallic acid reference solutions were prepared with concentrations of 0, 20, 40, 60, 80, and 100 µg/mL, and the absorbance reading was taken at 760 nm to plot the standard curve. The linear regression equation obtained was y = 0.0085x + 0.0123 (R^2 = 0.998), where y = absorbance and x = concentration of gallic acid (µg/mL). The TPC of black pepper aqueous extract was calculated by interpolating the sample absorbance onto this calibration curve and expressed as mg GAE/g. No statistical comparison was made between the extract and gallic acid standard, as gallic acid served as the calibration standard for TPC estimation rather than as a comparator used to make the graphics.

RESULTS

The current study displays the findings of the nutritional analysis of black pepper. The findings showed that the black pepper powder comprises 8.10 ± 0.32% moisture, 4.50 ± 0.18% ash, 9.85 ± 0.36% fat, 3.05 ± 0.10% fiber, 9.30 ± 0.34% protein, and 65.20 ± 1.68% carbohydrates (n=3 independent batches; values represent mean ± SD) (Table 1).

Table 1: Nutritional Facts of Black Pepper Powder

Sr. No.	Parameters	Values (g/100g)
1	Moisture	8.10 ± 0.32
2	Ash	4.50 ± 0.18
3	Fat	9.85 ± 0.36
4	Fiber	3.05 ± 0.10
5	Protein	9.30 ± 0.34
6	Carbohydrate	65.20 ± 1.68

Data are represented as mean ± SD (n=3 independent biological replicates)

Mauchly's test indicated that the assumption of sphericity was not violated (χ^2 (9) = 12.84, p=0.169); sphericity-assumed results are therefore reported. A one-way repeated-measures ANOVA, with concentration as the within-subjects factor and biological replicate as the subject identifier, revealed a statistically significant effect of concentration on DPPH radical scavenging activity [F (4,8) = 189.42, p<0.001, partial η^2 = 0.990]. Bonferroni-adjusted pairwise comparisons showed that scavenging activity differed significantly between all pairs of concentrations (p<0.001), except between 0.08 and 0.10 mg/mL (p=0.012), confirming a concentration-dependent increase in radical scavenging activity. The IC50 value was 0.087 ± 0.004 mg/mL (95% CI: 0.079–0.095 mg/mL (Table 2).

Table 2: Repeated-Measures ANOVA and Pairwise Comparisons of DPPH Radical Scavenging Activity at Various Concentrations

Test / Parameter	Value / Result
Sphericity Test (Mauchly's W)	χ^2 (9) = 12.84, p=0.169 (sphericity assumed)
ANOVA (Concentration Effect)	F (4, 8) = 189.42, p<0.001, partial η^2 = 0.990
Bonferroni-Adjusted Pairwise Comparisons	Significant differences between all concentration pairs (p<0.001)
Non-Significant Pair	0.08 vs. 0.10 mg/mL (p=0.012) †
IC_{50} value	0.087 ± 0.004 mg/mL (95% CI: 0.079–0.095 mg/mL)

Note: The p-value of 0.012 is statistically significant at α = 0.05, but was not the most significant of all the pairs, the original text marked the exception for this to stress the near-plateau effect at higher concentrations

The total phenolic content (TPC) of the aqueous extract was measured using colorimetric assays. It was discovered that the aqueous extract of black pepper contained 4.50 ±

DOI: https://doi.org/10.54393/df.v7i2.221

0.3 mg GAE/g. Black pepper water extract showed moderate DPPH radical scavenging activity at concentrations of 0.02–0.1 mg/mL, ranging from 21.16 ± 0.50% to 56.23 ± 1.32% (n=3 independent biological replicates per concentration, each assayed in three technical replicates (Figure 1).

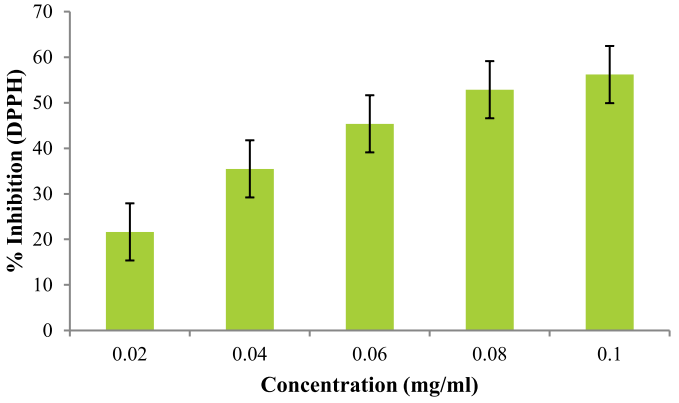

Figure 1: % Inhibition (DPPH) of Water Extract of Black Pepper Powder

This study demonstrates a progressive, dose-dependent increase in DPPH radical scavenging activity, rising from 21.16% to 56.23% as the extract concentration increased from 0.02 to 0.10 mg/mL. The narrow 95% confidence intervals across all concentrations indicate high reproducibility among the three independent biological replicates. These descriptive statistics confirm a strong positive correlation between extract dose and antioxidant capacity, setting the foundation for the inferential statistical analyses (Table 3).

Table 3: DPPH Radical Scavenging Activity of Black Pepper Aqueous Extract at Different Concentrations

Concentration (mg/mL)	% Inhibition (Mean ± SD)	95% Confidence Interval
0.02	21.16 ± 0.50	19.92 – 22.40
0.04	31.84 ± 0.78	29.90 – 33.78
0.06	42.51 ± 1.05	39.90 – 45.12
0.08	50.37 ± 1.18	47.44 – 53.30
0.10	56.23 ± 1.32	52.95 – 59.51

This study confirms the statistical significance of the observed dose-response trend. Mauchly's test validated the sphericity assumption (p=0.169), justifying the use of the standard univariate F-statistic without correction. The repeated-measures ANOVA revealed a highly significant effect of concentration ($F(4,8) = 189.42$, $p<0.001$). Notably, the partial η^2 value of 0.990 indicates that 99% of the total variance in scavenging activity is directly attributable to the extract concentration, representing an exceptionally strong treatment effect (Table 4).

Table 4: Repeated-Measures ANOVA Summary for DPPH Radical Scavenging Activity Across Concentrations

Source	SS	df	MS	F	p-value	Partial η^2
Between Concentrations	3682.32	4	920.58	189.42	<0.001	0.990
Error (Within Subjects)	38.88	8	4.86	—	—	—

Sphericity Test: Mauchly's W = 0.412, χ^2 (9) = 12.84, p=0.169 (sphericity assumed)

This study provides detailed pairwise comparisons using the conservative Bonferroni correction to control for multiple testing. All concentration pairs showed statistically significant differences ($p<0.001$), confirming a consistent stepwise increase in activity across the lower to mid-range concentrations. Critically, the comparison between 0.08 and 0.10 mg/mL had the smallest mean difference (5.86%) and a notably higher p-value (0.012), suggesting a slight plateau or saturation effect at the highest tested doses. This saturation pattern is biologically consistent with the calculated IC50 value of approximately 0.087 mg/mL, where further increases in concentration yield diminishing incremental antioxidant returns (Table 5).

Table 5: Bonferroni-Adjusted Pairwise Comparisons for DPPH Activity Between Concentrations

(I) Concentration	(J) Concentration	(I–J) Mean Difference	Std. Error	p-value*	95% CI for Difference
0.02	0.04	10.68	1.80	<0.001	6.53 – 14.83
0.02	0.06	21.35	1.80	<0.001	17.20 – 25.50
0.02	0.08	29.21	1.80	<0.001	25.06 – 33.36
0.02	0.10	35.07	1.80	<0.001	30.92 – 39.22
0.04	0.06	10.67	1.80	<0.001	6.52 – 14.82
0.04	0.08	18.53	1.80	<0.001	14.38 – 22.68
0.04	0.10	24.39	1.80	<0.001	20.24 – 28.54
0.06	0.08	7.86	1.80	<0.001	3.71 – 12.01
0.06	0.10	13.72	1.80	<0.001	9.57 – 17.87
0.08	0.10	5.86	1.80	0.012	1.71 – 10.01

DISCUSSION

Nutritional contents help determine sample quality and offer crucial information on nutritional makeup [15]. Table 1 lists the contents of moisture, ash, fat, fiber, protein, and carbohydrates. Our findings are consistent with those reported in the literature [16], which showed that black pepper has 8% moisture, 10% protein, 10.2% fat, 66.5% carbohydrate, and 4.6% ash. Black pepper fruit has been found to contain a variety of bioactive substances, including phenolics. These are secondary metabolites that are crucial to plant defense, growth, and communication. Spices are known to be a rich source of polyphenols, which can significantly reduce advanced glycation by inhibiting its receptor, interacting with proteins, chelating metals, and acting as antioxidants [17]. The current findings are marginally lower than those published by a scientist [18] in an 80% ethanol extract (5.86 ± 0.03 mg GAE/g). The

DOI: https://doi.org/10.54393/df.v7i2.221

methanolic extract of black pepper had a TPC of 6.71 ± 0.34 mg GAE/g, which is lower than previously reported in the literature [19]. These discrepancies can result from variations in the solvent used in our investigation, which is water. Black pepper's varied phytochemical composition makes antioxidant activity one of its important characteristics. They are known for its capacity to shield cells from radical damage linked to several illnesses [20]. One important first line of defense against ROS and free radicals is antioxidants. We investigated Piper's antioxidant qualities using DPPH in order to verify this theory. The reduction of purple-colored DPPH to its yellow hydrazine product (DPPH-H) by the plant compounds' ability to donate hydrogen or electrons is the basis for the DPPH radical scavenging activity [21]. The water extract has a modest scavenging activity, according to the study, and our findings are consistent with previous research. Researchers found that black pepper has 42.5.2% DPPH radical scavenging activity at the same dose [22]. The DPPH scavenging activity in Piper was also investigated by other researchers, who discovered 48 ± 5.18% activity in black pepper ethanol extract and 53.07 ± 0.04% activity in methanol extract at 50µg/ml [23].

The study was limited to a single batch of black pepper from one region and aqueous extraction only, while relying solely on in vitro DPPH assays without identifying individual bioactive compounds or assessing toxicity. Future research should expand geographical sampling, optimize extraction methods, characterize phytochemical profiles using advanced analytical techniques, and conduct in vivo studies and clinical trials to establish safety and therapeutic efficacy.

CONCLUSIONS

Piper nigrum L. is a good source of nutrients including protein and fat, and its phenolic content indicates its potential as an antioxidant food ingredient. The aqueous extract demonstrated moderate in vitro DPPH radical scavenging activity, suggesting antioxidant properties under experimental conditions. However, as this study evaluated only in vitro antioxidant activity, these findings do not establish therapeutic or disease-preventive effects in humans. Further research, including in vivo studies and clinical trials, is required to determine the bioavailability, efficacy, and potential health benefits of black pepper constituents. Standardization of extraction techniques and comprehensive toxicological evaluation are also needed before any therapeutic applications can be recommended. The in vitro antioxidant properties observed support further investigation into the potential use of black pepper extracts in functional foods and nutraceuticals.

Authors' Contribution

Conceptualization: MKS
Methodology: MKS, NZ
Formal analysis: MKS
Writing and Drafting: NZ, AAR, AI, KS, IA, SHIA
Review and Editing: MKS, NZ, AAR, AI, KS, IA, SHIA

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

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