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Comparative analysis of four Black Pepper grades based on Piperine yield, Antioxidant activity, and testing for Antidandruff potential

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Abstract

The present study addresses the extraction and isolation of piperine alkaloid from four different grades of black pepper obtained from the local market. Among the samples analysed, grade II exhibited the highest piperine yield and demonstrated strong radical scavenging activity, with an IC_{50} value of 7.596 µg/mL, using ascorbic acid as standard. Thin layer chromatography (TLC) analysis confirmed the purity of the isolated alkaloid, with an Rf value 0.63 matching with the standard reference. Additionally, the antidandruff potential of purified piperine extracts was evaluated, revealing a significant zone of inhibition against *Malassezia furfur* in all grades. The present investigation introduces a reliable method to enhance the quality of black pepper by focusing on its piperine content, which not only contributes to its pungency but also offers valuable antioxidant and antidandruff properties.

Keywords: Piperine; Antioxidant; Antidandruff; Black Pepper

1. Introduction

Black pepper (*Piper nigrum* L.), a tropical vine from the family Piperaceae, is globally recognized for its fruit. It produces small, spherical fruits with a wrinkled surface, known for their spicy taste and distinct aroma. *Piper nigrum* is one of the key spices widely used in food, medicine and cosmetic etc. Its pungent flavour is primarily due to the alkaloid Piperine and its isomers [1], which produces several physiological effects, while its aroma is identified due to its essential oil. The biological role of this spice are well documented in the literature, highlighting its antibacterial, antidepressant, anti-inflammatory, anti-spasmodic, gastric ailments, insecticidal properties etc. [2, 3, 4].

Piperine ($C_{17}H_{19}NO_3$) is a principle phytochemical compound in *P. nigrum* [5]. Research indicates that the pungent compound is paving a way for development of bioactive compound for various disease treatments [2, 6, 7], with concentrations ranging from 97.25% to 98.57 % as reported by Acharya et al [8]. Additionally, Piperine plays a significant role in enhancing drug availability [9].

Antioxidant activity is a key feature of medicinal plants due to their diverse phytochemical composition. They are recognized for their ability to protect cells from radical damage associated with several disorders [10, 11, 12]. Exogenous sources of antioxidants further aid in mitigating oxidative stress thereby enhancing cellular defence against radical-induced damage [10, 11]. As a result, plant derived antioxidants are widely explored for their rich phytochemical content, which helps maintain a balance between endogenous radical scavengers and free radicals.

Dandruff is a common scalp condition characterized by excessive shedding of skin cells and is primarily caused by yeast *Malassezia furfur* [13]. Conventional antidandruff shampoos contain active ingredients such as zinc pyrithione, salicylic acid, ketacanazole etc. [14]. However, these treatments come with certain limitations including the recurrence of

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dandruff and potential side effects. As a result, herbal formulations with natural antifungal properties are explored as a safer and more effective alternatives. Studies focusing on antifungal agents derived from plant sources are gaining attention for their potential to provide long term relief from dandruff while minimizing adverse effects.

The literature review revealed that while phytochemical properties of Black Pepper have been extensively studied, there is a lack of comparative analysis on Piperine yield from different grades of Black Pepper available in the local market. Additionally, the antidandruff potential of Piperine, also remains limited. Therefore, the objective of the present study is to evaluate the Piperine yield among different samples of black pepper collected from local market and to assess their antioxidant and anti-dandruff activities.

2. Materials and Method

2.1. Collection of Plant Materials

Four different grades of dried fruits of *Piper nigrum* (Black pepper) were purchased from a local grocery store in Mumbai as shown in figure (1).

Figure 1 Different grades of dried fruits of *Piper nigrum*

2.2. Preparation of seed extracts

10 g of powder of each grade was soaked in 300ml isopropanol. The extract was left to stand for 48 hours at room temperature with intermittent shaking. Whatman filter paper (number 1) was used to filter the extract. The extracts were used for phytochemical investigations: tests for alkaloids, glycosides, phenolic and tannins [15].

2.3. Isolation of Piperine

Soxhlet extraction using 20g of dried seed powder in 300 ml of isopropanol solvent was performed for 8 hours. The solution in the round bottom flask was concentrated using rotary evaporator. Approximately 15ml of 10% alcoholic sodium hydroxide was added to the concentrated solution at the room temperature. Next day, crystals of Piperine are observed at the bottom of the flask. The residue is collected, dried, and stored in the fridge till further use.

2.4. Thin layer chromatography

Precoated silica gel was used as a stationary phase with a solvent system Toluene: Ethyl acetate (7:3). Dragendorff's reagent was used as a visualizing agent. The Piperine samples of all grades along with standard (obtained from ICT lab., Matunga) were run on TLC and were analyzed.

2.5. Antioxidant activity

1gm powdered black pepper was extracted with methanol using a shaker at room temperature for 2 hours. The extract was centrifuged for 15 minutes at 8000 rpm. The supernatant was collected and was assayed for DPPH radical scavenging method. The different amounts of supernatants (of each grades) with 1 ml methanolic extract were taken and added 3ml DPPH. The test tubes were kept in dark for 30 minutes. The absorbance was measured at 517 nm using spectrophotometer with proper controls and L-ascorbic acid taken as standard. The assay was performed in three replicates. The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

$$\% \text{ Radical scavenging activity } = \frac{\text{Absorbance(control) – Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

IC_{50} of extracts was computed from a plot of percentage of DPPH free radical scavenging free inhibition versus extract concentration.

2.6. Antidandruff activity

2.6.1. Isolation of fungus

The sample was collected from an affected individual's scalp and inoculated into sterile Sabouraud dextrose agar (SDA) medium. The plates were incubated for 3 to 5 days. The white fungal growth observed on plates was identified through microscopic analysis and further confirmed by the Microbiology Department of the college.

2.6.2. Antifungal assay

The antifungal activity of Black pepper extracts against *Malassezia furfur* was investigated by disc diffusion method. A drop of olive oil was applied to the agar medium before uniformly spreading the organism over the surface of the plate. Paper discs soaked in purified Piperine extracts were placed along with a control. The plates were then incubated at 37°C for 24 and 48 hours, and the results were recorded after the incubation period.

3. Results and Discussion

3.1. Phytochemical analysis

The isopropanol extracts were analyzed for secondary metabolites with all grades testing positive for alkaloids and tannins (Table 1). However, the remaining tests yielded variable results across different grades. In grades 2 and 4, along with alkaloids and tannins, the presence of steroids and terpenoids were also observed. These findings indicate that alkaloids are the predominant constituents in the isopropanol extracts. Similar results have been reported using different solvents, such as methanol [16], dichloroethane [17], and glacial acetic acid [18]. The use of isopropanol in the present study proves to be an effective solvent for Piperine extraction, complementing the solvents previously identified by researchers.

Table 1 Phytochemical analysis of isopropanol extracts of different grades of Black Pepper

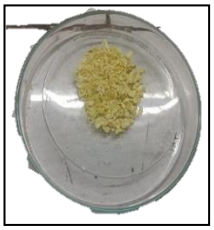
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Alkaloids	++	++	++	++
Glycosides	--	--	--	--
Flavonoids		--		
Tannins	+	+	+	+
Saponins	--	--	--	--
Steroids	--	+	--	+
Terpenoids	+	+	--	+

Note: '+' indicates presence and '—' indicates absence

3.2. Isolation of Piperine

Piperine was extracted from different grades of black pepper using isopropanol with variations in colour and texture as mentioned in Table (2). The yield of Piperine, a key determinant of spice quality, was measured for each grade. 20 grams of black pepper yielded 68% for Grade I, 90% for Grade II, 36% for Grade III, and 51% for Grade IV. Among these, Grade II exhibited the highest quality, followed by Grade I, while Grade IV was moderate, and Grade III was of the lowest quality. Similar findings were reported by Mollik et al [16] and Delia [19] when using methanol as the solvent system.

Table 2 Comparative analysis of Piperine isolated from different grades of Black Pepper

Grades	Color	Texture	% of Piperine	Isolated Piperine
I	Corn Yellow	Cluster	68.6	
II	Flexen Yellow	Cluster	90.6	
III	Yellow	Powdery	36.3	
IV	Lemon Yellow	Papery	51.5	

3.3. TLC analysis

The purity of the Piperine was confirmed by TLC analysis. The isolated Piperine, along with the standard, was run on a silica plate as shown in Figure (2). The calculated Rf values for the four grades were 0.60, 0.63, 0.65, and 0.61, while the standard Piperine had an Rf value of 0.63. These results confirmed the purity of grade II. Low-intensity bands were also observed in other grades alongside the Piperine band, indicating the possible presence of impurities. This study validates the use of isopropanol as an effective isolation solvent, offering an alternative to ethanol or methanol, as demonstrated by Mollik et al. [16]. Additionally, Dragendorff's reagent proved to be a more convenient detection method compared to anisaldehyde-sulfuric acid [20], or vanillin reagent [16].

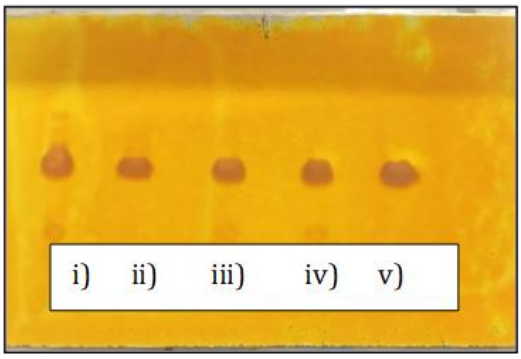


Figure 2 TLC Profile of purified Piperine from different grades (i to iv) and standard (v) of Black pepper using Dragendroff's reagent

3.4. Antioxidant assay

The antioxidant potential of black pepper extracts was assessed through IC_{50} values, which represent the concentration of the sample required to scavenge 50% of DPPH free radicals (Figure 3). IC_{50} is a widely recognized parameter for evaluating antioxidant activity [21]. A lower IC_{50} value indicates a stronger antioxidant capacity. In this study, Grade II exhibited the lowest IC_{50} value (7.596 µg/L), demonstrating superior antioxidant potential compared to other samples, including ascorbic acid (9.6 µg/L). The remaining grades displayed IC_{50} values exceeding 10 µg/L, suggesting relatively lower antioxidant activity compared to the standard. These findings align with previous research indicating that black pepper extracts possess notable free radical scavenging activity [22]. Additionally, the antioxidant effect increased with higher extract concentrations, supporting the dose-dependent nature of antioxidant efficacy [23]. Based on these results, black pepper extracts emerge as promising candidates for plant-derived antioxidant compounds with potential applications in nutraceuticals and functional foods.

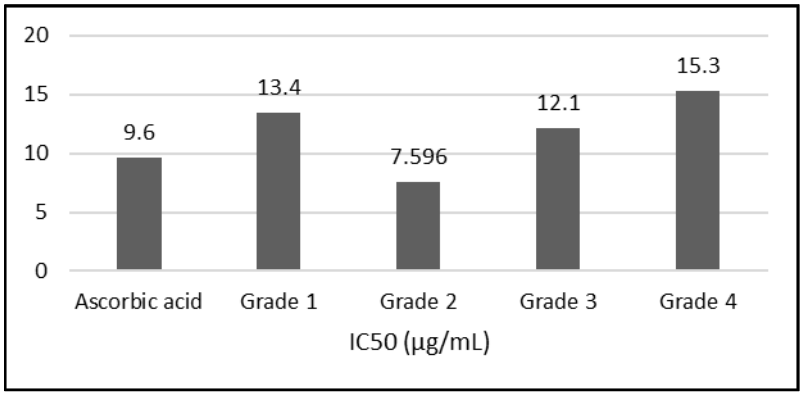


Figure 3 IC_{50} values of different grades of Black Pepper along with Ascorbic acid

3.5. Antidandruff activity

The antifungal activity of purified Piperine extracts are tabulated below:

Table 3 Comparison of antifungal activity of purified Piperine from different grades black pepper and antidandruff shampoos on the basis of zone of inhibition

Piperine extracts	Diameter in cm	Antidandruff Shampoos	Diameter in cm
Grade I	1.5	Brand a	2.3
Grade II	2	Brand b	2.5
Grade III	1.8	Brand c	2.5
Grade IV	1.5		

Several studies have reported the antifungal activity of plant extracts and its essential oil against dandruff causing fungus *Malassezia furfur* [13, 24, 25]. However, there are few reports of using purified piperine extracts for anti-dandruff activity. The disc diffusion method was used to assess its effectiveness against the fungus by measuring the zone of inhibition. Among all the grades tested, grade II showed the highest activity followed by grade III and others. When compared with antidandruff shampoos, enhanced activity was observed with shampoos. This observation aligns with the findings of Pingili et al [25] when comparing crude plant extracts with antidandruff shampoos. The results indicate significant antifungal activity of piperine extracts, which can be further studied to elucidate its precise mechanism of action against *Malassezia furfur*.

4. Conclusions

This study demonstrates that isopropanol is an effective method of extracting piperine from black pepper of different grades. Grade II found to be better quality in terms of piperine yield and antioxidant activity. TLC analysis confirmed the purity of isolated alkaloid, which also showed significant antifungal activity against *Malassezia furfur*, highlighting

its potential as an antidandruff agent. Optimizing the extraction process can enhance its medicinal value, making it suitable candidate for pharmaceutical and cosmetic applications.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest to disclose.

References

[1] Govindarajan VS, Stahl WH. Pepper chemistry, technology, and quality evaluation. C R C Critical Reviews in Food Science and Nutrition. 1977; 9(2):115–225.

[2] Srinivasan K. Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Crit Rev Food Sci Nutr. 2007;47(8):735-48.

[3] Meghwal M, Goswam TK. Chemical Composition, Nutritional, Medicinal and Functional Properties of Black Pepper. Open Access Scientific Reports. 2012;1(2).

[4] Sharma A, Gautam S, Jadhav SS. Spice extracts as dose-modifying factors in radiation inactivation of bacteria. J Agric Food Chem. 2000Apr;48(4):1340-4.

[5] Butt MS, Pasha I, Sultan MT, Randhawa MA, Saeed F, Ahmed W. Black pepper and health claims: a comprehensive treatise. Crit Rev Food Sci Nutr. 2013;53(9):875-86.

[6] Chavarria D, Silva T, Magalhães e Silva D, Remião F, Borges F. Lessons from black pepper: piperine and derivatives thereof. Expert Opin Ther Pat. 2016;26(2):245-64.

[7] Takooree H, Aumeeruddy MZ, Rengasamy KRR, Venugopala KN, Jeewon R, Zengin G, Mahomoodally MF. A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. Crit Rev Food Sci Nutr. 2019;59(sup1):S210-S243.

[8] Acharya S G, Momin A H, Gajjar A V. Review of piperine as a bioenhancer, American journal of pharmatech research. 2012: 2(2), 32-44.

[9] Bai, YF, Xu H. Protective action of piperine against experimental gastric ulcer. Acta Pharmacol. Sin. 2000, 21: 357–359.

[10] Rahman M, Bajgai J, Fadriquela A, Sharma S, Trinh Thi T, Akter R et al. Redox effects of molecular hydrogen and its therapeutic efficacy in the treatment of neurodegenerative diseases. Processes. 2021a, 9(2):308.

[11] Rahman M, Bajgai J, Fadriquela A, Sharma S, Trinh TT, Akter R et al. Therapeutic potential of natural products in treating neurodegenerative disorders and their future prospects and challenges. Molecules. 2021b, 26(17):5327.

[12] Zahin M, Bokhari NA, Ahmad I, Husain FM, Althubiani AS, Alru ways MW et al. Antioxidant, antibacterial, and antimutagenic activity of Piper nigrum seeds extracts. Saudi J Biol Sci. 2021, 28(9):5094–5105.

[13] Arora P, Nanda A, Karan M. Screening of plant essential oils for antifungal activity against malassezia furfur. Int.journal of pharmacy & pharmaceutical sciences. 2013, 5(2): 37-39.

[14] Vijayakumar R, Muthukumar C, Kumar T, Saravanamuthu R. Characterization of Malassezia furfur and its control by using plant extracts. Indian Journal of Dematology. 2006, 51(2):145-148.

[15] Khandelwal KR 2008. Practical Pharmacognosy. Pragati books Pvt Ltd.

[16] Mollik M, Rahman H, Al-Shaeri M, Ashraf G, Alexiou A, Gafur A. Isolation, characterization and in vitro antioxidant activity screening of pure compound from black pepper (Piper nigrum). Environmental Science and Pollution Research, 2022, 29:52220-52232.

[17] Madhavi B, Anisetti RN, Banji D, Madhu M, Ramani R, Swetha D. Extraction, Identification, formulation and evaluation of Piperine in alginate beads. Int J Pharm Pharmaceut Sci. 2009, 1:156-161.

[18] Kanaki, N, Dave M, Padh H, Rajani M. A rapid method for isolation of piperine from the fruits of Piper nigrum Linn. Journal of natural medicines. 2008, 62:281-3.

[19] Delia Nica-Badea. Separation. Identification and Estimation of Piperine as Major Constituent from Black Pepper, by Thin Layer Chromatography Coupled with GC-MS. REV. CHIM. (Bucharest). 2014, 65 (6): 730-733.

[20] Paarakh PM, Sreeram DC, D SS, Ganapathy SP. In vitro cytotoxic and in silico activity of piperine isolated from Piper nigrum fruits Linn. In Silico Pharmacol. 2015 Dec, 3(1):9.

[21] Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J Agric Food Chem. 1998, 47: 425–4.

[22] Kumar A, Sasmal D, Sharma N. Immunomodulatory role of piperine in deltamethrin induced thymic apoptosis and altered immune functions, Environmental Toxicology and Pharmacology.2015, 39(2):504-514.

[23] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995, 28:25–30.

[24] Lee, Jeong-Hyun and Jae-Sug Lee. Chemical composition and Antifungal Activity of Plant Essential Oils Against Malassezia furfur. Kor. J. Microbiol. Biotechnol. 2010, 38 (3): 315-321.

[25] Pingili M, Vanga S, Raparla R. Antifungal activity of plant extracts against dandruff causing organism Malassezia furfur. International Journal of Bioassays 2016, 5:5047-5049.