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Phytochemical analysis of *Piper nigrum*

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ABSTRACT

Medicinal plants contain varieties of bioactive compounds such as alkaloids, terpenoids, tannins, flavonoids, steroids that possess anti-inflammatory, antioxidant, antibacterial, anti-diabetic functional properties and also protects from many chronic diseases. In the present study the *Piper nigrum* seeds were screened for qualitative phytochemical analysis. *P. nigrum* seed powder was extracted with distilled water and ethanol. The solvent free extracts were then subjected for phytochemical screening. The study confirms that *P. nigrum* possesses a number of important phytochemical constituents with therapeutic activities.

Keywords: Piper nigrum, medicinal plant, phytochemicals, bioactive compounds, therapeutic activities.

1. INTRODUCTION

Herbs and spices have been a crucial part of human nutrition since the beginning of mankind. They have not only been used to increase the flavour, colour, and aroma of food, but also have been recognized for their preservative features and medicinal properties. [1,2,3,4]

Black pepper scientifically known as *Piper nigrum* is one of the most commonly used spices. It is also known as Pippali in Sanskrit, Kali Mirch in Urdu, White pepper, Peppercorn, Madagascar pepper, Green pepper, pepper in English.[5,6] Due to its trade in international market, it is known as "The King of Spices".[7,8,9] Geographically, P. nigrum is cultivated in moist and hot conditions.[10,11]

Black pepper is a climbing vine. It is perennial and grows well in the shade with support from trees or poles. The glabrous woody climbers grow up to 10 m or more height.[12,13,14] Black pepper plant has 10–20 primary adventitious roots developed from the base of the mature stem.[13,15] Leaves are simple, alternate, with 2 to 5 cm long grooved petiole, variable leaf length of 8–20 cm and breadth of 4-12 cm.[15] Presence of piperine and volatile oils in black pepper contributes to its aroma and pungency.[16,17,18,19]

Time of harvest and processing techniques vary for White and black peppers. White pepper is obtained by removing the pulp from ripe fruit, while black pepper is produced by drying unripe fruit to wrinkled form which still contains the pulp.[19,20,21,22,23,24] Both white and black pepper have a wide range of applications, like spices, preservatives, insecticides, and also in herbal medicine.[19,21,25]

Studies on black pepper bioactivities have reported a very high spectrum of pharmacological activities like antioxidant, antiplatelets,[26] antitumor,[27] anti-asthmatics,[28] analgesic,[29] anti-inflammatory,[30] antihypertensive,[31,32] anti-diarrheal, antispasmodic, antipyretic, anxiolytic, antidepressants,[33] hepato-protective,[34] antipyretic, immuno-modulatory, anti-apoptotic, antibacterial, anti-thyroids, antimutagenic, anti-metastatic, anti-spermatogenic, insecticidal , antifungal and larvicidal activities etc. Additionally, piperine is known for its ability to increase the bioavailability of drugs, and thus enhance their therapeutic potential.[35,36,37,38,39,40]

2. MATERIALS AND METHODS

2.1. Collection and drying

Fresh peppercorn of *P. nigrum* was collected from local market, Patna. The black pepper was washed thoroughly three times with sterile distilled water. The materials were air dried under hot air oven at 55°C for 3 hours. Clean raw material was grinded well in the grinder. Grinder content was then filtered with thin cloth piece to collect fine powder. Powdered samples were then stored in separate airtight containers until the time of the extraction (Fig-1).

Fig.1: Powdered sample of *Piper nigrum*

2.2. Chemicals and Reagents

High purity commercially available chemicals of analytical grade were used during the investigation of Phytochemical constituents.

2.3. Extract Preparation

100gms of powdered *P. nigrum* was soaked in double distilled water and ethanol separately for 72 hours with occasional shaking. Afterwards both the extracts were filtered using Whatman filter paper No. 1 and Buchner funnel. The solvents were removed under pressure in rotatory evaporator at 50˚C and the dried *P. nigrum* seed powder was stored at 4˚C for further study. The nature and colour of P. nigrum in both the solvent is shown in Table-1.

Table-I: Nature and colour of *P. nigrum* seed extract

Solvent	*Piper nigrum*	
	Texture	**Colour**
Distilled water	Crystal	Brown
Ethanol	Sticky	Yellow brown

3. PHYTOCHEMICAL ANALYSIS

The aqueous and ethanolic extract of *P. nigrum* seed were subjected to various qualitative test for the identification of various plant constituents present in the species.

3.1. Test for Phenols

To 1ml of extract, 3-4 drops of 10% ferric chloride solution was added. Bluish black colour appearance shows the presence of phenols.

3.2. Test for Carbohydrates

- **Fehling's Test:**1ml of extract was heated with equal quantities of Fehling's solution A & B. Formation of a brick red precipitate indicates the presence of sugar.
- **Benedict's Test:** Extracts were treated with Benedict's reagent & heated gently. Orange red precipitate shows the presence of reducing sugar.

3.3. Test for Alkaloids

Both the extracts were treated dissolved individually in dil. HCl. After filtration these acidified extracts were then subjected to the following test:

- **Mayer's Test:** To 1ml of filtered acidified extract, 1ml of Mayer's reagent (Potassium mercuric iodide Sol.) was added. Formation of Wheatish Yellow coloured precipitate shows the presence of alkaloids.
- **Wagner's Test:** To 1ml of filtrate 2ml of Wagner's reagent (Iodine in Potassium iodide) was added. Reddish brown precipitate indicates the presence of alkaloids.
- **Dragendroff's Test:** To 1ml of filtrate 1ml of Dragendroff's reagent (Potassium bismuth iodide solution) was added. Formation of Orange red precipitate shows the presence of alkaloids.
- **Hager's Test:** To 1ml of filtrate 3ml of Hager's reagent (Saturated Picric acid) was added. Formation of yellow colour shows the presence of alkaloids.

3.4. Test for Glycosides

- **Balijet Test:** 1ml of the extracts were treated with 1ml of Sodium picrate solution. The formation of Orangish yellow colour indicates the presence of Glycosides.

3.5. Test for Saponins

- **Froth Test:** Both the aqueous and the ethanolic extracts were taken separately and it was diluted with 20ml of Distilled water & was then shaken in graduated cylinder for 15minutes. Formation of 1cm layer of foam shows the presence of saponins.

3.6. Test for Tannins

- **Lead acetate Test:** Both the aqueous and the ethanolic extract of the sample were treated with 1% sol. of Lead acetate. Formation of white precipitate indicates the presence of tannins.
- **Ferric chloride Test:** To 1ml of plant extract, 5% 1ml Ferric chloride sol. was added. Appearance of dark black or green colour shows the presence of tannins.

3.7. Test for flavonoids

- **Lead acetate Test:** Test samples were treated with few drops of Lead acetate solution. Formation of yellow colour precipitate shows the presence of flavonoids.
- **Alkaline Reagent Test:** The extracts were treated with few drops of Sodium hydroxide solution. Formation of deep yellow colour which becomes colourless after the addition of dilute acid indicates the presence of flavonoids.

3.8. Test for Proteins & Amino acids

- **Xanthoproteic Test:** The test samples were heated with few drops of conc. Nitric acid. Appearance of yellow colour precipitate shows the presence of protein.
- **Biuret Test:** To 1ml of 40% Sodium hydroxide sol. 2 drops of 1% $CuSO_4$ sol. was added. Blue colour was produced then 1ml of test sample was added. Appearance of pinkish or purple violet colour indicates the presence of protein.
- **Ninhydrin Test:** The small quantity of samples was heated with 2 drops of freshly prepared 0.2% Ninhydrin reagent (0.1%solution in n-butanol). Formation of the blue colour indicates the presence of protein, peptides or amino acids.

3.9. Test for Gum & Mucilage

To 500µl of sample extracts, 1ml of absolute alcohol was added. Formation of white/cloudy precipitate shows the presence of gums & mucilage.

3.10. Test for Oils:

- **Spot Test:** The small quantity of extracts was pressed between the filter paper. Oil stains on paper shows the presence of fixed oils.

3.11. Test for Cholesterol

To 2ml of test sample, 2ml of chloroform with 10 drops od acetic anhydride was added and then was treated with 2-3 drops of conc. H_2SO_4. Appearance of red rose colour shows the presence of cholesterol

3.12. Test for Terpenoids

2ml of chloroform was added to 5ml of test sample (evaporated on water bath) and then 3ml of conc. H_2SO_4 was added. Appearance of grey coloured solution indicates the presence of terpenoids.

3.13. Test for Phytosterols

- **Salkowski Test:** To 1ml of test sample, 2ml of chloroform and equal layer of conc. HCl were added. The colour of upper layer changes to reddish and the HCl part showed yellow with green fluorescence and this appearance indicates the presence of steroids in the plant sample.

4. RESULT & DISCUSSION

The phytochemical constituents of aqueous and ethanolic extracts of *P. nigrum* are tabulated in Table-II and the preliminary phytochemical screening for different tests in *P. nigrum*was shown in Fig-2.

Table-II: Qualitative screening of *Piper nigrum*

Tests	***Piper nigrum***	
	Aqueous	**Ethanolic**
Phenols	+	+
Carbohydrates		
Fehling's Test	+	-
Benedict's Test	-	-
Alkaloids		
Mayer's Test	+	+
Wagner's Test	+	+
Dragendroff's Test	+	+
Hager's Test	+	+
Glycosides		
Balijet Test	+	+
Saponins		
Froth Test	-	-
Tannins		
Lead acetate Test	+	+
Ferric chloride Test	+	+
Flavonoids		
Lead acetate Test	+	+
Alkaline Reagent Test	-	+
Proteins& Amino acids		
Xanthoproteic Test	+	+
Biuret Test	+	+
Ninhydrin Test	+	+
Gums & Mucilage	-	-
Fixed Oils		
Spot Test	-	-
Cholesterol	+	-
Terpenoids	-	-
Phytosterols		
Salkowski Test	+	+

+ indicates presence of phytochemicals and – indicates absence of phytochemicals.

Fig.2: Preliminary Phytochemical screening of *Piper nigrum*

The present study demonstrated that *P. nigrum* seeds are rich in several phytochemical constituents such as alkaloids, carbohydrates, phenols, tannins, flavonoids, terpenoids, glycosides, phytosterols, proteins and amino acids. It was identified that both the carbohydrates and cholesterol was present in aqueous extract while it is absent in ethanolic extract. Many constituents of plants are known to possess wonderful biological activities. Alkaloids are naturally occurring nitrogenous compounds which are widely used as chemotherapeutic agents, CNS stimulant and anaesthetics. Terpenoids are known to have various pharmacological activities such as antifungal, anti-inflammatory, antiparasitic, antifungal, anti-hyperglycaemic, antimicrobial, immuno- modulatory and anti-parasitic. Tannin rich plants are commonly used in several diseases as a healing agent. Phenols present in plant act as free radical scavengers. Flavonoids on the other hand, work as an antioxidant and enhances the effect of Vitamin C. Steroids plays an important role in pharmacy as they have compounds like sex hormones and are used for drugs production.

5. CONCLUSION

The result of this study showed that both aqueous & ethanolic samples of *P. nigrum* seed consist of varieties of useful phytochemical constituents. Due to the presence of secondary metabolites, *P. nigrum* shows a wide range of biological activities like anti-diabetic, antioxidant, antibacterial, analgesic, antiviral, immuno- modulatory and anti-inflammatory activities. As the phytochemical analysis of the plant is important to pharmaceutical companies for the drug discoveries for the treatment of various diseases. It also reveals that P. nigrum contains various medicinally important bioactive compounds and justifies its use as a convectional medication for the treatment of several diseases.

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7. CONFLICT OF INTEREST

No conflict of interest

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