



Evaluation of antibacterial activity and phytochemical analysis of white pepper (*Piper nigrum L.*) and extraction of essential oil

Swathi Govindaraj¹, Saruka Sundaram², Sowmiya Palanisamy³

¹Assistant professor Biotechnology, Bannari Amman Institute of Technology, Sathy

²B.Tech Biotechnology, Bannari Amman Institute of Technology, Sathy

³B.Tech Biotechnology, Bannari Amman Institute of Technology, Sathy

ABSTRACT

Medicinal plants have various health benefits and disease preventing properties. Phytochemicals are the medicinally active substances found in most of the plants. In this study, the antibacterial activity of white pepper (*Piper nigrum L.*) against some disease causing bacteriae such as *E.coli*, *Bacillus subtilis* have been evaluated by agar well diffusion method. Various tests have been done to detect the presence of saponins, tannins, alkaloids, flavonoids and phenolic compounds. The antibacterial activity against *E.coli*, *Bacillus subtilis* is due to the presence of phytochemical compounds in white peppercorns (*Piper nigrum L.*). The spicy, warm and aromatic essential oil with nutritive values have been extracted by steam distillation.

Keywords: Antibacterial activity, Essential oil, Phytochemical analysis, *Piper nigrum L.*, Silver nanoparticles, Steam distillation.

I. INTRODUCTION

Pepper (*Piper nigrum L.*) is the most common and valuable spice in the world with numerous benefits for human health. Black peppercorns are normally used by large population of people for its flavour, spice and aromatic nature in food. It is also being used in large scale for its medicinal properties in the preparation of homemade medicines then and now. Black and white peppercorns are both the fruits of the pepper plant but they are processed differently.

Black pepper is picked when they are almost ripe and sundried in which the outer layer turns black in colour whereas the white peppercorns are picked when they are fully ripen and the outer layer is removed before or after drying and only the inner seeds are used. White pepper is more hotter than the black pepper and it is used in cooking food items that should retain white in colour and also used for its enhanced flavour.

In this study, the medicinal values and bioactive compounds of white pepper (*Piper nigrum L.*) has been evaluated by detecting its antibacterial activity against disease causing bacteriae such as *E.coli* and *Bacillus*



subtilis. Medicinal plants are naturally available renewable source and it is easily absorbed by our body and donot have any adverse effects. White pepper serves as a best medicine for upset stomach, bacterial infection, arthritis, joint pain, lowering the blood pressure and in maintaining the heart health. It also helps to maintain good cholesterol in the body and improves brain function. White pepper helps to prevent the occurrence of cancer. Essential oil extracted by the steam distillation from white pepper is used to aid the digestive and nervous system.

II. MATERIALS AND METHODS

2.1. Collection and drying

Fresh white peppercorns are collected from the spice store, Sathyamangalam, Erode district. It is washed with distilled water and sun dried for 3 hours. It is then grinded into a fine powder and stored in an air tight container under temperature until used.

2.2. Soxhlet extraction

40 grams of white pepper powder is extracted with 200 ml of ethanol (56 °C- 60 °C), methanol (65 °C- 70 °C) and chloroform (76 °C- 78 °C) in soxhlet extractor until the clear extract is obtained. The extract is distilled to eliminate the solvent and to obtain a pasty form. It is stored in sample containers until use.

2.3. Inoculum preparation

Single colony of *E.coli* and *Bacillus subtilis* is inoculated in 5ml of nutrient broth and incubated at 37 °C until the turbidity forms.

2.4. Antibacterial activity by agar well diffusion method

Muller-Hinton agar is used to determine the antibacterial activity of *Piper nigrum L.* Two clean sterile petri plates were taken and Muller-Hinton agar is poured. To the prepared Muller-Hinton agar plates the bacterial inoculum is spreaded using a sterile swab. The wells are punched on the agar plates and the ethanol, methanol and chloroform extracts were added to both the plates. The plates are incubated at 37 °C for 24 hours. The antibacterial activity of white pepper against *E.coli* and *Bacillus subtilis* is evaluated by the formation of zone of inhibition on the plates.

2.5. Phytochemical analysis

2.5.1. Test for alkaloids

10 ml of extract of each solvent is taken in a test tube.



2.5.1.1. Mayer's test

A drop of mayer's reagent is added to the test tube. Formation of white precipitate or turbidity indicates the presence of alkaloids.

2.5.1.2. Wagner's test

A drop of wagner's reagent is added to the test tube. Presence of alkaloids in the extracts is determined by the formation of reddish brown precipitate.

2.5.2. Test for flavonoids

2.5.2.1. Sodium hydroxide test:

To 5ml of extract a few drops of dilute sodium hydroxide was added and the intense yellow colour appeared in the plant extract becomes colourless on the addition of dilute hydrochloric acid which indicates the presence of flavonoids.

2.5.2.2. Lead acetate test:

10 ml of sample is taken in a test tube and heated with 5 ml of lead acetate for 2 - 3 minutes. Then the diluted ammonia is added. Formation of yellow colour indicates the presence of flavonoids.

2.5.3. Test for tannins

2.5.3.1. Ferric chloride test

To the 10 ml of sample 0.1% of ferric chloride is added. Presence is indicated when the solution turns green.

2.5.3.2. Gelatin test

5 ml of sample is heated with required amount of gelatin in the steam bath for 5 minutes. Formation of white colour indicates the presence of tannins.

2.6. Extraction of essential oil

Extraction of white pepper oil is done by steam distillation method. In this method, crushed white peppercorns is distilled with water as a solvent at 110 °C. The distillate is then transferred to the separating funnel and 5 ml of hexane is added and shaken. Now the distillate is allowed to stand for some time until two layers of aqueous and organic phases are seen. The water (aqueous layer) is more denser and is seen on the bottom and the organic phase containing hexane and oil appears at the top. The organic phase is collected and it is heated for the hexane to evaporate leaving the essential oil.



Volume of essential oil extracted (ml)

Yield of essential oil (ml/g) = -----

Amount of sample used (grams)

III. RESULT AND DISCUSSION

3.1. ANTIBACTERIAL ACTIVITY

Bioactive molecules derived from medicinal plants has a great potential in therapeutics and medicine. Piperine is an alkaloid present in all the plants of the family piperaceae. It is now used in all the herbal medicines. It is slightly soluble in water and highly soluble in chloroform and ethanol. The antibacterial activity of ethanol extract is higher against *E.coli* and *Bacillus subtilis*. The chloroform and methanol extracts shows comparatively lesser antibacterial activity against *E.coli* and *Bacillus subtilis*.

Table 3.1: Determination of antibacterial activity

STRAINS OF BACTERIA	Zone of inhibition (mm)		
	Chloroform extract	Ethanol extract	Methanol extract
<i>E.coli</i> (Gram negative)	5.5	8.5	7.0
<i>Bacillus subtilis</i> (Gram positive)	5.2	8.0	6.5

3.2. PHYTOCHEMICAL ANALYSIS

Table 3.2. Qualitative test for phytochemicals

TESTS		ETHANOL EXTRACT	METHANOL EXTRACT	CHLOROFORM EXTRACT
Alkaloids	Mayer's test	+	+	+
	Wagner's test	+	+	+
	Sodium	+	-	+



Flavonoids	hydroxide test			
	Lead acetate test	+	-	+
Tannins	Ferric chloride test	+	+	+
	Gelatin test	+	+	+

3.3. EXTRACTION OF ESSENTIAL OIL

The yield of essential oil obtained from *Piper nigrum L.* is 0.25 ml/g. Oleoresin is the naturally occurring compound and is a mixture of oil and resin. It is present in almost all the plants. In *Piper nigrum L.* oleoresin contains alkaloids, piperine and chavicine which contributes for its pungent taste.

IV. CONCLUSION

Piper nigrum L. is the native plant of South India. It has various medicinal properties and acts as an anti inflammatory, anti bacterial, anti depressant, anti oxidant. It has also been used as an ayurvedic medicine from thousands of years.

REFERENCES

- [1] S.P. Malu, Go. Obochi, Antibacterial activity and medicinal properties of ginger, Global journal of pure and applied sciences, 15(3), 2009, 365 -368.
- [2] M.A. Dib, N. Djabou, Identification of phenolic compounds and antimicrobial activity of roots of *Arbutus unedo L.*, Asian journal of chemistry, 22(5), 2010, 4045 - 4053.
- [3] Mital Patani, Jitendra Jain, Bhavna Marya, Mandev Patel, Formulation and evaluation of Indomethacin bilayer sustained release tablets, International Journal of Pharmacy and Technology, 3(1), 2011, 1867 - 1875.
- [4] Sluer, R. Troller, Caebi, Antibacterial and anti-inflammatory kinetics of Curcumin as a potential antimucositis agent in cancer patients, Nutrition and cancer, 64(7), 2012, 975 - 981.
- [5] Thien Hien Tran, Le Ke Ha, Duy Chinh Nguyen, The study on extraction process and analysis of components in essential oils of black pepper (*Piper Nigrum L.*), World Journal of Agricultural Sciences, 7, 56, 2018.