



Phytochemical Analysis and Comparative Study of Anti-Bacterial Activity of Pepper

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Abstract

Black pepper (*Piper nigrum* L.) native of south India popularly known as “king of spices”. Pepper is mostly used in the curry recipes as masalas and also as ingredient in the prescriptions of folk medicine, Ayurveda and traditional medicinal systems. The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. In the present study, we have evaluated the antibacterial effect of the Ethanol, and water extracts of widely used Pepper variety in South India, such as Panniyur 6 and Panniyur 7 against two bacterial human pathogens such as *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumoniae* was tested. The antibacterial activity was measured by agar well diffusion method. Piperine showed antibacterial activity against all tested bacteria with zone of inhibition ranged from 8-14mm. Maximum zone of inhibition was against Gram positive bacteria *Bacillus subtilis* (14mm) on ethanol extract. The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent.

Keywords: Black Pepper; Panniyur; Phytochemicals; Anti-Bacterial Activity

Introduction

The term spices refer to aromatic or pungent vegetable substances used for flavouring foods and have several commercial uses according to (ISO). Since ancient times people used spices for preventing food deterioration and pathogenic diseases. Phytochemical investigations of the aerial parts of the plants have tartaric acid, acetic acid, citric acid, succinic acid, gums, pectin, sugars, tannins, alkaloids, flavonoids, glycosides and sesquiterpenes [1-4].

Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices have also been Exploited [5].

The antimicrobial activity of is documented an alarming interest continues to the present [6]. Pepper is also used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neuropathy, melanoderma and leprosy due to the presence of vola-

tile compounds, tannins, phenols and other unknown substances [2,7,8]. The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit.

According to alkaloids play a significant role in plant physiology, agriculture, host-plant resistance, entomology, the diet and medicine. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper.

Black pepper is native to the Malabar coast of India, and the Malabar pepper is extensively cultivated there and in other tropical regions. Pepper Panniyur is the first Black Pepper variety developed by black pepper research institute in Kerala Agricultural University, Kannur. Panniyur crop is mainly cultivated in major parts of Kerala, Karnataka, Tamil Nadu. Panniyur-1, Panniyur-2, Panniyur-3, Panniyur-4, panniyur-5, Panniyur-6, Panniyur-7 and Panniyur-8 are the different pepper varieties of panniyur. Panniyur-6 and panniyur-7

are the high yielding pepper varieties among these in Kerala. Panniyur-6 is a high yielding clone coming under medium maturity group with an average yield of 6460 kg green pepper/ha and 2127 kg drypepper/ha. The vine is vigorous in growth characterized by more number of spikes/vine with bold and medium sized berries. One of the attractive of this variety is the high setting percentage (99%) and compactly arranged berries of medium spike length (7.92 cm). The most distinguishing feature by which Panniyur-7 (culture 1558) can be identified is by the purple pigmentation of the shoot tip in contrast to the greenish yellow or faint pink coloured shoot tips of other cultures. It tolerates drought and adverse climatic conditions. The unique feature of this culture is that it has got a very long straight spike with mean spike length of 19.4cm.

Materials and Methods

- **Collection of samples:** Two samples of pepper namely Panniyur 6 and Panniyur 7 was collected from farmers. These seeds were dried in the sunlight and then powdered. Both varieties of pepper seeds were identified and confirmed by the authorities of Kerala Agricultural University, Mannuthi.
- **Preparation of extract:** The experiment was done in Distilled water and 80% ethanol. 7gm of both pepper varieties were weighed using a weighing machine and transferred to different conical flasks respectively. To both conical flask, pour 100 ml distilled water, 80% ethanol and 100ml of ether solution to each conical flask using measuring cylinder. Close the top portion of both conical flasks tightly and kept in dark condition for 3 days. After 3 days the solutions were double filtered using No. 1 filter paper and is stored in dark condition.

Phytochemical tests

- **Test for alkaloids:** 0.5 gm of aqueous and ethanolic extract stirred with 4 ml of 1% dilute hydrochloric acid separately. It was boiled and filtered.
- **Mayer's test:** 1ml of the filtrate was treated with a few drops of Mayer's reagent. Turbidity or precipitation indicated the presence of alkaloids.
- **Wagner's Test:** 1ml of extract, add 2ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.
- **Test for saponins:** 0.5 gm of aqueous and Ethanolic extract was boiled and the mixture was filtered separately. To 2.5 ml of the filtrate, 10 ml of distilled water was added in a test tube. It was shaken well for a few minutes and was allowed to stand for sometime. Frothing along with the formation of honeycomb indicated the presence of saponins.

- **Test for tannins:** 3g of aqueous and ethanolic extract taken in 3 boiling tubes was added to sim of distilled water, which was filtered and a few drops of 10% ferric chloride solution was added to it. A bluish green color indicated the presence of Tannins.
- **Test for terpenoids:** 5ml of each extract was mixed in 2ml of chloroform, and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of Terpenoids.
- **Test for steroids:** 0.5 ml of each extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer. A reddish brown color ring with a slight greenish fluorescence was taken as the indication for the presence of steroids.
- **Test for amino acids:** Ninhydrin Test: To the sample extract, few drops of ninhydrin reagent was added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-black color indicated the presence of protein.
- **Test for carbohydrates:** Benedict's Test: Take 5 ml of Benedict's reagent. Add 8 drops of carbohydrate solution. Boil over a flame or in a boiling water bath for 2 minutes. Let the solution cool down.
- **Molisch's Test:** Take 2 ml of solution in a test tube. Add two drops of Molisch's reagent. Mix it well. Under the cooling conditions incline the test tube and add 2 ml concentrated sulphuric acid slowly along the sides of test tubes.

Determination of anti-bacterial activity (Agar Well diffusion method)

Nutrient Agar plates were prepared and inoculated with test organism by spreading the bacterial inoculums on the surface of the media with the help of sterile swab. Wells (8mm in diameter) were punched in the agar by using cork borer into agar plates containing the bacterial inoculums. Here we are testing Anti-Bacterial property using three cultures namely *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumonia*. We have prepared 4 culture plates of each organism.

Results

In the present study, phytochemical tests indicate the presence of alkaloids, saponins, terpenoids and steroids. And the antibacterial effect of Pepper is showed in Table 1. Highest antibacterial activity showed in ethanolic extract than water extract against all test bacteria with zone of inhibition ranged from 8mm-18mm.

Si.no	Test organism	Water extract (Panniyur 6)	Ethanol extract (Panniyur 6)	Water extract (Panniyur 7)	Ethanol extract (Panniyur 7)
1	<i>Bacillus subtilis</i>	13mm	14mm	12mm	13mm
2	<i>Klebsiella pneumoniae</i>	9mm	9mm	9mm	8.5mm
3	<i>Escherichia coli</i>	8mm	8.5mm	8mm	8.5mm

Table 1: Result of Antibacterial activity.

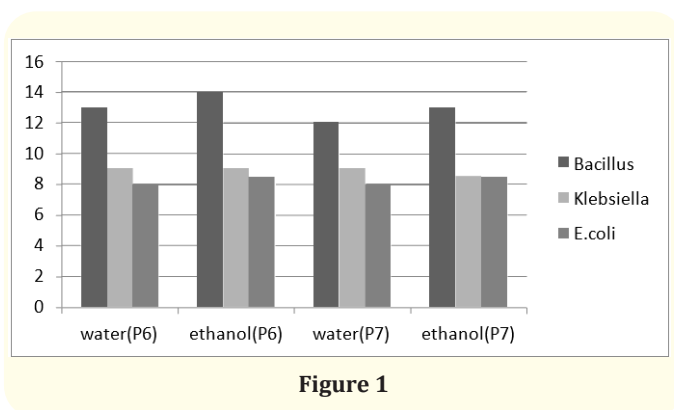


Figure 1

Discussion

In the present study it was revealed that Gram positive bacteria (*Bacillus subtilis*) are more susceptible towards the pepper extracts than gram negative bacteria. More sensitivity is shown in ethanol extract than water extract. The variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. Piperine an alkaloid the major constituent of piperamides present in the skin and seed of the black pepper is responsible for the antimicrobial activity. Spices we used in our daily diet can provide protection towards bacteria. We conclude that the extracts of black pepper can be used as antibacterial agents.

Conclusion

According to the study, the antibacterial activity of *Piper nigrum* ethanol extract is due to the presence of phytochemical compounds like tannins and alkaloids.

Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents those can be used in the synthesis of useful drugs. The pepper have shown a promising effect in therapeutics. Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family, such as *P. Nigrum*. It is the major constituent of piperamides

present in the skin and seed of the black pepper is responsible for the antimicrobial activity. It has also been used in some forms of traditional medicine and as an insecticide.

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