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## Comparative study of anti-microbial and phytochemical analysis of *Piper longum* and *Piper nigrum*

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### Abstract

The present research deals with the comparative analysis of Anti-Microbial and Phytochemical properties of *Piper longum* and *Piper nigrum*. Six isolates of microorganisms were used to check the antimicrobial properties. Agar well diffusion method and Agar disc diffusion method was employed. The volatile oil content was absent in both plant materials. Water extractive value for *P. nigrum* was about 7.6% and *P. longum* was 12%. alcohol soluble extractive value for *P. nigrum* was only 2% and *P. longum* was found to be 7.6%. Both plant species are rich in phytochemical constituents such as alkaloids, steroids, flavonoids etc. The present result suggests that alcoholic extract of *P. nigrum* is a curing agent for diseases caused by *E. coli*.

**Keywords:** *Piper longum*, *Piper nigrum*, antimicrobial, phytochemical constituents

### Introduction

According to the ancient Indian physician Charaka, there are no such plants which are absolutely useless<sup>[1]</sup>. Various therapeutic plants those described in ancient texts have shown great potential as leads for medicinal properties against many health issues and drug discovery<sup>[2, 3]</sup>. In rural areas, the majority of people mainly depend on herbal medicines for the treatment of different types of diseases<sup>[4]</sup>. Several plants mentioned in the Vedas, has obtained considerable importance in present-day research due to the wide range of pharmacological significance.

The two plant species were selected from the genus Piper i.e., *P. longum* and *P. nigrum* for the present study. *P. longum* is also known as Indian long piper or Tippali. The medicinal and nutritional properties of *P. longum* have described in the ancient Indian text books of Ayurveda. *P. longum* is a flowering vine belongs to the family Piperaceae and their dried fruit is used as a spice. The plant grows in evergreen forests and cultivated in the southern part of India. Limestone soil and heavy rainfall areas are suitable for the large scale cultivation of *P. longum*. The fruit comprises enormous amount of alkaloids and associated compounds, the most abundant of which is piperine and the recent researches revealed the content present in *P. longum* has Anticancer as well as Antioxidant properties<sup>[5]</sup>.

*P. nigrum* or Black pepper is another species from the family Piperaceae, and used in traditional medicine for digestive issues. *P. nigrum* usually cultivated in sheltered positions and it prefers a neutral soil with plentiful organic matter. The present study reveals significant records of antimicrobial and phytochemical analysis of *P. longum* and *P. nigrum*.

### Materials and Methods

#### Collection and Extraction of plant material

Dried fruits of *P. longum* and *P. nigrum* were collected from the Kollam district of Kerala, India. The powdered samples were sealed in airtight container to avoid the effect of humidity and then stored for further analysis. Powdered plant material was extracted successively with ethanol and chloroform in Soxhlet extractor apparatus.

#### Test Microorganisms

There are six isolates of microorganisms were used for the present study, which includes *E. coli*, Coagulase negative *Staphylococci*, *Pseudomonas aeruginosa*, *Klebsiella Sp*, *Proteus Sp*. and *Staphylococcus aureus*. The isolates were obtained from Kerala Government Public Health Laboratory, Trivandrum.

**Determination of anti-microbial activity**

Two different methods were employed for the determination of antimicrobial activities, an Agar well diffusion method for the alcohol extract and Agar disc diffusion method for antibiotic susceptibility.

**Agar well diffusion method [6]**

In this method the bacterial inoculums were uniformly spread using sterile cotton swab on a sterile Muller Hinton agar media to get lawn culture. 50 microliter of extract dilutions added to each of 5 wells (7 mm in diameters), the plates were incubated at 37 °C for 24 hours.

**Agar disc diffusion method [7]**

The sterile discs dipped in extracts of varying concentration are placed over Mullen Hinton agar having the lawn culture of tested bacteria. The lawn culture of tested bacteria for negative and positive control disc is also kept over it. Two antibiotic discs amoxyclav and ciprofloxacin aseptically deposited on the surface of incubated plates, each containing a specific test microorganism after 24 hours of incubation at 37 °C, positive results were established by the clear zone of inhibition around active extracts.

**Physico chemical parameters**

The following physico-chemical parameters such as Moisture content, Volatile oil content, Water soluble extract value, Alcohol soluble extract value were determined by using the standard methodology mentioned in Ayurvedic Pharmacopoeia of India [8-10].

**Qualitative Analysis for Phytochemical constituents**

Qualitative tests for various secondary metabolites like alkaloids, steroids, flavonoids etc. were carried out by using the methanol extracts of the samples and by following standard procedures [11].

**Result and Discussion**

**Antimicrobial activity of plant materials**

The alcoholic extract of the *Piper longum* showed no antimicrobial activity towards the microorganisms used in the study (Table 1). But the alcoholic extract of *Piper nigrum* showed antimicrobial activity that is high zone of inhibition (22mm) compared to positive control ciprofloxacin (26mm). The antibiotics which are used as positive control showed zone of inhibition against the tested bacteria (Table 2). Similar work by Ram *et al.*, in 2010 [12] showed that aqueous extract of *P. nigrum* showed activity against *S.aureus*.

**Table 1:** Antimicrobial activity of *Piper longum*

Name of Microorganism	Zone of inhibition of the extract (mm)					Zone of inhibition of Antibiotics 9mm	
	Master Dilution	1	2	3	Negative control	Amoxyclav 10 mg/ml	Ciprofloxacin 10 mg/ml
<i>E. coli</i>	R	R	R	R	R	-	26
<i>Klebsiella Sp.</i>	R	R	R	R	R	-	15
<i>Proteus Sp.</i>	R	R	R	R	R	-	7
<i>P.aeruginosa</i>	R	R	R	R	R	-	33
<i>S.aureus</i>	R	R	R	R	R	28	-
Coagulase negative <i>Staphylococci</i>	R	R	R	R	R	23	-

\*R\*: Resistant

**Table 2:** Antimicrobial activity of *Piper nigrum*

Name of Microorganism	Zone of inhibition of the extract (mm)					Zone of inhibition of Antibiotics 9mm	
	Master Dilution	1	2	3	Negative control	Amoxyclav 10 mg/ml	Ciprofloxacin 10 mg/ml
<i>E. coli</i>	22	R	R	R	R	-	26
<i>Klebsiella Sp.</i>	R	R	R	R	R	-	15
<i>Proteus Sp.</i>	R	R	R	R	R	-	7
<i>P.aeruginosa</i>	R	R	R	R	R	-	33
<i>S.aureus</i>	R	R	R	R	R	28	-
Coagulase negative <i>Staphylococci</i>	R	R	R	R	R	23	-

\*R\*: Resistant

**Physico-chemical parameters of plant materials**

The moisture content of *P. nigrum* was only a trace amount

whereas in *P. longum* was about 4%. The volatile oil content was absent in both plant materials. (Table 3)

**Table 3:** Moisture and volatile oil content of the plant materials

Sl no.	Experiment	Percentage (%)	
		<i>P. nigrum</i>	<i>P. longum</i>
1.	Moisture content	Traces	4%
2.	Volatile oil content	Nil	Nil

The solubility of samples was determined by the percentage of water extractive and alcohol extractive. Water extractive value for *P. nigrum* was about 7.6% and *P. longum* was

12%. alcohol soluble extractive value for *P. nigrum* was only 2% and *P. longum* was found to be 7.6%. (Figure 1)

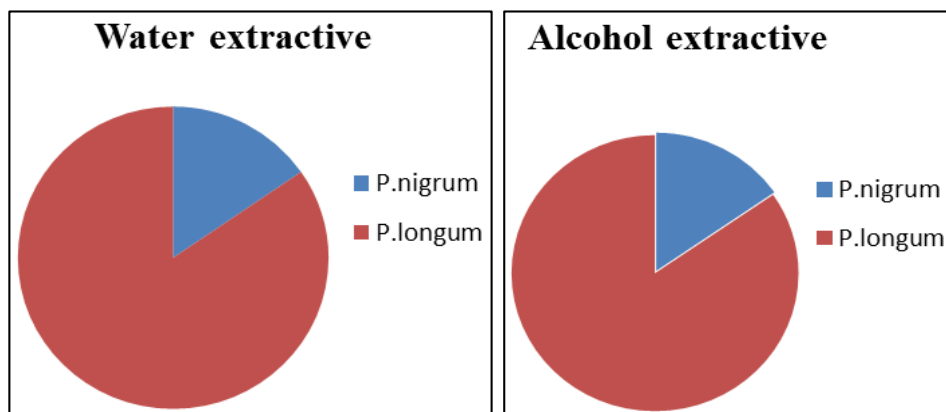


Fig 1: Water and Alcohol extractive value of Plant materials

**Qualitative Analysis**

The phenol was absent in *P. nigrum* where as it was present in *P. longum*. Flavonoid was present in *P. nigrum* and absent in *P. longum*. The remaining constituents such as steroids, alkaloids and tannins were present in both plant samples. (Table 4)

Table 4: Qualitative analysis

SI No.	Plant Constituents	Inference	
		<i>P. nigrum</i>	<i>P. longum</i>
1	Steroids	Present	Present
2	Phenols	Absent	Present
3	Alkaloids	Present	Present
4	Flavonoids	Present	Absent
5	Tannins	Present	Present

**Conclusion**

The results obtained from this comparative analysis documented the antimicrobial and phytochemical properties of the two selected plant species *P. longum* and *P. nigrum*. From the qualitative analysis, it is recognized that both plant species are rich in phytochemical constituents and *P. nigrum* showed a high zone of inhibition against *E. coli*. Nowadays drinking water pollution by *E. coli* is very severe, so the alcoholic extract of *P. nigrum* may be used against the gram negative *E. coli* and it may be useful for the curing of diseases caused by *E. coli*.

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