

Antimicrobial activity of Piper fruits

Mohib Khan* and Mustafa Siddiqui

Department of Pharmacognosy and Phytochemistry

MESCO College of Pharmacy

Hyderabad -500 006, Andhra Pradesh, India

*Correspondent author, E-mail: mohibkhan@sify.com

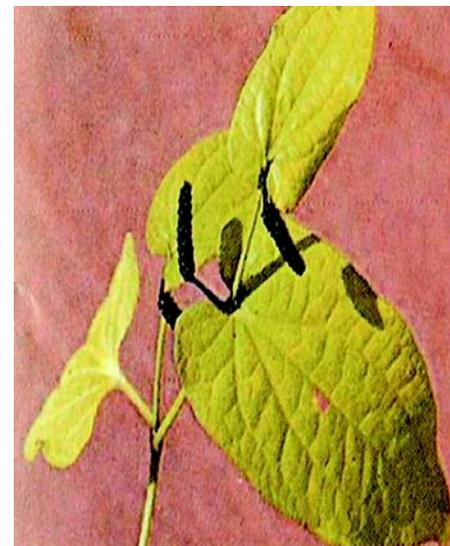
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Abstract

In this study an attempt is made to evaluate the antimicrobial activity of various extracts of fruits of different Piper species available in the Aurangabad-Marathwada (Maharashtra State) region. Twenty eight extracts prepared from the fruits of four species, viz. *Piper cubeba* Linn. f., *P. retrofractum* Vahl syn. *P. chaba* Hunter non Blume, *P. longum* Linn. and *P. nigrum* Linn. were evaluated against bacterial pathogens, such as *Staphylococcus albus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus megaterium* and one fungus, *Aspergillus niger*. Compared to Streptomycin all the extracts exhibited a good antibacterial activity. Some of the extracts showed antifungal activity as well.

Keywords: Antimicrobial activity, Piper fruits, *Piper cubeba*, *Piper retrofractum*, *Piper longum*, *Piper nigrum*.

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Piper longum

Introduction

Piper Linn., belonging to family Piperaceae is a very large genus of shrub, rarely herbs and trees, distributed throughout the tropical and sub-tropical regions of the world. About 30 species of the genus in India and 700 species in the world have been reported, of which, *P. nigrum*, the Black Pepper and *P. betle* Linn., the Pan or Betel are widely cultivated¹. Five species are used as herbal ingredients of Asian medicines and they are *P. betle*, *P. cubeba* Linn. f. (Cubebs), *P. retrofractum* Vahl syn. *P. chaba* Hunter non Blume (Java Long Pepper), *P. longum* Linn. (Indian Long Pepper) and *P. nigrum* Linn.². The leaf juice of *P. betle* is used as eye drop³. *P. cubeba* is used as antibacterial⁴, expectorant⁵ and as gastroprotective⁶. *P. longum* is used as bioavailability enhancer⁷, digestive and in the treatment of bronchitis⁸ and also as hepatoprotective

agent⁹. Scientists have received US patent on obtaining a diabetes mellitus therapeutic agent from *P. longum*¹⁰. *P. nigrum* is used as nerve tonic, and in the treatment of constipation, itching and flatulence¹¹. Some of the Piper species contain a piperidine type alkaloid, piperine, which is a central nervous system depressant¹². Most of the piper fruits contain volatile oil¹³.

All the oils present are mostly phenolics and are antibacterial because they interfere with selective permeability of cell membranes but these terpenes can not discriminate between human cell membrane and microbial cell membrane so can not be used against systemic infections. Therefore, it was thought worthwhile to see the *in vitro* effect of extracts of four piper species available in Aurangabad, Maharashtra state region.

Materials and Methods

Collection of fruits and microorganisms

The plants used in the study were identified at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. After authentication, same fruits were collected from Shabbar Dawasaz, Pan Dariba, Aurangabad.

The pure cultures of *Staphylococcus albus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus megaterium* and the fungus, *Aspergillus niger* were obtained from the Department of Botany, Government Institute of Science, Aurangabad.

Preparation of fruit extracts

The fruits were milled separately into coarse powder using a mechanical grinder. The ground materials (10g) were successively extracted with carbon tetrachloride, benzene, chloroform, ethyl

acetate, acetone, ethanol and distilled water and pooled separately. The extracts were concentrated separately under vacuum, and the resulting dried extracts were weighed and used for the study (Table 1).

Antimicrobial activity

The activities were evaluated using three different culture medium: (i) Nutrient agar (for Gram positive bacteria); (ii) MacConkey agar (for Gram negative bacteria) and (iii) Potato dextrose agar

Table 1 : Antimicrobial activity of various extracts of different *Piper* spp. fruits

| Test plant/drug | Solvent | <i>E. coli</i> | <i>B. megaterium</i> | <i>S. albus</i> | <i>S. typhi</i> | <i>P. aeruginosa</i> | <i>A. niger</i> |
|------------------------|----------------------|----------------|----------------------|-----------------|-----------------|----------------------|-----------------|
| <i>P. cubeba</i> | Carbon tetrachloride | + | + | + | - | + | * |
| <i>P. retrofractum</i> | Carbon tetrachloride | + | + | + | - | + | + |
| <i>P. longum</i> | Carbon tetrachloride | + | + | + | - | + | - |
| <i>P. nigrum</i> | Carbon tetrachloride | + | + | + | - | + | + |
| <i>P. cubeba</i> | Benzene | + | + | + | + | + | + |
| <i>P. retrofractum</i> | Benzene | + | + | + | - | + | + |
| <i>P. longum</i> | Benzene | + | + | + | + | + | - |
| <i>P. nigrum</i> | Benzene | + | + | + | + | + | ++ |
| <i>P. cubeba</i> | Chloroform | + | - | + | + | + | ++ |
| <i>P. retrofractum</i> | Chloroform | + | - | + | + | + | + |
| <i>P. longum</i> | Chloroform | - | - | + | + | + | + |
| <i>P. nigrum</i> | Chloroform | - | - | + | + | + | + |
| <i>P. cubeba</i> | Ethyl acetate | + | - | + | + | + | - |
| <i>P. retrofractum</i> | Ethyl acetate | + | - | + | + | + | - |
| <i>P. longum</i> | Ethyl acetate | + | - | + | + | + | + |
| <i>P. nigrum</i> | Ethyl acetate | - | - | + | + | + | + |
| <i>P. cubeba</i> | Acetone | + | - | + | + | + | + |
| <i>P. retrofractum</i> | Acetone | - | - | + | + | + | - |
| <i>P. longum</i> | Acetone | * | - | + | + | + | + |
| <i>P. nigrum</i> | Acetone | - | - | + | + | + | + |
| <i>P. cubeba</i> | Ethanol | ++ | + | + | + | + | ++ |
| <i>P. retrofractum</i> | Ethanol | + | + | + | + | + | + |
| <i>P. longum</i> | Ethanol | + | + | * | + | + | + |
| <i>P. nigrum</i> | Ethanol | + | + | + | ++ | + | + |
| <i>P. cubeba</i> | Distilled water | + | + | + | + | - | - |
| <i>P. retrofractum</i> | Distilled water | + | + | + | + | - | - |
| <i>P. longum</i> | Distilled water | + | + | + | + | - | - |
| <i>P. nigrum</i> | Distilled water | + | + | + | + | - | - |
| Streptomycin | | +++ | +++ | +++ | +++ | +++ | |
| Griseofulvin | | | | | | | +++ |

Disc diameter = 4 mm; Diameter of zone of inhibition (mm) indicated by sign +++, ++, +, - and *.

Where +++ = ≥ 15 mm; ++ = 10-14 mm; + = 5-9 mm; - = no inhibition; and * = not tested.

(for fungus). About 25 ml medium per plate was used. Pure isolates of different microbes obtained from the Department of Botany, Government Institute of Science, Aurangabad was inoculated in the plate using pour plate technique.

The *in vitro* antimicrobial activity of various extracts of different Piper fruits was evaluated by the disc diffusion method¹⁴. The extracts were reconstituted in dimethyl sulphoxide and applied on paper discs. Each disc containing 40 µg extract were tested against *Staphylococcus albus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus megaterium* and *Aspergillus niger*. The activities of the extracts were compared with Streptomycin and Griseofulvin. The plates were incubated at 37°C for 48 hours. The diameters of the zones showing complete inhibition were measured.

Results and Discussions

The solvents with their increasing order of polarity were used for the extraction¹⁵. A total of 28 extracts at a single concentration (40 µg/disc) from the fruits of four species of *Piper* were tested for inhibitory activity against both Gram positive bacteria, such as *S. albus* and *B. megaterium* and Gram negative bacteria, like *E. coli*, *S. typhi* and *P. aeruginosa* and a fungus *A. niger* (Table 1).

Twenty seven extracts of different solvents showed activity against *S. albus*, 24 extracts against *P. aeruginosa*, 22 extracts against *E. coli* and *S. typhi*, 16 extracts showed activity against *B. megaterium*, and 18 extracts showed activity against *A. niger*.

Alkaloids and terpenoids present in the crude drugs are the chief chemical moieties of all piper fruits and their presence may be attributed to the medicinal properties. The results of the present study reveal that all the fruits studied contained antimicrobials.

Conclusion

The results revealed that ethanolic extract of all species is more active against all bacteria and fungi studied during present work.

Out of seven species available in Maharashtra, only four could be collected. If all piper fruits *P. betle*, *P. cubeba*, *P. retrofractum*, *P. longum* and *P. nigrum* and two unnamed species further screened phytochemically at a large scale and studied at the same time for antimicrobial and other pharmacological activities then phytopharmacological significance of these species could be drawn.

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