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Antibacterial activity of *Piper nigrum* – an in vitro study

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ABSTRACT

The present study was carried out to evaluate the antibacterial activity and phytochemical analysis of *Piper nigrum*. Ethanol and aqueous extracts were obtained and their antibacterial activity was carried out using disc diffusion method with gram positive and gram negative strains. The result indicated that ethanol extract was most active while aqueous extract was highly inactive. Preliminary phytochemical screening revealed the presence of terpenoids, alkaloids, flavonoids and carbohydrates. This study showed that ethanol extract of *P. nigrum* could be a potential source of new antibacterial agents

Key words: Antibacterial, medicinal plant, secondary metabolites.

INTRODUCTION

Development of drug resistant in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from plant sources. Hence scientists have shown revival interest for the management of drug resistant infections. Therefore in recent years attempts have been made to use spices extracts as an effort to find out their disease curing properties. *Piper nigrum* (Piperaceae) commonly called "Black pepper", a native to Malabar and Sumatra, has round smooth woody stems which are branched, swelling at each joint, leaves are dark green, white flowers, and fruits are globular, wrinkled and red when ripe. It has been traditionally used for fever, neurological, broncho-pulmonary and gastro intestinal disorders (Joseph Laurie, 1979; Raj *et al*., 1978). Considering the pharmacological and traditional use of this medicinal plant, the present study focused to study their antibacterial activity against gram positive and gram negative bacteria and phytochemical analysis.

MATERIAL AND METHODS

Plant collection and extraction

Piper nigrum were purchased from local market, Tamil Nadu, India. It was taxonomically identified and voucher specimen was prepared and deposited at the department herbarium, Loyola College. Collected material was washed thoroughly, shade dried in open air and grounded into powder.

Aqueous extraction

For aqueous extraction, 10 g of dried powder was placed in distilled water and boiled for 5 hr at intervals of 1 hr it was filtered through muslin cloth and centrifuged at 5000 × g for 15 min. The supernatant was collected after 5 hr the supernatant was concentrated, sterilized, stored at 4°C.

Solvent extraction

10 g of air dried powder was soaked in 100 ml of ethanol in a conical flask for 24 hr repeatedly with intermittent shaking. It was filtered through

muslin cloth and centrifuged at 5000 × g for 15 min. The supernatant was collected and the solvent was evaporated.

Phytochemical analysis

The presence of phytochemicals alkaloids (Draggendorff's), flavonoids (Shibat'as reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4-dinitro-phenyl hydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test) were evaluated according to the methods described by Edeogal *et al*., (2005).

Preparation of test organism

The included bacterial strains were *Klebsiella pneumonia* (ATCC 15380), *Proteus vulgaris* (MTCC 1771), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). All bacteria were obtained from the Microbiology lab, Christian Medical College, Vellore, Tamil Nadu, India and very carefully identified using standard microbiological method. All bacterial strains were maintained a MHA slants, stocks were stored at -20°C until use.

Antibacterial test

Antibacterial activity was screened using a minimum modification of the disc diffusion method originally described by Bauer (Bauer et al., 1966). Plant extracts were dissolved in 20 % DMSO in water. MHA plates were swabbed with a sterile cotton swap with overnight broth cotton of respective bacteria. Sterile filter paper disc (6 mm in diameter) impregnated with the plant extracts (2.5 mg/disc) were placed on the cultured plates and incubated at 37°C. The solvent without extracts served as negative control. Standard antibiotics of streptomycin 30 µg were used as positive controls. After 24 h of incubation the diameter in mm of the inhibitory clear zones around the disks were recorded. Three independent trials were conducted for each concentration.

RESULTS AND DISCUSSION

Piper nigrum exhibited significant activity against gram positive and gram negative bacteria are summarized in table 1. In this study ethanol extract are highly active towards the tested bacterial strain and its inhibition zone are similar to streptomycin. Similarly Vijayan *et al*., (2003) reported antibacterial activity of *Piper nigrum* (acetone extract) against *E.coli, S. aureus, B. subtilis*. Most of the tested bacterial strains were sensitive in ethanol extract. This may be due to the active components were soluble in organic solvents. Aqueous extract are highly in active to the tested bacterial strains.

The phytochemical analysis of ethanol extract had showed the presence of flavonoids, terpenoids, alkaloids, and Carbohydrate. Previous literature of its chemical constituents revealed the presence of piperine, an alkaloid, constitutes of 3-9% and 3-5% (dry wt) and it is reported to cause antipyretic and anti-inflammatory activity (Virinder, *et al*., 1997). The most important of their bioactive

Table 1: Antibacterial activity of the extracts of *P. nigrum*

Bacteria	Diameter of zone of inhibition (mm)		
	Ethanol	Aqueous	Streptomycin (30 µg/disc)
Klebsiella pneumonia	15	-	15
Proteus vulgaris	13	-	15
Enterococcus faecalis	13	-	14
Pseudomonas aeruginosa	15	-	14
Escherichia coli	15	-	15
Bacillus subtilis	15	-	15
Staphylococcus aureus	13	-	14

compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds and this antibacterial activity may be due to presence of secondary metabolites (Edeogal *et al*., 2005). Our study concludes that *P. nigrum* (ethanol extract) may posses antibacterial components and it is possible for elucidation and identification of its active components for the development of new antibacterial drugs.

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