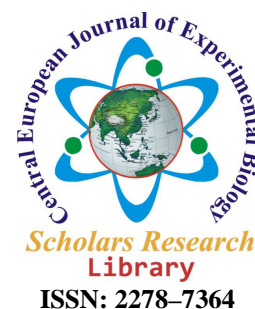




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Central European Journal of Experimental
Biology, 2014, 3 (2):36-41

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ISSN: 2278-7364

Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens

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ABSTRACT

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science to have medicinal effects. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The main objective of the study is to screen the antibacterial activity of pepper (*Piper nigrum* L.) against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Proteus sp.* and *Pseudomonas aeruginosa*. Agar well diffusion method was performed and found that all the bacteria showed sensitive to methanol and chloroform extract of pepper except *Pseudomonas aeruginosa* which showed resistant to both the extracts. The pepper plant extracts were screened for their phytochemical content. Qualitative phytochemical tests were used to detect the presence of alkaloids, tannins, flavonoids, cardiac and cardiac glycosides. Presence of these phytochemicals in the medicinal plants indicates the presence of antibacterial properties against the *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Proteus sp.* This study scientifically validates the use of plants as a potent antibacterial agent.

Keywords: Antibacterial activity, *Piper nigrum*, Agar well diffusion method and Phytochemicals.

INTRODUCTION

The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. Herbs and spices are an important part of the human diet. They have been used for thousands of year to enhance the flavour, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative and medicinal value, which forms one of the oldest sciences. Yet it was only in recent years that modern science has started paying attention to the properties of spices [1].

Medicinal and spice plants are renewable raw materials. Their production is an alternative to the overproduction of traditional crops in agriculture. They also have an increasing economic importance. Spices can be defined as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground form that contribute flavour, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages [1]. Spices include leaves (coriander, mint), flower (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stem (cinnamon), rhizomes (ginger, turmeric) and other plant parts [2].

Although, as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances' being derived from a plant does not mean it is always harmless. One drug used for one ailment could actually be detrimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, mutagens and abortifacient [2]. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno pharmacologists, botanists, microbiologists and natural-products chemists are combing the Earth for photochemical and "leads" which could be developed for treatment of infectious diseases.

The discovery of medicinal plants in different parts of the world is important to agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants represents rich source from which antimicrobial agents may be obtained. The different parts used include root, stem, flower, leaves, fruit, twigs exudates and modified plant organs. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The antimicrobial activities of plant extracts may reside in a variety of different components. The beneficial medicinal effects of the plant materials typically result from the combinations of secondary product present in the plant [3].

Piper nigrum L., sometimes called Indian Long Pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant, and has a similar, though generally hotter, taste. The root and fruit of *Piper nigrum* are used in palsy, gout and lumbago. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive [4]. They have a pungent pepper - like taste and produce salivation and numbness of the mouth. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz., cough, bronchitis, asthma etc; as counter-irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative. Besides fruits, the roots and thicker parts of stem are cut and dried and used as an important drug in the Ayurvedic and Unani systems [5].

It is native to India and has been a prized spice since ancient times. The volatile of pepper has been shown to have antimicrobial activity. It had been used to treat vertigo, chronic indigestion, colon toxins, obesity, sinusitis, congestion, fever, paralytic, arthritic disorder and also advised in diarrhoea and cholera [6]. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenol compounds, flavonoids, steroids, resins, and fatty acids which are capable of producing definite physiological action on disease causing organisms. The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in treatment of several diseases [7]. In the present study, we have evaluated the antibacterial effect of the ethanol and chloroform extracts of widely used spice in South India, such as *Piper nigrum* (Black Pepper) against five bacterial human pathogens such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus* sp. was tested.

MATERIALS AND METHODS

2.1. Collection and drying

Fresh peppercorn of *Piper nigrum* was collected from a stationery store, Chidambaram, Cuddalore district. The black pepper was washed thoroughly three times with sterile distilled water. The materials were air dried under hot air oven at 55°C for 3 hours and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of the extraction.

2.2. Extract preparation

Forty g of powdered black pepper was extracted successively with 200 ml of ethanol (56 - 60°C) and chloroform (76 - 78°C) in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a sterile plastic container.

2.3. Microbial cultures used and Inoculum preparation

Five different clinical bacterial isolates viz., *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were collected from Rajah Muthiah Medical College and Hospital, Annamalai Nagar, Annamalai University. Bacterial inoculum was prepared by inoculating a single colony of test organism in 5 ml of Nutrient broth and incubated at 37°C for 3 - 5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standard solution.

2.4. Determination of antibacterial activity (Agar Well Diffusion Method)

Muller Hinton agar plates were prepared and inoculated with test organisms by spreading the bacterial inoculum on the surface of the media with the help of sterile swab. Wells (8 mm in diameter) were punched in the agar by using cork borer. Extracts with different concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml and 4 mg/ml) were added. Along with the sample extraction, solvent Chloroform and Ethanol, 40 µl of each as control was added. The plates were incubated at 37°C for 18 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and recorded in mm.

2.5. Phytochemical analysis [8]

2.5.1. Test for saponins

Two g of the powdered sample was boiled in 20 ml of distilled water in a water bath. Ten ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent froth. The following was mixed with 3 drops of Olive oil and shaken vigorously. Then observed for the formation of emulsion.

2.5.2. Test for tannins

A quantity of 0.5 g of the dried powdered sample was boiled in 20 ml of water and filtered. A few drops of 0.1% Ferric chloride was added and observed for brownish or bluish black colour.

2.5.3. Test for alkaloids (Meyer's test)

A quantity of 0.5 g of the dried powdered sample was boiled in 20 ml of water and filtered. To a few drops of the filtrate, a drop of Meyer's reagent was added by t1 side of the test tube. A creamy or white precipitate indicates the test is positive.

2.5.4. Test for flavonoids

A portion of the powdered sample was heated with 10 ml of Ethyl acetate over a steam bath for 3 minutes and then mixture was filtered. 4ml of the filtrate was shaken with diluted Ammonia. Yellow coloration indicates the presence of flavonoid.

2.5.5. Test for cardiac glycosides

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% FeCl₃. This was under laid with conc. H₂SO₄. A brown ring obtained at the interface indicated the presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form just above ring and gradually spreads throughout this layer.

2.5.6. Test for phenolic compounds (Ferric chloride test)

Three hundred mg of extract was diluted to 5 ml of distilled water and filtered. To the filtrate 5 % of ferric chloride was added. Dark green color indicates the presence of Phenolic compounds.

RESULTS AND DISCUSSION

Many plant-derived molecules have shown a promising effect in therapeutics. Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae. Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family, such as *P. nigrum* and *P. longum*. Piperine is the trans stereoisomer of 1-piperoylpiperidine. It is also known as (E, E)-1- piperoylpiperidine and (E, E)-1- [5-(1, 3- benzodioxol-5-yl)-1-oxo-2, 4-pentdienyl] piperidine. Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine). It has also been used in some forms of traditional medicine and as an insecticide.

The antibacterial activity of ethanol extract of *Piper nigrum* showed against *Escherichia coli*, *Salmonella typhi*, *Proteus* sp. and *Staphylococcus aureus* where as *Pseudomonas aeruginosa* showed resistant and results were given in the Fig. 1. Chloroform extract of *Piper nigrum* showed high antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Proteus* sp. and *Staphylococcus aureus* where as *Pseudomonas aeruginosa* showed resistant and the results were given in the Fig. 2.

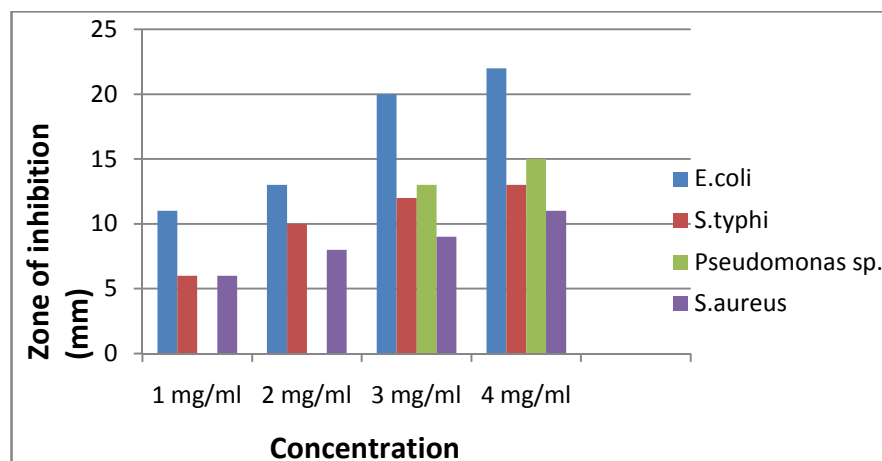


Fig - 1: Antibacterial activity of *Piper nigrum* ethanol extract

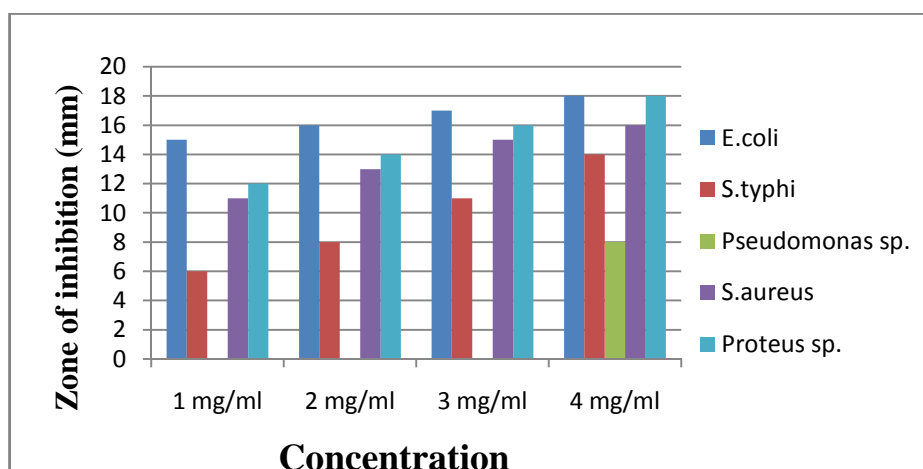


Fig - 2: Antibacterial activity of *Piper nigrum* chloroform extract

Phytochemical analysis of ethanol extract of *Piper nigrum* showed the presence of tannins and alkaloids and results were given in the Table - 1. Phytochemical analysis of chloroform leaf extract of *Piper nigrum* showed the presence of tannins, alkaloids and flavonoids and the results were given in the Table - 2.

Table-1: Phytochemical analysis of *Piper nigrum* ethanol extract

S.No	TEST	INFERENCE	RESULT
1	Saponins	No Frothing	Absent
2	Tannins	Brownish Green color was appeared	Present
3	Alkaloids	Creamy white precipitate was appeared	Present
4	Flavonoids	No yellow color	Absent
5	Cardiac glycosides	Greenish and violet ring appeared	Present
6	Phenolic compounds	No dark green color	Absent

Table-2: Phytochemical analysis of *Piper nigrum* chloroform extract

S.No	TEST	INFERENCE	RESULT
1	Saponins	No Frothing	Absent
2	Tannins	Brownish Green color appeared	Present
3	Alkaloids	Creamy white precipitate appeared	Present
4	Flavonoids	No yellow color	Present
5	Cardiac glycosides	Greenish and violet ring appeared	Present
6	Phenolic compounds	No dark green color	Absent

Saranraj and Sivasakthivelan [9] reported that piperine is widely used in various herbal cough syrups for its potent antitussive and bronchodilator properties. It is used in anti inflammatory, anti malarial, anti leukemia treatment. Recent medicinal studies have shown it is helpful in increasing the absorption of certain vitamins, selenium and beta-carotene, also increase the body's natural thermogenic activity.

Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases. Therefore, there is exigency to discover new and efficient antimicrobials from other source such as plant. In the present study an attempt was made to screen different extracts prepared from dried fruit of *Piper nigrum* for its antimicrobial action against Gram positive and negative bacteria. Ahmed Chaudhry *et al.* [10] focused on antibacterial potential of aqueous decoction of black pepper (*Piper nigrum* L.), bay leaf (*Laurus nobilis* L.), aniseed (*Pimpinella anisum* L.), and coriander (*Coriandrum sativum* L.) against 176 bacterial isolates belonging to 12 different genera of bacterial population isolated from oral cavity of 200 individuals. The disc diffusion technique was employed. Overall aqueous decoction of black pepper was the most bacterial-toxic exhibited 75% antibacterial activity as compared to aqueous decoction of bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10 µl/disc. The aqueous decoction of coriander did not show any antibacterial effect against tested bacterial isolates.

In this present study, the extracts of *Piper nigrum* were tested against pathogenic bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Proteus* sp. by Agar well diffusion method. The ethanol extract of *Piper nigrum* showed maximum inhibition against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Proteus* sp. 1mg/ml of ethanol extract showed resistant against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The chloroform extract of *Piper nigrum*, showed maximum inhibition against *Escherichia coli* and *Staphylococcus aureus* and *Salmonella typhi*. *Pseudomonas aeruginosa* was resistant to chloroform extract of *Piper nigrum*. The antibacterial activity of *Piper nigrum* ethanol extract is due to the presence of phytochemical compound like tannins and alkaloids. The Chloroform extract of *Piper nigrum* plant leaves possess antibacterial activity due to the presence of phytochemical compound like Tannins, Alkaloids and Flavonoids.

Reshmi *et al.* [11] studied on isolation of piperidine from *Piper nigrum* and its antiproliferative activity. The present study aimed to extract the phytochemical compounds in different solvent system in *Piper longum*, *Piper nigrum* and *Piper cubeba* as well as testing their antibacterial and antitumour activity. HPTLC analysis of *Piper nigrum* sample showed six alkaloid bands two alkaloid bands were similar to Piperine standard 1 and 2, the other alkaloid may be piperidine, piperettine and piperanine. *Piper longum* sample contain three alkaloid bands one band was similar to Piperine standard 1, the other may be piperlongumine and piperlonguminine and no alkaloid band was found in *Piper cubeba*. The anti bacterial activity was tested against gram positive and negative organism using Agar well diffusion method. High activity was found in *Piper nigrum* ethanol extract against the organism *Salmonella typhi*. The alkaloid piperidine was purified by refluxion method to check the antitumor activity which shows 51.38% of inhibition at 5 µg/ml concentration that conforms the compound piperidine to be used as anticancer drug for further mechanistic works.

Ram Kumar Pundir *et al.* [12] studied a total of six extracts of two spices namely black pepper and turmeric in three solvents for their antibacterial and antifungal activity. The antibacterial activity was measured by Agar well diffusion method and antifungal activity by poisoned food technique. All the extracts showed antibacterial activity against all the test bacterial isolates. In aqueous extract, black pepper and turmeric showed good inhibitory activity against *Staphylococcus aureus* with zone of inhibition 25mm to 30 mm and 26 mm to 28 mm respectively. In ethanolic extract, black pepper extract showed antibacterial activity against all test bacteria with zone of inhibition ranged between 15 mm and 22 mm while turmeric showed activity with zone of inhibition ranged between 13 mm and 24 mm. In methanolic extract, the diameter of zone of inhibition ranged between 12 mm and 28 mm in black pepper and 13 mm and 22 mm in turmeric. Based on this finding, these extracts may be an alternative to chemical preservatives and used as natural antimicrobial preservatives to reclaim the shelf-life of food.

Mital Patani *et al.* [13] determined the antibacterial activity of *Piper nigrum* and *Mallotus philipinesis* against a wide variety of pathogenic bacteria. Acetone and dichloromethan extracts of *Piper nigrum* showed mild to moderate activities against most of the tested bacteria. Dichlorom ethane has very potent activity than acetone. Methanol and ethyacetate extract of *Mallotus philipinesis* methanol has potent antibacterial activity. Present study shows that both the extracts of *Piper nigrum* and *Mallotus philipinesis* have good antibacterial activity, but when compared with bacteria, Gram positive bacteria are more susceptible to the extracts than the Gram negative bacteria. The mechanism of antibacterial action appears to be loss of control over cell membrane permeability.

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