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Antibacterial Action of Crude Solvent Extracts of *Piper officinarum*

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Antimicrobial activities of various solvent extracts of *Piper officinarum* were determined against a wide variety of pathogenic bacteria. Crude extracts of *Piper officinarum* shows mild to moderate activities for most of the treated bacteria. Ethyl acetate extract, chloroform extracts and methanol extracts showed antibacterial effect against most of the tested organisms. It has been expected that the present work on antimicrobial screening of the plant materials will help researchers who wish to work in designing clinical drugs concerning the killer diseases.

Key Words: Antibacterial activity, *Piper officinarum*.

INTRODUCTION

Many organisms can cause several diseases and now, in this world of modern science, man can face any challenge against any disease. But in spite of the tremendous advancement of medical science and technology, diseases are the leading health problem particularly in the under privileged population in the remote rural areas in the developing countries. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine[1,2]. The wide spread use of herbal remedies and health care preparations, such as those described in ancient texts like 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 antibacterial activities should be tested against an appropriate microbial model.

Piper officinarum sometimes called pippal modha (and also called as sarku by girijans at Paderu forest area Andhra Pradesh, India) and also known as Javanese long pepper or balivenese long pepper is a succulent herb in the family Piperaceae confirm the activity and to ascertain the parameters associated with it. It is, cultivated for its roots, which is usually dried and used as an ingredient in the medicine. It is a close relative of the black pepper plant and has a similar, though generally hotter, taste. 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, namely cough, bronchitis, asthma, heart complaints, vitality *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[3-11].

Due to the fact that the plant *Piper officinarum* is very useful, as found by above mentioned reports and the fact that little information cited in the literature[12] is available on the biological activities, there is a need to find out more about the potentiality of this plant as an antibacterial agent. The present study is, therefore, designed to assess the potency of different solvents extracts of *Piper officinarum* on some selected microorganisms.

EXPERIMENTAL

Analytical balance, hot air oven, autoclave, laminar air flow, incubator, micropipettes required in present study.

Sources of the plant material: The plant *Piper officinarum* was collected from Paderu area, Andhra Pradesh, India. Organisms used in the present study were collected from MTCC, Chandigarh, India. All solvents used for this study were distilled and purified in Chemistry Department, V.S.M. College. Antibacterial activity was investigated with the help of Microbiology Department of V.S.M. College, Ramachandrapuram.

The antimicrobial activities of various solvent extracts of *Piper officinarum* were determined against a wide variety of pathogenic bacteria. Crude extracts of *Piper officinarum* showed mild to moderate activities against most of the tested bacteria. Ethyl acetate extracts of *Piper officinarum* showed relatively better antibacterial effect against most of the test organisms. *n*-Hexane extracts of *Piper officinarum* were found to be inactive against most of the test organisms. It has been expected that the present work on antimicrobial screening of the plant materials will help the scientists to continue work on clinical investigations concerning the killer diseases.

The roots of *Piper officinarum* were collected from the Paderu forests Andhra Pradesh, India. They are chopped into pieces. They are properly cleaned with water and dried. After 2-3 days of drying, the roots are pulverized into fine powder by grinding machine. Fine powder thus obtained is stored in air tight containers.

Procedure: The organic solvents used are chloroform, methanol and ethyl acetate. After 2-3 days of powder incubation in the desired organic solvent, they are filtered by the filter paper. The filtrate was taken into the round bottom flask of the distillation unit. It is kept on the heating mantle and the power is switched on. The distillation process is started and the solvent in the flask will be boiled and the vapours are collected. After complete vapourization of the organic solvent in the flask, the remnant was taken as the crude root organic solvent extract. This extract was collected in an air tight container.

Preliminary anti bacterial test of the crude extract: The cultures used for the antibacterial testing are Gram positive *viz.*, *Bacillus subtilis* MTCC 21, *Staphylococcus aureus* MTCC 96 and *Streptococcus thermophilus* MTCC1938. Gram negative *viz.*, *Escherichia coli* MTCC 40, *Klebsiella pneumonia* MTCC39, *Proteus vulgaris* MTCC426.

RESULTS AND DISCUSSION

The steps involved for the preliminary antibacterial test are as follows:

Revival of the pure cultures: Take the lyophilized pure culture of bacteria. Prepare sterile nutrient broth and sterile nutrient agar slants. Break open the vials and add 100 µL of sterile nutrient broth and mix it. Take a loopful of the culture and streak on the solidified nutrient agar slants. They are incubated at 37 ºC for 24 h.

Preparation of 24 h pure cultures: Prepare six conical flasks of nutrient broth of 10 mL each. They are sterilized by autoclaving at 121 ºC for 15 min. After autoclaving they are cooled. Take a flask inoculates a loopful of the desired slant culture and incubate the flask 37 ºC for 24 h.

Antibacterial testing of the crude extracts

Steps involved are as follows: Prepare one Muller Hinton agar plate and one nutrient agar plate. To this add 0.1 mL of the inoculums, spread it. They are kept in an incubator for 10 min for the setting of the cultures on the plates. Now with a cork borer wells/cups are made. To this add 10 µL of the desired organic solvent root extract. These are placed in an incubator at 37 0 ºC for 18-24 h. After the incubation period zone of inhibition was seen. Measure the zone of inhibition.

Preparation of stock solution of root extracts of *Piper officinarum*: Weigh 0.005 Gms of crude root extracts of *Piper officinarum*. Dissolve in 1000 µL of different organic solvent extracts (ethyl acetate, chloroform and methanol). This gives the stock solution of *Piper officinarum*.

Preparation of working standard solutions of *Piper officinarum*: Take 100 µL of the stock solution. Add to fresh vials. Make up to 1000 µL by adding 900 µL of desired solvent. This will give a concentration of 400 µg/µL.

Process of studying the antibacterial activities of the desired concentrations of the crude root extracts.

The process involves the following steps:

Preparation of the liquid cultures of the desired organism: Prepare 6 flasks of nutrient broth each containing 10 mL of the media. Sterilize by autoclaving at 121 ºC for 15 min. After they get cooled inoculate a loopful of culture from the nutrient agar slant. They are incubated at 37 ºC for 18-24 h.

Preparation of lawn cultures of microorganisms on nutrient agar and Muller Hinton agar: Prepare 150 mL of nutrient agar and Muller Hinton agar in 250 mL conical flasks. They are plugged and autoclaved at 121 ºC for 15 min. After sterilization they are poured into 7 sterile Petri plates. After they get solidified the desired liquid culture was swabbed in both horizontal and vertical direction. They are left for few minutes in incubator. A lawn culture of desired organism was obtained.

Loading with the organic solvent root extract: On to the solidified agar plates make wells/cups by using cork borer. To these cups add 10 µL of working standards of desired organic solvent root extracts. They are incubated in the normal position at 37 ºC for 18-24 h. After the incubation period zone of inhibition was measured.

TABLE-1
ANTIBACTERIAL ACTIVITIES OF DIFFERENT ROOT EXTRACTS OF *Piper officinarum*

Test organism	Diameter of zone of inhibition (mm)			
	EER	CER	MER	STK
Gram positive				
Bacillus subtilis	10	7	9	8
Staphylococcus aureus	6	4	5	6
Streptococcus thermophilus	4	2	1	7
Gram negative				
Escherichia coli	12	9	2	6
Klebsiella pneumonia	8	4	6	8
Proteus vulgaris	5	5	3	7

EER = Ethyl acetate extract of root; CER = Chloroform extract of root; MER = Methanol extract of root; STK = Standard kanamycin (10 µg/disc)

As can be seen from Table-1, ethyl acetate, chloroform and methanol extracts obtained from roots of *Piper officinarum* showed mild to moderate activity against most of the tested organisms. The results are compared with that of kanamycin as a standard antibiotic. Of the three extracts, ethyl acetate extract showed more antibacterial activity against Gram positive and Gram negative bacteria (10 mm, 12 mm). Sensitivity of the bacteria to the three different extract is as follows:

Bacillus subtilis: It is highly sensitive to ethyl acetate and methanol extract compared to chloroform extract.

Staphylococcus aureus: It is moderately sensitive to all the three extracts.

Streptococcus thermophilus: It is moderately to sensitive to ethyl acetate and chloroform extract and resistant to methanol extract.

Escherichia coli: It is highly sensitive to ethyl acetate, chloroform extracts and resistant to methanol extract.

Klebsiella pneumonia: It is moderately sensitive to all the three extracts.

Proteus vulgaris: It showed moderate sensitivity to ethyl acetate and chloroform extracts and mild sensitivity to methanol extract.

On an overall consideration, ethyl acetate extract (EER) of roots of *Piper officinarum* showed more antibacterial activity as compared to methanol and chloroform extract.

Conclusion

It may, therefore be concluded from above investigation that the crude extracts obtained from roots of *Piper officinarum* may be used enough as drug to treat disease caused by those of bacteria which showed sensitivity to the above mentioned samples. However further specific studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents.

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