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Antibacterial Activity Of Chloroform Extract From Piper Longum

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ABSTRACT

The aim of the present study was to assess the antibacterial activity of chloroform extract of Piper longum. Piper longum is one of the oldest known herbal remedies famous for its vast variety of therapeutic properties. Chloroform extract of Piper longum was tested for antibacterial activity against Staphylococcus aureus, Vibrio chlorea, Escherichia coli, Pseudomonas aeroginosa and Proteus mirabilis. Disc diffusion technique and Minimum inhibitory concentration was followed for screening antibacterial activity. The chloroform extracts to different concentrations ranges 5, 10, 15 and 20 µl/ml was used for above assays. After incubation at 24 hours, the zone of Inhibition and optical density was measured by mean of triplicates standard deviation. The chloroform extract of Piper longum was more effective against the Gram positive strain S. aureus with a highest zone of inhibition percentage of 26.1±0.28 at the concentration of 20µl/ml. The extract at different concentrations showed varying degree of antibacterial activity against the microorganisms tested. The results indicated that chloroform extract of Piper longum contained biomolecules showing high inhibitory activity against the pathogens.

Keywords: Piper longum; Chloroform extract; Antibacterial.

INTRODUCTION

India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, the ethnopharmacology and traditional system of medicine are re-emerging to offer an attractive discovery engine. Medicinal plants and its products are a source of many potent and newer powerful herbal drugs (Srivastava et al., 1996). Natural products continue to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug is designed. The active principles of many drugs found in plants are secondary metabolites

(synthesized during secondary metabolism of the plant) (Ghani, 1990; Dobelis, 1993). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et
al., 2000). There is an essential need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Therefore, search for medicinal plants with potential secondary metabolites have been extensively investigated as a source of medicinal agents.

Piper longum is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, fever, hemiplegia, headache, earache, muscle pain, respiratory disorder and digestive trouble. The roots of this plant are also used to treat cough, jaundice, thrust, arthritis, cephalgia, facial paralysis, otalgia, hemicranias and liver disorders (Sankaranarayanan, 2009).

MATERIALS AND METHODS

Plant material

The plants were collected from Kancheepuram district, Tamilnadu, India. The plant was identified with the help of available literature and authenticated by Dr. S. Sankaranarayanan, Head of the department, Department of Medicinal Botany, Sri Sairam Siddha Medical College, Tambaram, Chennai. Collected plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight containers.

Bacterial strains

Microorganisms used for the determination of antibacterial activities were Gram positive; Staphylococcus aureus and Pseudomonas aeroginosa, Gram negative; Proteus mirabilis, Escherichia coli, Vibrio chlorea. Both bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, India. Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37 °C.

Preparation of Chloroform leaf extract

Dried leaf powder of the plants (500 g) was extracted with 1litre of chloroform at room temperature after 24 hrs of soaking. The extracts were collected, filtered, centrifuged at 4000 rpm for 10 minutes and the supernatant was collected, concentrated in a vacuum rotary evaporator and used for analysis.

Agar disc diffusion assay

The antibacterial activity was studied using the disc-diffusion method followed by Sathyabama et al. (2011). Bacteria were grown overnight on Muller Hinton agar plates. Five young colonies were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately 3×108 colony forming units (CFU). The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90 ° to ensure an even distribution of the inoculums. The medium was
allowed to dry for about 3 min before adding a sterile paper disc of 5 mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact. 5, 10, 15 and 20 µl of the chloroform leaf extract of Piper longum individually were introduced on each disc (Three replicates) and 7% chloroform alone served as a negative control. The plates were incubated at 37 °C for 24 h; inhibition zones were measured and calculated.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by dilution method (Brantner, and Grein,
1994). The strains were grown in Mueller Hinton broth to exponential phase with an A560 of 0.8, representing 3×108 CFU/ml. Different dilutions of the chloroform leaf extract of Piper longum individually were prepared to give concentrations at 5, 10, 15 and 20 µg/ml respectively. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 108
CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% chloroform were used as bacterial controls, 4.5 ml of uninoculated Mueller Hinton broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at A560 nm.

RESULTS AND DISCUSSION

On testing, all the bacteria were sensitive to chloroform extract of Piper longum.
Chloroform extract of Piper longum was more effective against the Gram positive strain S. aureus and Pseudomonas aeroginosa than Gram negative microorganism Escherichia coli, Vibrio chlorea and Proteus mirabilis. The results were indicated that antibacterial activity of chloroform extract of Piper longum maximum control gram positive strain by disc diffusion technique at the concentration of 20µl/ml (Table-1). Plants are important source for
the development of new molecules antibacterial agents. The first step towards this goal is the in vitro antibacterial activity assay (Sumathy et al., 2013). The present findings corroborate with the report of antibacterial activity by J. gendarussa aqueous stem extract shown significant antibacterial activity against S. flexneri 26.20mm, P. mirabilis (24.50mm), E. coli (21.40mm), B. subtilis (20.25mm), S.paratyphi A (19.50mm) and S. typhimurium (17.20mm) (Subramanian et al., 2012). The similar result was reported Sugumaran et al.

(2013) Chloroformic extract of J. gendarussa root responded against E. coli and the present result helpful in assessing chemical constituents in the plant material that may lead to their quantitative estimation and also
in locating the source of pharmacologically active chemical compounds.

The Minimum Inhibitory Concentration of Chloroform extract of Piper longum was
treated against both gram positive and gram negative bacteria sugh as Vibrio chlorea, E. coli, Proteus mirabilis, and gram positive and P. aeruginosa and S. aureus at the range of 5-20 µl/ml. The MIC value of inhibition bacterial density when increasing the concentration of Piper longum chloroform extract were treated against both gram positive and negative bacteria (Table-2). Similarly the Minimum inhibitory concentrations of the extracts of Piper longum were found to be significant against Shigella boydii, Shigella flese, Shigella dysenteriae, Escherichia coli, Proteus vulgaris, Salmonella typhi, Hafnia alvei, Staphylococcus epidermidis, Streptococcus pyogenes, Staphylococcus aureus (Kumar et al., 2012). Hence it is concluded that the chloroform extracts of Piper longum showed inhibition of bacterial growth even at low concentrations. The MIC value of Piper longum is the lowest against both Staphylococcus aureus and Pseudomonas aeroginosa than other bacteria. Chloroform extracts of Piper longum shows significant ($p<0.005$) bactericidal activity. According to the results of antibacterial assay, the chloroform extracts of Piper longum might be
used as antibacterial agents against both gram positive and negative bacteria which affect human.

CONCLUSION

In this study demonstrated Chloroform extracts of Piper longum possess broadspectrum of antibacterial activity against both grampositive and gram-negative bacteria. Bioactive substances from Chloroform extracts of Piper longum employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. Isolation, identification and
purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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