In vitro screening of *P. sarmentosum* for antibacterial activity using Agar well diffusion method

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Abstract

Piper sarmentosum Roxb is a herbaceous plant belongs to the family Piperaceae which is commonly used in Southeast Asian cuisine. It can be found in Southeast Asia's tropical regions, Northeast India, and South China, as well as the Andaman Islands. Traditional Asian medicines make use of the plant's leaves. The phytochemical compounds such as naringenin, as well as amides and other compounds, have been discovered in the leaves through phytochemical analysis. Traditional medicinal properties such as anti-tuberculosis, anti-plasmodial, wind-cold cough, fever, rheumatism, diarrhoea, dysentery, postpartum foot edoema, stomachache, toothache, diabetes, and traumatic damage may be due to the presence of these compounds. The objective of the study was to determine the presence of antibacterial activity in the crude methanolic extracts of *P. sarmentosum*. In this preliminary investigation, the leaves were used and the crude extracts were subjected to screening against four strains of bacteria species such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*, using standard protocol of agar well diffusion method. Gentamycin was used as the standard as the positive control. The antibacterial activities were assessed by the presence or absence of inhibition zones. *P. sarmentosum* have potential antibacterial activities. *E.coli* was more sensitive than *B. subtilis, S. aureus* and there is no zone of inhibition against the bacteria *K. pneumoniae*. This finding forms a basis for further studies on screening of extracts of *P. sarmentosum* for knowing the medicinal property of the plant.

Keywords: *P.sarmentosum*, well diffusion assay, methanolic extract, inhibition zones.

Introduction

Today, life threatening diseases has increased all over the world. Hence, discovering a new effective drug is vital for the treatment of complex diseases. Synthetic drugs are less efficient and have many side effects. So, an alternative medicine from nature is very important. Medicines from the plant are an excellent, cheapest, safer,

abundant, and easily available with no side effect. Huge number of medicines were isolated from the plant for the medical practices (Amador, Jimeno, Paz-Ares, Cortes-Funes, & Hidalgo, 2003). The effect of phytochemical constituents in pharmacological study for the treatment of several diseases has been increased dramatically over the past years (Katiyar, Singhvi, Kushwaha, Ramji, & Suryanarayana, 2009). The use of plant as medicinal purposes were first recorded in about BC 2600 from the Sumerians and Akkaidians (Samuelsson, 1999).

Pathogenic organisms like bacteria, fungi and virus are creating fatalistic impact in humans and other organisms like plants and animals. Under this circumstance, there is a need for the development of a novel drug, as an antimicrobial agent from plant or natural product (Baron & Finegold, 1994). The discovery of antibiotics is one of the biggest finding for saving many lives against bacterial infection, but the misuse and abuse of antibiotics has a negative impact on the human beings (Ojala et al., 2000). Due to this reason there is an increased demand for antimicrobials developed from plants and hence may be considered as a reliable source for the development of antimicrobial agent with fewer side effects.

Piper sarmentosum Roxb., a traditional medicinal and herbaceous plant found in South East Asian countries such as India, Malaysia, Thailand, and China's south eastern coastal provinces, including Fujian, Guangdong, and Guizhou. This plant belongs to the family Piperaceae. The aerial parts of the plant are eaten in various forms as a vegetable, and the whole plant or parts are used as folk remedies to treat a variety of ailments, including wind-cold cough, fever, rheumatism, diarrhoea, dysentery, postpartum foot swelling, stomachache, toothache, diabetes, and traumatic injury, alone or in combination with other herbs. So the current research is primarily focused on the efficacy of the extract against infectious disease-causing organisms through the antibacterial analysis.

Materials and Methods

Collection of plant materials

Live accessions of the species was collected from the natural locality at North Bay and maintained at the Field Gene Bank of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Kerala. Saplings were procured from JNTBGRI and reared through vegetative propagation and maintained in uniform conditions at the conservatory of the Department of Botany, University of Kerala for experimental studies. Taxonomic characterization of the accession has been carried out and voucher specimens have been deposited in the Herbarium of University of Kerala Botany Department (KUBH 9793). Passport data on original collection from Andaman is at JNTBGRI. The plant materials for the experiments have been procured from the accession maintained at the Department of Botany, University of Kerala.

Preparation of plant extract

Leaves of *P.sarmentosum* collected from the Department of Botany, University of Kerala conservatory and were utilized for the experiments. Collected leaves were thoroughly washed, chopped and air dried at room temperature to constant weights. The air dried samples were ground to powder. The crude plant extract was prepared by using Soxhlet apparatus. About five grams of powdered plant material was uniformly packed into thimble and extracted with 100 ml solvent. Five different solvent, viz., petroleum ether, chloroform, ethyl acetate, methanol, and distilled water were used for extraction. The process of extraction continued as the solvent in the siphon tube of the extractor became colorless. After that, the extract was taken in a rotary evaporator (Rotavapsuperfit continental (P) Ltd., Mumbai), to separate the extract and solvent. Then, the extract was taken in a Petri plate and kept in a hot air oven at 40-50^0 C until the evaporation of total solvent. Dried extract were kept under refrigerator at 4^{0}C for further phytochemical analysis.

Antibacterial analysis

The methanolic extract from the leaf of the *P.sarmentosum* were subjected to antibacterial analysis

Microorganisms

The Microorganisms like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* were obtained from the Institute of Microbial Technology, Chandigarh, India and from the Public Health Laboratory, Thiruvananthapuram.

Antibacterial assay (Well-Diffusion assay) (Smania Jr, Monache, Smania, & Cuneo, 1999)

Mueller-Hinton agar (15-20ml) was poured onto Petriplates of same size and allowed to solidify. Each agar plate was punched to make 4 wells (20 mm apart from one another) with a sterile cork borer of 8mm size. Standardized inoculums of the test microorganism were uniformly spread on agar surface of each plate using sterile cotton swab. 50 & 100 µl (stock: 100mg/ml) of sample and one positive (80µg of Gentamycin) were poured with micropipette in the bores. The plates were allowed to standby for 30 minutes. The plates were incubated at 37°C for 24h. After incubation, plates were observed for clear zone formation and zone of inhibition was measured in mm. Gentamycin (80µg) was used as positive control.

Result

Antibacterial analysis

The antibacterial activity of the extract from the leaf of the *P.sarmentosum* was examined by using agar well diffusion assay. The effect of the antibacterial activity were checked against the selected Gram positive and Gram negative bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*

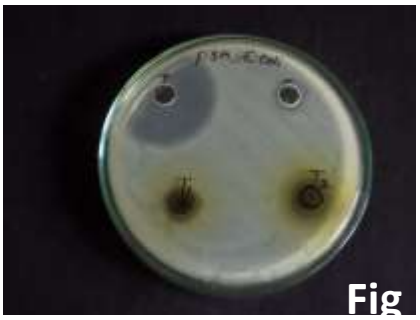
The antibacterial activity of extract was evaluated based on the zone of inhibition against the bacterial growth. The results of zone of inhibition by extract against the selected bacterial strains were presented in Table 1. The antibacterial activity of methanolic extract showed increased zone of inhibition with increased concentration (mg/ml). The gentamycin is used as the standard as the positive control, all the tested bacteria were susceptible to Gentamycin.

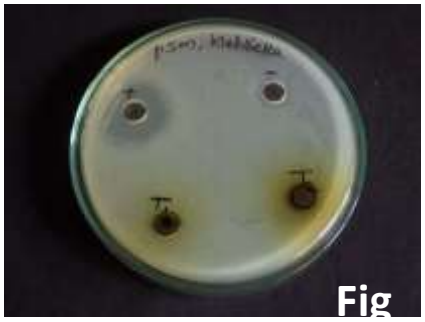
The results of antibacterial activity of methanolic extract showed that *E.coli* was more sensitive than *B. subtilis, S. aureus* and there is no zone of inhibition against the bacteria *K. pneumoniae* (Fig- a, b, c & d). The screening of antibacterial activity from the leaf extract showed that, the methanolic leaf extract of *P.sarmentosum* showed a promising antibacterial activity against the selected bacteria.

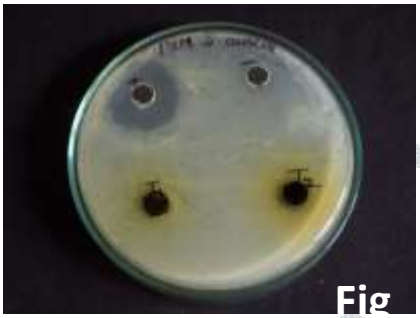
Table 3.1: Antibacterial analysis in *P.sarmentosum* methanolic leaf extract

Sample	**PSM (Methanolic extract)**		
Organisms	+10 µg (mm)	50 µg (mm)	100µg (mm)
Bacillus subtilis	29.10±0.18	10.16±0.12	13.00±0.18
Staphylococcus aureus	30.14±0.26	9.60±0.18	11.24±0.14
Escherichia coli	22.20±0.10	13.30±0.26	15.10±0.24
Klebsiella pneumonia	20.14±0.28	-	-

Values are Mean ± SE of 3 replicates







Antibacterial analysis in *P.sarmentosum* against the selected bacteria *a-Escherichia coli , b-Klebsiella pneumonia , c-Staphylococcus aureus , d-Bacillus subtilis*

Discussion

Antibacterial activity

Plants have a natural resistance against the bacterial disease (Barnabas & Nagarajan, 1988). The plants are inevitable and indispensable resources of natural products for the health of human beings well-being. Plants have a great potential for producing new drugs. (Nascimento, Locatelli, Freitas, & Silva, 2000). The plant derived drugs have an increased interest is developed because they are safer and cheaper than costly synthetic drugs. So, the screening of medicinal plant is very important to identify the biological activity of particular medicinal pant. Through this screening, develop several new drugs to cure many diseases (Silver & Bostian, 1993).

Two Gram positive and two Gram negative bacteria were used to analyze the effect of antibacterial activity of the extract. The antibacterial analysis in extract showed that, the extract showed an active inhibition against bacteria *E.coli* and showed a moderate inhibition against the growth of *B. subtilis*.

Usually Gram negative bacteria are more resistant than Gram positive bacteria because the Gram negative bacteria has an outer layer consist of the lipopolysaccharide layer along with proteins and phospholipids which function as barrier and they prevent the entry of antibiotic or drug (Tomas-Barberán, Msonthi, & Hostettmann,

1988).The similar results were obtained in the methanolic extract, which is less efficient in Gram negative bacteria like *E.coli* and *K.pneumonia.* The *K.pneumonia* was completely inhibited the methanolic extract. On the other hand the extract had better antibacterial activity against all Gram positive bacteria. The same results were obtained in *Piper longum*, showed an active inhibition against Gram positive bacteria than the Gram negative bacteria (Srinivasa Reddy, Jamil, Madhusudhan, Anjani, & Das, 2001).

The phytochemicals present in medicinal plants such as alkaloid, flavonoid, tannin and phenolic compound reported to have many medicinal pharmacological property like antimicrobial, antioxidant and cytotoxicity (Barnabas & Nagarajan, 1988). The antibacterial activity in the methanolic extract showed that extract may possess presence of these phytochemicals at high concentration which might be the reason for the antibacterial activity of the plant.

Conclusion

The antibacterial examination of the extract suggests that the *P.sarmentosum* leaf can be an effective extract for resisting infectious disorders, and this study supports that these plants have diverse anti-infective agents against these bacteria.

References

Amador, M. L., Jimeno, J., Paz-Ares, L., Cortes-Funes, H., & Hidalgo, M. (2003). Progress in the development and acquisition of anticancer agents from marine sources. *Annals of Oncology*, *14*(11), 1607-1615.

Barnabas, C., & Nagarajan, S. (1988). Antimicrobial activity of flavonoids of some medicinal plants. *Fitoterapia*, *3*, 508-510.

Baron, E. J., & Finegold, S. (1994). Methods for testing antimicrobial effectiveness, Baily & Scotts diagnostic microbiology. *NewYork: Mosby*.

Katiyar, R., Singhvi, N., Kushwaha, R., Ramji, L., & Suryanarayana, N. (2009). VA-mycorrhizal association in arjuna and jamun trees in forest of Bhandara region, Maharashtra, India. *International Journal of Plant Sciences (Muzaffarnagar)*, *4*(1), 229-232.

Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*(4), 247-256.

Ojala, T., Remes, S., Haansuu, P., Vuorela, H., Hiltunen, R., Haahtela, K., & Vuorela, P. (2000). Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *Journal of Ethnopharmacology*, *73*(1-2), 299-305.

Samuelsson, G. (1999). Drugs of natural origin, Apotekarsocieteten: Swedish Pharmaceutical Press.

Silver, L. L., & Bostian, K. (1993). Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrobial agents and chemotherapy, 37*(3), 377.

Smania Jr, A., Monache, F. D., Smania, E. d. F. A., & Cuneo, R. S. (1999). Antibacterial activity of steroidal compounds isolated from Ganoderma applanatum (Pers.) Pat.(Aphyllophoromycetideae) fruit body. *International Journal of medicinal mushrooms, 1*(4).

Srinivasa Reddy, P., Jamil, K., Madhusudhan, P., Anjani, G., & Das, B. (2001). Antibacterial activity of isolates from Piper longum and Taxus baccata. *Pharmaceutical biology, 39*(3), 236-238.

Tomas-Barberán, F. A., Msonthi, J. D., & Hostettmann, K. (1988). Antifungal epicuticular methylated flavonoids from Helichrysum nitens. *Phytochemistry, 27*(3), 753-755.