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RESEARCH ARTICLE

Anti-staphylococcal Potential of Active Fraction from Methanol Extract of *Polyalthia longifolia* var. Pendula

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ABSTRACT

The aim of this study was to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active fraction isolated from methanol extract of *Polyalthia longifolia* against 70 clinically isolated *Staphylococcus* strains. Two different fractions (Fraction 1 and fraction 2) were isolated from methanol extract of *P. longifolia* and studied for anti-staphylococcal activity by agar well diffusion method. Fraction 2 showed considerable anti-staphylococcal activity; hence it was selected for MIC and MBC studies by 96 well microtitre plates. Rifampicin was used as positive control. Fraction 2 was highly active against most of the strains studied which was comparable with standard drug rifampicin. Our results confer the utility of this plant fraction in developing a novel broad spectrum anti-*Staphylococcus* agent.

HIGHLIGHTS

- In the present investigation, two fractions of methanolic extract of *Polyalthia longifolia* was used to check its antibacterial potential against 70 clinically isolated *Staphylococcus* strains.
- Anti-staphylococcal efficacy of fraction 2 was more potent than fraction 1 against most of the strains.

Keywords: *Polyalthia longifolia,* anti-staphylococcal activity, MIC, MBC, plant extract

Gram-positive bacteria are a diverse group of organisms that are a major source of morbidity and mortality in patients with immunocompetent and immunocompromised hosts (Wang *et al*. 2021). *Staphylococcus aureus* is commonly cited as being a major hospital-acquired pathogen. These strains pose major problems worldwide as a cause of nosocomial infection and have emerged as a cause of community-acquired infections (Shin *et al*. 2021). Vancomycin is considered as the last-line treatment against a variety of serious infections caused by MRSA (Pires *et al*. 2020). However, reports of vancomycin-resistant strains have generated great concerns regarding the treatment to overcome these infections and the emergence of VRSA is a serious public health concern and is likely to have a dramatic negative impact on many current medical practices (Mahros *et al*. 2021). The increase in isolates of *S. aureus* with resistance to methicillin and decreased susceptibility to vancomycin has created an urgent need for the development of new anti-staphylococcal agents (Saeed *et al*. 2020). Resistance is a major concern with any new agent and will become even more important in the future as new classes of drugs are established. There are essentially two routes of drug discovery, firstly, synthesizing entirely new chemicals and evaluating

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them for a particular pharmaceutical use. Secondly, identifying the chemical or biological origin (natural product) and evaluate it for direct or indirect use as a template for the development of a new drug. Many studies tried to find alternative ways to reduce and prevent the problem of antibiotic resistance in bacteria (Wu *et al*. 2019). Natural chemical compounds can be a new treatment option for multidrug resistant organisms. Plant derived drugs are widely used for the treatment of various diseases. Screening of the natural products in a search for new anti-staphylococcal agents that would be active against this organism is the need of the hour (Bisi-Johnson *et al*. 2017).

In this study two different fractions were isolated from methanol extract of *P. longifolia* and studied for anti-*Staphylococcus* activity by agar well diffusion method and the most active fraction was further studied for MIC and MBC determination against 70 clinically isolated *Staphylococcus* strains.

MATERIALS AND METHODS

Chemicals and reagents

Muller Hinton Agar No. 2 and Muller Hinton broth were purchased from Hi-media, Mumbai, India. Hexane, methanol and dimethylsulphoxide (DMSO) were obtained from Merck, India. All reagents used were of analytical grade.

Bacterial Strains

In the present study, seventy strains of *Staphylococcus* were isolated from different clinical specimens in Department of Microbiology, Cancer Research Institute, Ahmedabad, Gujarat, India and Sanjivani Pathology Laboratory Rajkot, Gujarat, India. All the isolates were identified based on morphology and biochemical parameters (Prescott 2002).

Plant material

Fresh leaves of *P. longifolia* (Sonn.) Thw. var. pendula were collected from Rajkot, Gujarat, India. The plant was compared with voucher specimen deposited by Dr. P.S. Nagar (PSN4) at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in airtight bottle.

Extraction

The dried powder of *P. longifolia* (Leaves) was defatted with hexane and then extracted in methanol for 24 h on rotary shaker by cold percolation method (Kaneria *et al*. 2009; Nyayiru Kannaian *et al*. 2020). Ten grams of dried powder was added to 100 ml of solvent in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 190-220 rpm for 24 h. Then the extract was filtered with 8 layers of muslin cloth. The filtrate was centrifuged at 5000 g for 10 min. The supernatant was collected and the solvent was evaporated. The dried extract was stored at 4°C in airtight bottles.

Fractionation of methanol extract of *Polyalthia longifolia* leaves

Fractionation of the methanol extract was done by solvent-solvent partition (Tang *et al*. 2010). Five grams of methanol extract of *P. longifolia* was dissolved in hot methanol (200 ml). Slight precipitation obtained was discarded as methanol insoluble matter. The methanol-soluble fraction was filtered and collected. It was concentrated to about 50 ml volume and ethyl acetate was added to it till faint turbidity was obtained. Then it was allowed to settle down in a refrigerator. The settled gelatinous reddish mass and supernatant was separated and collected separately. The supernatant was further concentrated and ethyl acetate step was repeated till reddish gelatinous mass obtained. All the settled mass was collected together and dissolved in methanol. It was concentrated further to dryness and designated as Fraction I (FS-I). The collected supernatant was concentrated further to near dryness and then dissolved in methanol. Then chloroform was added to it and cooled. Light yellow waxy sediment was separated and light buff colored supernatant was collected. This supernatant was concentrated further to dryness and designated as Fraction II (FS-II).

Preparation of the extract for anti-staphylococcal assay

Plant extracts were dissolved in 100% dimethylsulphoxide (DMSO) for anti-staphylococcal study. Concentration of the extracts was 15 mg/ml.

Antibacterial assay by agar well diffusion method (Perez *et al.* 1990)

Fraction-1 and fraction-2 of *P. longifolia* were selected for anti-staphylococcal study against ten strains of clinically isolated staphylococci. A loop full of each strain was inoculated in 25 ml of Muller-Hinton broth in a conical flask and then incubated at room temperature on a rotary shaker for 24 h in order to activate the test bacteria. The final cellular concentration was 1×10^8 cfu/ml. The molten Mueller-Hinton agar no. 2 was inoculated with 200 µl of the inoculum and poured into the sterile petri plates. Care was taken to ensure proper homogenization. After media were solidified a well was made in the plates with the help of a cup-borer (8.5 mm). The well was filled with 100 µl of extract (1.5 mg/well) and the plates were incubated overnight at 37°C. Bacterial growth was determined according to the diameter of the zone of inhibition. The experiments were performed 3 times and mean values are presented. For all bacterial strains, DMSO was used as negative control.

Preparation of the extracts and antibiotic for MIC and MBC study

Twofold serial dilutions of the extracts (8000–62.5 µg/ml) and rifampicin (160-1.25 µg/ml) were prepared in DMSO.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Microbroth dilution method, using 96 well microtitre plates, was performed to evaluate MIC of the plant extracts (Andrew 2001). An inoculum suspension was prepared in Mueller–Hinton broth. The inocula were adjusted to each bacterial strain to yield a cell concentration of 10^8 CFU/ml. A final volume of 200 µl was achieved in each well (180 µl bacterial suspension and 20 µl plant extract/antibiotic). Two control wells were maintained for each test batch. These included test control (well containing extract/antibiotic and the growth medium without inoculum) and organism control (the well containing the growth medium and the inoculum). The lowest concentration (highest dilution) of the extract/antibiotic that produced no visible bacterial growth (no turbidity) when compared with the control wells were regarded as MIC. However, the MBC was determined by subculturing the test dilution on to a fresh drug-free solid medium and incubated further for 18–24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

RESULTS AND DISCUSSION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. *Polyalthia longifolia* (Sonn.) Thw. var. pendula is commonly cultivated all over India. It is a tall, ornamental, evergreen tree. Different parts of this plant have been reported by many authors for their medicinal uses. Different biological activities have been studied of extracts/isolated compounds of *P. longifolia* like anti-bacterial, cytotoxicity, anti-fungal, anti-inflammatory, hepatoprotective, anti-ulcer, anti-leishmanial, anti-malarial etc. (Tanna *et al*. 2009; Chanda *et al*. 2011; Kwansa-Bentum *et al*. 2019; Kirubakari *et al*. 2020). Anti-cancer efficacy of secondary metabolites of this plant was also studied (Manjula *et al*. 2010; Sashidhara *et al*. 2010).

Results of anti-staphylococcal activity of fractions of methanol extract of *P. longifolia* are presented in Table 1. Vancomycin and rifampicin were used as positive control. Resistance, intermediate and

Table 1: Antibacterial screening of two fractions from methanol extract of *P. longifolia* against ten clinically isolated staphylococci

Plants	Extractive Yield (%)	Zone of inhibition in mm									
		S1	S2	S3	S4	S50	S51	S52	S62	S63	S32
P. longifolia (Fraction 1)*	44.49	12	10	10	10	9	11	10	9	9	10
P. longifolia (Fraction 2)*	35.6	20	17	18	18	16	23	16	16	18	20
Vancomycin	—	13	13	13	13	10	8	10	15	11	8
Rifampicin	—	21	29	27	25	19	19	32	26	30	18

susceptible staphylococcal strains to vancomycin were considered if the zone of diameter was ≤10 mm, 11-14 mm and ≥15 mm respectively. Resistance, intermediate and susceptible staphylococcal strains to rifampicin were considered if the zone of diameter was ≤20 mm, 21-25 mm and ≥26 mm respectively. Four strains were resistant to vancomycin and five strains were intermediate. Only one strain was susceptible to vancomycin. Five strains were susceptible, four strains were intermediate and only one strain was resistant to rifampicin. Our results showed rifampicin was more active than vancomycin against staphylococcal strains studied. Among two fractions of methanol extract of *P. logifolia*, fraction 2 (PLF2) showed potential anti-staphylococcal activity as compare to fraction-1. So, PLF2 was selected for MIC and MBC studies against seventy clinically isolated *Staphylococcus* strains. Rifampicin (Ri) was used as positive control. The concentrations of MIC and MBC for plant extracts and rifampicin were 125-8000 µg/ml and 1.25-160 µg/ml respectively. The MIC was interpreted as the lowest concentration that inhibited visible microbial growth, whereas the MBC was interpreted as the lowest concentration that can completely remove the microorganisms. MIC and MBC was expressed in terms of µg/ml. The MIC values were evaluated after 24 h of incubation and MBC values were obtained from further analysis of the MIC results.

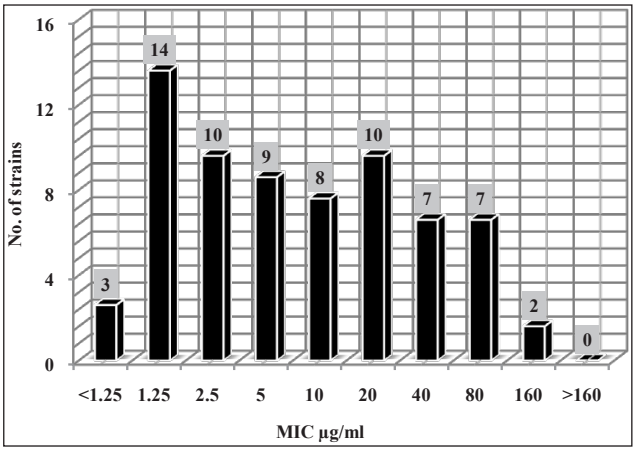

Fig. 1: MIC of Rifampicin against 70 *Staphylococcus* strains

Results of MIC and MBC of PLF2 and Ri are presented in Fig. 1 to 4. The range of MIC and MBC values of PLF2 was between <62.5-1000 and <62.5-4000 µg/ml respectively. The range of MIC and MBC values of Ri was between <1.25-160 and <1.25 - >160 µg/ml respectively. Present results showed that PLF2 possess good antibacterial activity against *Staphylococcus* strains. PLF2 showed lower MBC value than Ri against strain No. 22 and 54. These results showed the potency of PLF2 which was higher than the standard antibiotic studied.

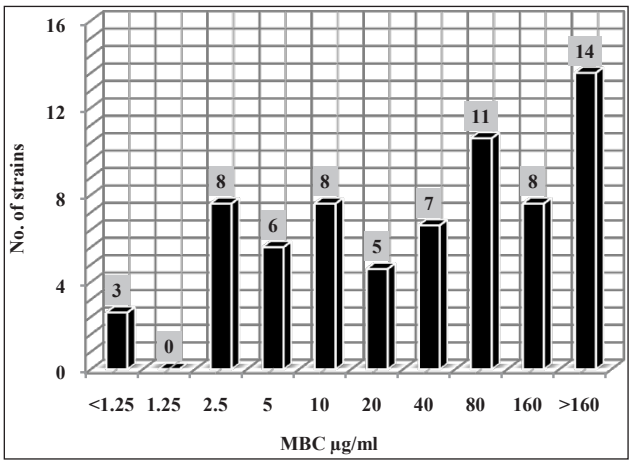

Fig. 2: MBC of Rifampicin against 70 *Staphylococcus* strains

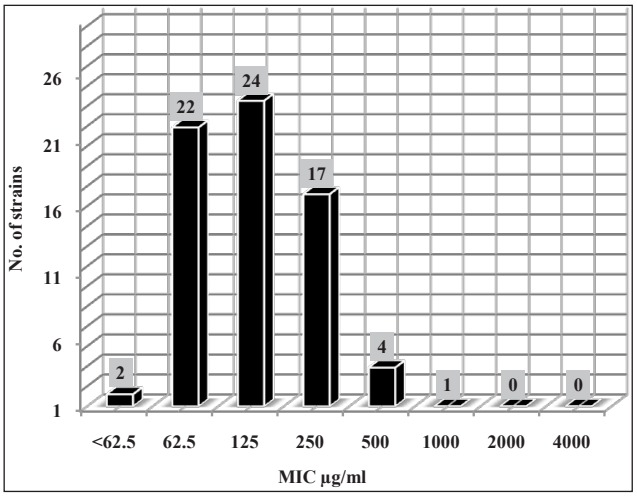

Fig. 3: MIC of PLF2 against 70 *Staphylococcus* strains

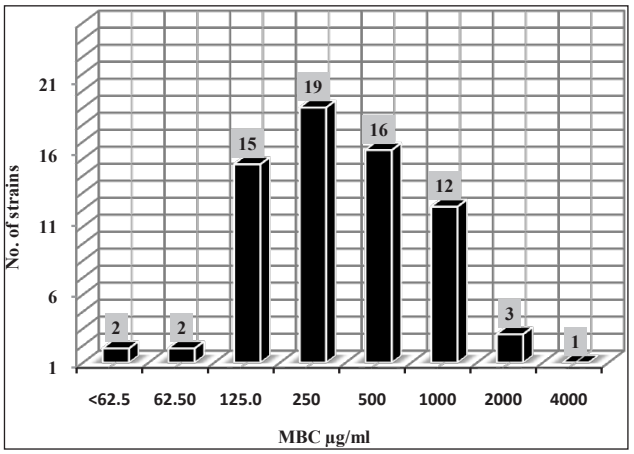

Fig. 4: MBC of PLF2 against 70 *Staphylococcus* strains

There are many reports for antibacterial potency of *P. longifolia* by many researchers. Marthanda Murthy *et al*. (2005) isolated diterpenoids from hexane extract of *P. longifolia* and it showed significant antibacterial and antifungal activities. Faizi *et al*. (2003) isolated alkaloids from root extract of *P. longifolia* and it showed good antibacterial activity with 0.2-20 µg/ml MIC value. Sashidhara *et al*. (2009) studied antibacterial activity of diterpenoid isolated from ethanol extract of *P. longifolia* leaves against *S. aureus* and Sporothrix schenckii with 6.25 µg/ml MIC value. Diterpenoids from methanol extract of *P. longifolia* leaves and berries showed antibacterial activity with 7.8 and 500 µg/ml MIC value (Faizi *et al*. 2008). Present study also showed potent anti-staphylococcal efficacy of fraction 2 isolated from methanol extract of *P. longifolia* leaves against multi-drug resistant staphylococci.

CONCLUSION

The use of plants to heal infectious diseases has been extensively applied by people. Data from literature as well as results of the present study reveal great potential of fraction 2 of *Polyalthia longifolia* for therapeutic treatment to control multidrug resistant *S. aureus*, a major threat to human health. Potent anti-staphylococcal efficacy of fraction 2 from methanol extract of *Polyalthia longifolia* is of great interest and requires further investigation. These preliminary studies are highly interesting as they open new avenues for further studies which would support the validation of the traditional use of this plant in the treatment of antibiotic resistant pathogens. The *in vivo* effects of this extract need to be investigated to fully establish the effectiveness against staphylococcal infections.

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