

Antibacterial activity of *Polyalthia longifolia* var. *angustifolia* stem bark extract

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Abstract: *Polyalthia longifolia* var. *angustifolia* stem bark extracts were evaluated against six important pathogenic bacteria viz. *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella sp.* and *Staphylococcus aureus*. The powdered stem bark extracts were successively extracted with petroleum ether, chloroform, methanol and water using Soxhlet apparatus. The antibacterial activity study was performed by both agar well diffusion and serial dilution methods. The petroleum ether extract was found to exhibit highest activity against all tested bacteria. The inhibitory effect is very similar and comparable with that of standard drug.

Keywords: Antibacterial, *Polyalthia longifolia* var. *angustifolia*, stem bark.

INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies¹. The increasing prevalence of multi-drug resistant strains of bacteria and recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to search for new effective therapeutic agents. Now a day, the development of alternative antimicrobial drug from medicinal plants for the treatment of various diseases has become necessary. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against

cancer, as well as viral and microbial infections². Although hundreds of plants species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated³.

Polyalthia is a large genus of shrubs and trees distributed in tropic and subtropic regions⁴. It belongs to the family Annonaceae. The similar variety of the plant i.e. *Polyalthia longifolia* var. *pendula* has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis⁵. A number of biologically active compounds have been isolated from this plant^{6,7,8}. The plant extract and isolated compounds were studied for various biological activities like antibacterial activity, cytotoxicity, antifungal activity^{9,10,11,12}. Further screening of this medicinal plant may result in the discovery of novel effective compounds. A survey of literature revealed that no methodical reports on anti bacterial activity of

various extracts of *P. longifolia* var. *angustifolia* are available. Therefore it was thought worthwhile to explore this indigenous plant for its activity against different microorganisms.

MATERIALS AND METHODS

Experimental design:

The stem bark extracts of *Polyalthia longifolia* var. *angustifolia* (PLA) were prepared with a series of non polar to polar solvents by hot extraction method¹³ in soxhlet assembly and were screened for antibacterial activity by agar well diffusion method¹⁴ against clinical strains of a few pathogenic bacteria. The extract showing best activity was then used for assay of minimum inhibitory concentration by tube dilution method¹⁵.

Collection of plant material:

The stem barks of PLA were collected in bulk during the month of November from the Khandagiri hills of Bhubaneswar, Orissa. The specimen was identified by Dr. P. C. Panda, Senior Scientist, Regional Plant Resource Centre, Bhubaneswar, Orisa. A voucher specimen (No. SPS-3) has been deposited in the Pharmacognosy Division of University Department of Pharmaceutical Sciences, Utkal University for future reference.

Preparation of plant extracts:

Freshly collected stem barks of PLA were washed under tap water, dried at a temperature of 40°C for 48 h and powdered with the help of mechanical grinder. The coarsely powdered drug (500 g) was packed in a Soxhlet apparatus and extracted successively with petroleum ether, chloroform, acetone and methanol for 24 h. Finally the marc was added to distilled water and extraction was performed by heating at 80°C for 20 minutes to get aqueous extract. All the extracts were filtered and distilled on water bath and finally concentrated at low temperature (40-45°C) under reduced pressure in a rotatory evaporator and preserved in desiccators for further use.

Test micro organisms:

Bacterial strains were obtained from Department of Microbiology, Orissa Agriculture University and Technology, Bhubaneswar, Orissa. The bacteria were sub cultured and once again identified by standard methods of identification¹⁶. Pure cultures of these bacteria were maintained at 4°C on nutrient agar medium.

Antibacterial assay:

The antibacterial activity of extracts was studied by agar well diffusion method¹⁴ with slight modification. Molten nutrient agar (25 ml) was poured into pre

sterilized Petri plates and allowed to solidify at room temperature. The plates were then seeded with 0.1ml (105-106 cells/ml) of overnight bacterial culture. Subsequently 8 mm wide wells were bored within these agar plates using a sterile cork borer. 10 mg of respective extracts was mixed with 5 gm of petroleum jelly to get stock mixture of 2000 µg/gm extract concentration. The wells were aseptically filled with 1gm of this mixture and labeled accordingly.

The plates were incubated over night at 37 ° C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, a negative control was maintained where petroleum jelly without extract was used. Standard antibiotic like Chloramphenicol was also maintained as positive control (Table 3). The experiment was carried out three times and mean values are presented.

Determination of minimum inhibitory concentration (MIC):

MIC of petroleum ether extract was determined by tube dilution method. Successive tubes filled with 15 ml nutrient broth containing 1000µg/ml, 900µg/ml, and 800µg/ml up to 50µg/ml respective concentrations of PLA stem bark extract were inoculated with 100 µl of the bacterial suspension containing 108 CFU/ml of respective test organisms. The tubes were incubated at 37 °C in an incubator and observed for change in turbidity after 24 h. A tube containing nutrient broth without extract was taken as control. The least extract concentration which inhibited the growth of the test organisms was taken as MIC.

Statistical Analysis:

Mean value and standard deviation were calculated for each test bacteria. Data were analyzed by one-way ANOVA and p values were considered significant at p <0.05.

RESULTS

The results of the preliminary phytochemical screening revealed presence of steroids, triterpenoids, tannins, phenolic compounds, gums and flavonoids in different extracts.

In the present study PLA stem bark extracts prepared in various organic solvents were screened for antibacterial activity. The inhibitory effect of the extract was compared with standard antibiotics like Chloramphenicol. Results of the study are listed in Tables 1, 2 and 3. Maximum percentage yield was obtained for methanolic extract followed by aqueous, petroleum ether, chloroform and acetone extract (Table 1).

Results of antimicrobial assay (Table 3) suggest that of all the extracts assayed, petroleum ether extract was the most effective as it showed more significant

inhibition of all test bacteria which was comparable with that of standard drug. Methanolic extract displayed significant activity ($p < 0.01$) against all the test organisms and near potent as standard antibiotic. Significant inhibition of *E. coli* (9.85 mm), *Bacillus subtilis* (6.24 mm) and *Proteus mirabilis* (15.18 mm) were also found to be significantly inhibited by aqueous extract. Chloroform extract showed significant inhibition against *E. coli*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella sp.* and *Staphylococcus aureus* but not active against *Proteus mirabilis* and *P. aeruginosa*. Reasonable inhibition of *E. coli* (12.45

mm), *Salmonella typhi* (14.87 mm) and *Proteus mirabilis* (13.75 mm) and *Staphylococcus aureus* (11.3 mm) by acetone extract was also observed.

Results of assay of minimum inhibitory concentration of crude petroleum ether extract are shown in table 2. MIC values indicate that the petroleum ether extract was highly active against *B. subtilis* (7 $\mu\text{g/ml}$) followed by *E. coli* (10 $\mu\text{g/ml}$), *S. typhi* (72 $\mu\text{g/ml}$), *P. mirabilis* (76 $\mu\text{g/ml}$) *P. aeruginosa* (83 $\mu\text{g/ml}$), *Klebsiella sp* (143 $\mu\text{g/ml}$) and *Staphylococcus aureus* (67 $\mu\text{g/ml}$).

Table 1: Percentage yield of *Polyalthia longifolia* var. *angustifolia* stem bark

S. No.	Solvent	Yield (%)
1	Petroleum ether	6.45
2	Chloroform	2.35
3	Acetone	1.72
4	Methanol	13.65
5	Aqueous	8.4

Table 2: Minimum inhibitory concentration (MIC) of petroleum ether extract of *Polyalthia longifolia* var. *angustifolia*

S. No.	Test organisms	MIC ($\mu\text{g/ml}$)
1	<i>Escherichia coli</i>	10
2	<i>Bacillus subtilis</i>	7
3	<i>Salmonella typhi</i>	72
4	<i>Proteus mirabilis</i>	76
5	<i>Pseudomonas aeruginosa</i>	83
6	<i>Klebsiella sp.</i>	143
7	<i>Staphylococcus aureus</i>	67

DISCUSSION

The antibacterial activities of medicinal plants are attributed due to the presence of flavonoids, tannins and steroidal alkaloids^{17,18,19}. These reports and presence of flavonoids, tannins and steroidal alkaloids in different extract of PLA confirm its potential against all selected pathogens.

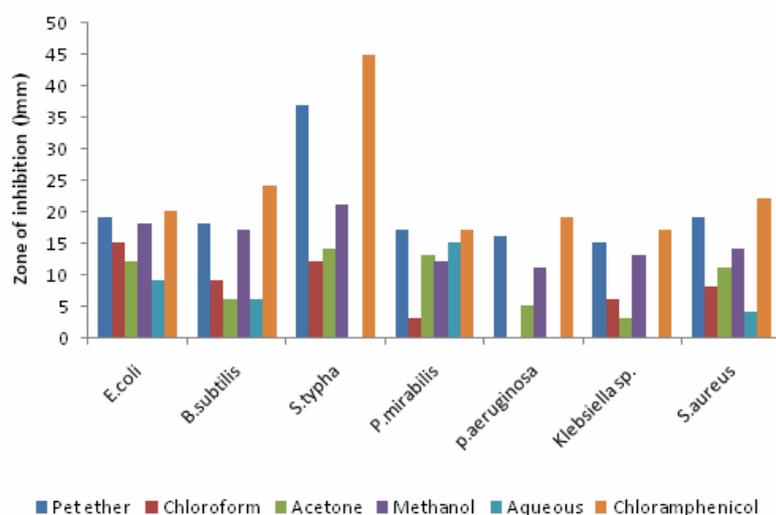
This study suggests that the stem bark extract of PLA have a board spectrum of antibacterial activity, although the degree of susceptibility could differ between different organisms. The antibacterial activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually

or in combination. Our results indicates the potential usefulness of PLA in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of bacterial diseases. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove costly in case of prolonged treatment. Therefore, there is need to develop new antibacterial agents which can satisfy the present demand.

Table 3: Antibacterial activity of stem bark extracts of *Polyalthia longifolia* var. *angustifolia*.

Micro-organisms	Zone of inhibition					
	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous	Chloramphenicol
<i>Escherichia coli</i>	19.33.25±0.42**	15.76±0.21**	12.45±0.73*	18.5±0.54**	9.85±0.3*	20.25±0.52
<i>Bacillus subtilis</i>	18.15±0.64**	9.24±0.46*	6.72±0.4	17.35±0.67**	6.24±0.47	23.56±0.44
<i>Salmonella typhi</i>	37.55±0.76**	12.61±0.32*	14.87±0.55*	21.64±0.45**	No Zone	45.95±0.61
<i>Proteus mirabilis</i>	16.89.2±0.82**	3.24±0.45	13.75±0.48**	12.2±0.76**	15.18±0.4**	17.12±0.35
<i>Pseudomonas aeruginosa</i>	17.35±0.57**	No Zone	4.75±0.65	11.75±0.56**	No Zone	18.66±0.46
<i>Klebsiella sp.</i>	15.5±0.54**	6.26±0.43*	3.16±0.35	13.5±0.5**	No Zone	16.85±0.73
<i>Staphylococcus aureus</i>	19.2±0.44**	8.33±0.85*	11.3±0.53**	14.5±0.75**	3.76±0.68	22.33±0.57

All values are expressed as mean ± SD (n=3). The data were statistically analysed by one-way ANOVA. P values less than 0.05 were considered significant. *: p < 0.05; **: p < 0.01.

**Fig. 1: Antibacterial activity of *Polyalthia longifolia* var. *angustifolia* stem bark**

CONCLUSION

Comparable activity of these extracts with standard antibiotic suggests that the petroleum ether extract of PLA stem bark can yield a highly potent form of novel antibacterial molecule due to presence of steroidal alkaloid. It is documented that steroid alkaloids show a wide spectrum of biological activities, among these antimicrobial effects. Further study for isolation and characterization of active molecule are in progress.

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