

Antibacterial potentiality and phytochemical analysis of mature leaves of *Polyalthia longifolia* (Magnoliales: Annonaceae)

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ABSTRACT

The present study evaluated the antibacterial potentiality of hot aqueous and methanol solvent extract of mature leaves of *Polyalthia longifolia* (Sonn.) Thwaites (Magnoliales: Annonaceae) against six reference bacteria viz. *Staphylococcus aureus* MTCC 2940, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 739, *Klebsiella pneumoniae* MTCC 530, *Proteus vulgaris* MTCC 426 and *Enterobacter aerogenes* MTCC 111. A sensitivity test performed with commercially available sensitivity test disks resulted in the appearance of multiple drug resistance phenotypes as all the tested bacteria are resistant to amoxycillin, cloxacillin, penicillin G and ampicillin. Most effective antibiotic against bacteria includes ofloxacin against *S. aureus* and *K. pneumoniae*; cephalexin against *P. vulgaris*; ciprofloxacin against *E. coli* and *B. subtilis*; kanamycin against *E. aerogenes*. When antibacterial potentiality of hot aqueous and methanol solvent extract of mature leaves of *P. longifolia* was evaluated against the bacteria, highest antibacterial activity was observed against *K. pneumoniae* in both the extracts followed by *E. coli* in hot aqueous extract and *B. subtilis* in methanol extract as evident from MIC values. Chromatographic analysis of the methanol extract of *P. longifolia* revealed the presence of steroids, alkaloids, biterpenoids, carbohydrates, amino acids, essential oil, phenolics and flavonoids as major phytochemicals.

Keywords: Antibacterial potentiality, antibiogram, drug resistance, phytochemical, *P. longifolia*.

1 INTRODUCTION

The increasing failure and side effects of popularly used chemotherapeutics and appearance of multiple drug resistance phenotypes in pathogenic bacteria led to the search of new compounds with antibacterial activity. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand on plant based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. India is the largest producer of medicinal herbs and traditional practitioners of this country use more than 6,000 medicinal plants in primary health care (Shariff *et al.* 2006). But only a few botanicals have moved from the laboratory to field use, because they are poorly characterized and most works are restricted to preliminary screenings.

Polyalthia longifolia (Sonn.) Thwaites (Order: Magnoliales; Family: Annonaceae) is an evergreen plant commonly used as an ornamental street tree due to its effectiveness in combating noise pollution. The antibacterial potentiality of *P. longifolia* was reported against some reference bacteria (Faizi *et al.* 2003; Murthy *et al.* 2005; Nair *et al.* 2005). Its aqueous extract also lowers the blood pressure and rate of respiration in experimental animals (Saleem *et al.* 2005). The hypoglycemic and antihyperglycemic activities of various solvent extracts of *P. longifolia* var. *pendula* leaf extracts were evaluated by Nair *et al.* 2007, in alloxan induced experimental diabetic rats.

The present study was aimed at evaluating the antibacterial potentiality of organic and aqueous extracts obtained from mature leaves of *P. longifolia* against six reference bacteria that were not previously studied. A detailed sensitivity pattern of the reference bacterial strains

was also made against commercially available antibiotics to determine the antibiotic resistance pattern. Other objective includes search of active phytochemicals present in methanol extract of mature leaves and to obtain a preliminary idea about the chemical nature of the active ingredient/ingredients involved in antibacterial potentiality.

2 METHODS

2.1 PLANT MATERIALS

Mature leaves of *Polyalthia longifolia* were collected from the Medicinal Plant Garden of Raiganj, Uttar Dinajpur, West Bengal, India, during the rainy season (mid-June to mid-July, 2006). The leaves were initially rinsed with distilled water and dried on paper towels in the laboratory at (37 ± 1) °C for 24h.

2.2 PREPARATION OF PLANT EXTRACT

Hot aqueous and methanol extract of mature leaves of *P. longifolia* was carried out from air dried plant materials according to the standard methodologies (Bhattacharjee *et al.* 2006). The extracts obtained (2000 µg/ml) were stored as a stock solution in a refrigerator at 4°C for further use. All the extracts were exposed to UV rays (200-400 nm) for 24h and checked frequently for sterility by streaking on nutrient agar plates (Chessbrough 2000).

2.3 TEST MICROORGANISMS

Six reference bacteria, viz. *Staphylococcus aureus* MTCC 2940, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 739, *Klebsiella pneumoniae* MTCC 530, *Proteus vulgaris* MTCC 426 and *Enterobacter aerogenes* MTCC 111 were used during the present study and were obtained from Microbiology Department of Raiganj

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University College. The bacteria were grown in nutrient broth (Hi media, M002) at 37°C and maintained on nutrient agar slants at 4°C.

2.4 ANTIBIOTICS

The following antibiotic sensitivity test discs (Span Diagnostics Limited, Surat, India), at the given concentrations were used to determine antibiotic sensitivity profile of reference bacteria: Amoxycillin (30µg), Gentamycin (10µg), Cloxacillin (10µg), Ofloxacin (5µg), Kanamycin (30µg), Tetracyclin (30µg), Ciprofloxacin (5µg), Penicillin G (10µg), Norfloxacin (10µg), Ampicillin (10µg), Tobramycin (10µg) and Cephalixin (10µg).

2.5 SENSITIVITY TEST

Antibiogram was done by disc diffusion method (Bauer *et al.* 1966; NCCLS 1993) with commonly used antibiotics. Antibiotic sensitivity was tested in Mueller-Hinton agar plates. The test microbes were removed from the slant aseptically with inoculating loops and transferred to separate test tubes containing 5.0mL of sterile distilled water. Sufficient inoculum's was added until the turbidity equaled 0.5 McFarland (10^8 CFU/mL). For each of the bacteria, one milliliter of the test tube suspension was added to the 15–20mL of nutrient agar and transferred to the agar plate (9 cm in diameter). After cooling the inoculated agars at room temperature for 25 min, antibiotic sensitivity test discs were placed on the surface of solid agar. The plates were incubated for 24h at 30°C for *B. subtilis* and at 37°C for other bacteria. The plates were examined thereafter, clear zones of inhibition formed around the discs were measured and antibiotic sensitivity was assayed from the diameter of the clear inhibition zones (in cm). Zone diameters were interpreted as sensitive, intermediate and resistant according to the manufacturer's instructions.

2.6 ANTIBACTERIAL BIOASSAY

The assay was conducted by agar well diffusion method (Perez *et al.* 1990). The bacterial strains grown on nutrient agar at 37°C for 18h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (10^8 CFU/ml). The suspension was used to inoculate 90 mm diameter Petri plates. After solidification of the agar, wells (6 mm diameter) were punched and filled with 30 µl each of 2000 µg/ml aqueous and methanol extracts. The dissolution of the organic extracts (Methanol) was aided by 1% (v/v) Dimethylsulphoxide (DMSO) and that of the aqueous extracts with water. DMSO was taken as control for the methanol extracts and sterile distilled water was taken as control for aqueous extract which did not affect the growth of microorganisms. The plates were incubated for 24h at

30°C for *B. subtilis* and at 37°C for other bacteria. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were repeated thrice.

2.7 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC was determined by dilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). The cultures were diluted in Mueller-Hinton broth at a density adjusted to turbidity of 0.5 MacFarland standards. Equal volume (0.5 ml) of each extract (by serial dilutions from the suspension of hot aqueous and methanol plant extract stock solution) and nutrient broth were mixed in test tubes. Specifically 0.1 ml of standardized inoculum (5×10^5 CFU/ml) was added to each tube. The tubes were incubated at 37°C for 24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC.

2.8 PHYTOCHEMICAL ANALYSIS

The phytochemical analysis was carried out using the methanol extract with the standard methods of Harbone (1984) and Stahl (1984). The phytochemicals studied were – saponins [Frothing test], sapogenins [Antimony chloride spray; Acetone: Hexane 1:4 as solvent], steroid and terpenoids [Leibermann Burchard], flavonoids [Saturated alcoholic sodium acetate spray; Chloroform-Acetic acid-Water (90:45:6) as solvent], alkaloid [Dragendorff's test], essential oils [Vanillin- Sulphuric acid spray; Chloroform-Benzene (1:1) solvent], phenolics (Ferric chloride test) and amino acids (Nin hydrin test).

3 RESULTS

Antibacterial activity of specific concentration (30 µl of 2000 µg/ml extract) of aqueous and methanol extract of *P. longifolia* is presented in Table 1. Present study revealed that both hot aqueous and methanol extract of *P. longifolia* exhibited antibacterial activity against all the reference bacterial strains. Highest antibacterial activity was observed against *K. pneumoniae* in both the extracts followed by *E. coli* in hot aqueous extract and *B. subtilis* in methanol extract (Table 1). Similar types of observation were also noticed during the determination of MIC values (Table 1).

Table 1 Antibacterial activity of specific concentration (30 µl of 2000 µg/ml extract) of aqueous and methanol extract of *P.longifolia*

Type of extracts	Inhibition Zone diameter (cm)					
	<i>E.coli</i>	<i>S.aureus</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>K.pneumoniae</i>	<i>E.aerogenes</i>
Aqueous	1.83± 0.091	1.76± 0.09	1.56± 0.14	1.73± 0.20	3.23± 0.20	1.53± 0.03
Methanol	1.63± 0.14	2.76± 0.09	2.16± 0.17	2.9± 0.06	3.03± 0.09	2.46± 0.18
Control*	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
MIC values (mg/ml)						
Aqueous	75.5	71.0	82.5	63.5	20.5	84.2
Methanol	81.5	25.5	43.5	24.5	23.0	35.5

* Control set consisting of distilled water and DMSO.

Table 2 Antibiotic sensitivity profile of bacteria against commercially available antibiotics

Name of the antibiotics	Inhibition Zone diameter (cm)					
	<i>E.coli</i>	<i>S.aureus</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>K.pneumoniae</i>	<i>E.aerogenes</i>
AX (30µg)	1.0(R)	0.0(R)	1.2(R)	0.0(R)	0.0(R)	1.1(R)
AP (10µg)	0.8(R)	1.0(R)	0.0(R)	0.0(R)	0.0(R)	0.4(R)
RC (5µg)	2.7(S)	2.5(S)	2.1(S)	3.1(S)	1.8(S)	1.9(S)
CX (10µg)	0.0(R)	0.0(R)	0.9(R)	0.0(R)	0.0(R)	0.0(R)
GM (10µg)	2.2(S)	2.3(S)	1.8(S)	1.9(S)	1.4(IM)	2.2(S)
NX (10µg)	2.4(S)	1.7(S)	2.1(S)	0.0(R)	1.7(S)	2.4(S)
NZ (5µg)	2.0(S)	2.8(S)	2.3(S)	2.0(S)	1.8(S)	1.9(S)
PG (10µg)	0.0(R)	0.7(R)	0.0(R)	0.0(R)	0.0(R)	0.0(R)
TB (10µg)	0.0(R)	1.6(S)	1.6(S)	1.7(S)	1.4(IM)	2.3(S)
TE (30µg)	1.2(R)	0.0(R)	1.0(R)	1.7(S)	1.1(R)	1.4(IM)
PR (10µg)	1.4(IM)	2.4(S)	3.2(S)	0.0(R)	1.5(IM)	1.2(R)
KA ((30µg)	1.0(R)	1.1(R)	1.9(S)	2.6(S)	1.2(R)	2.7(S)

Abbreviations: AX = Amoxycillin, AP = Ampicillin, RC = Ciprofloxacin, CX = Cloxacillin, GM = Gentamycin, KA = Kanamycin, NX = Norfloxacin, NZ = Ofloxacin, PG = Penicillin G, TB = Tobramycin, TE = Tetracyclin, PR = Cephalexin, R=Resistant, S=Sensitive, IM=Intermediate.

Antibiotic sensitivity profiles of bacterial strains ARE listed in Table 2. The results showed that all the bacteria are resistant to amoxycillin, cloxacillin, penicillin G and ampicillin. The resistant antibiotics cannot be used as therapeutic agents for treating diseases related with reference bacteria. A comparison of data in the inhibition zones of pathogenic bacteria showed that gentamycin and ofloxacin were effective against all. Most effective antibiotic against bacteria includes ofloxacin against *S. aureus* and *K. pneumoniae*; cephalexin against *P. vulgaris*; ciprofloxacin against *E. coli* and *B. subtilis*; and kanamycin against *E. aerogenes* (Table 2).

The result of preliminary phytochemical analysis of the methanol extract of the mature leaves of *P. longifolia* indicated the presence of steroids ($R_f = 0.02, 0.08$ and 0.15), alkaloids ($R_f = 0.95$ and 0.97), biterpenoids ($R_f = 0.87$), amino acids ($R_f = 0.78$), essential oil($R_f = 0.98$), phenolics ($R_f = 0.91, 0.94$ and 0.96), and flavonoids($R_f = 0.99$), as major phytochemicals. However, absence of saponins and sapogenins are noticed following the application of different solvent systems and spraying reagents.

4 DISCUSSION

The emergence of antibiotic resistance has its roots in the injudicious use of antibiotics and the subsequent transfer of resistance genes and bacteria among animals, animal products and environment. Extra chromosomal genes associated with plasmids were found to be responsible for these antibacterial resistant phenotypes that may impart resistance to an entire antibacterial class (Dixon 2007). The present study also indicates the multiple drug resistance phenotypes as all the bacteria are resistant to amoxycillin, cloxacillin, penicillin G and ampicillin.

The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. The results of the present study support the use of *P. longifolia* for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances. The antibacterial activity of *P. longifolia* was higher than the antibacterial activity of ethyl acetate and n-butanol fractions of ethanol extract of *Bacopa monnieri* against *P. aeruginosa* (Ghosh *et al.* 2007); crude extract of *Baccharis microphylla*, *B. petiolata* and *B. santeliciis* against *S. aureus* and *B. subtilis* (Morales *et al.* 2008); acetone extract of *Ramalina farinacea* against *B. subtilis*, *P. vulgaris* and *S. aureus* (Tay *et al.* 2004); essential oil of *Abies balsamea* against *E. coli* and *S. aureus* (Pichette 2006). However the reported antibacterial potentiality of *P. longifolia* was lower than aqueous and methanol extracts of *Salvadora persica* against *S. aureus* and *P. aeruginosa* (Al-bayati & Sulaiman 2008); methanol extract of *Piper ribesoides* root extract against *S. aureus* (Zakaria *et al.* 2007), methanol extract of *Hibiscus sabdariffa* against *S. aureus*, *E. coli* and *K. pneumoniae* (Tolulope 2007); seed extract of *Moringa oleifera* against *E. coli*, *B. subtilis* and *S. aureus* (Jabeen 2008) and methanol extracts of *Tribulus terrestris* against *S. aureus*, *E. coli* and *P. aeruginosa* (Kianbakht and Jahaniani 2003) as evident from MIC values of the earlier studies. During the present study higher activity was recorded in methanol extract in comparison to aqueous extract (except for *E. coli* and *K. pneumoniae*). This may be attributed to two reasons; firstly, the nature and potentiality of biological active components (alkaloids, flavonoids, essential oil, biterpenoids etc), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number/amount of active constituents responsible for antibacterial activity.

Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores (Wink 1998). The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant; such as phenols (Kazmi *et al.* 1994), essential oils (Cosentino *et al.* 1999; Daferera *et al.* 2003) terpenoids (Habtemariam *et al.* 1993; Taylor *et al.* 1996), alkaloids (Omulokoli *et al.* 1997) and flavonoids (Batista *et al.* 1994). Preliminary

phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances.

5 CONCLUSIONS

In conclusion, *P. longifolia* offers potential antibacterial property against the reference strains. Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances, but the nature of active phytochemical responsible for antibacterial activity can not be ascertained. Further studies of the active principles involved and their mode of action, formulated preparations for enhancing potency and stability are needed to recommend *P. longifolia* in control of several bacteria associated diseases.

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7 REFERENCES

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