

Evaluation of Antibacterial Potential of Stem Bark of Ethnomedicinal Tree *Pittosporum dasycaulon* Miq

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Abstract

The ethnic people indigenous to Southern Western Ghats using stem bark of *Pittosporum dasycaulon* Miq. in their medicinal preparations to treat various skin diseases, leprosy and bronchitis and also used as antifungal and antibacterial agent. The dried stem bark of *P. dasycaulon* was serially extracted with petroleum ether, chloroform, acetone, methanol and water. The extracts were concentrated and their antibacterial activity was detected against thirteen bacterial strains by disc diffusion method. Minimum inhibitory concentration (MIC) of each extract was also determined. Chloroform and acetone extracts displayed a potential antibacterial activity, against all the tested gram-positive bacteria and eight gram-negative bacteria, compared to petroleum ether, methanol and aqueous extracts. Among the different fractions chloroform extract was most effective with maximum zone of inhibition (23.33 ± 0.58 mm) and lowest MIC value (≤ 0.325 mg/ml) against *Enterobacter aerogenes*. The aqueous extract was least effective against all the tested bacteria. The results of this investigation suggest that stem bark of *Pittosporum dasycaulon* can be used to discover broad spectrum antibacterial principles for developing new pharmaceuticals to control studied and many other human pathogenic bacteria responsible for severe illness.

Keywords

Pittosporum dasycaulon, Stem Bark, Ayurveda, Disc Diffusion, Minimum Inhibitory Concentration

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1. Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries¹. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the spectre of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting agents²⁻³. Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents⁴⁻⁵. Plant based antimicrobials represent a vast untapped source for

medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential⁶. Different extracts from traditional medicinal plants have been tested and many reports have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles⁷⁻⁹.

Primitive people have used plant derived preparations to cure a variety of human ailments including skin diseases¹⁰. In India, the use of herbal drugs is an important component of the traditional system of medicine. Many Indian plants have been used from time immemorial to treat various diseases

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and infections in traditional medicinal systems of India such as Siddha, Ayurvedha, Naturopathy, etc¹¹⁻¹². Even today, 85% of Indians use higher plants as effective antimicrobials for the treatment of various diseases¹³. A vast number of medicinal plants which are used in Indian traditional system have not been studied for their antibacterial properties scientifically including species of *Pittosporum*. In India, the bark of *Pittosporum* genus is used as anti-inflammatory, antispasmodic, antidote and for narcotic effects. Its bark is found to be useful in chronic bronchitis, bacterial and fungal diseases and is also administered in leprosy infections and rheumatic swellings¹⁴⁻¹⁶.

Pittosporum dasycaulon Miq. is a small evergreen tree, belongs to the family Pittosporaceae. The plant is distributed in the moist deciduous and evergreen forests of southern and central parts of Western Ghats above 800 m from mean sea level (MSL)¹⁶. Bark is bitter, aromatic and has powerful antioxidant potential¹⁷. The bark and stem of *P. dasycaulon* have been used in folk medicine as an antibacterial and antifungal agent to treat infection¹⁴. Essential oil obtained from the stem bark of this plant shows antibacterial activity¹⁸.

As an important wild source of medicine *Pittosporum dasycaulon* Miq. is yet to receive sufficient attention. Furthermore, little information about the antibacterial effect of this species is available. Therefore, the present investigation was aimed at evaluating the antibacterial potential of the extracts of stem bark of *Pittosporum dasycaulon* Miq. an ethnomedicinal plant endemic to Western Ghats¹⁶.

2. Material and Methods

2.1. Plant Material and Preparation of Extracts

Stem bark of *Pittosporum dasycaulon* Miq. was collected from Southern Western Ghats, Kerala, India, in 2010. The species was identified by Dr. S. John Britto, The Rapinat Herbarium and Centre for Molecular Systematics and voucher specimen (RHT58608) has been deposited in The Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

The plant material brought to the laboratory and washed with distilled water to remove the adhering dust particles. Extracts were prepared from shade dried ($29 \pm 2^\circ\text{C}$) and ground sample of the plant part collected. About 100 g powdered plant material was subjected to successive extraction using Soxhlet apparatus with petroleum ether, chloroform, acetone, methanol and water. After extraction with a solvent, the powdered plant material was air dried overnight at room temperature for complete evaporation of extracting solvent

before subsequent extraction with other solvent. The individual extracts were collected, filtered and concentrated using rotary evaporator (Buchi) under reduced pressure and dried in a vacuum oven which was collected in sterile container and stored at 4°C in air tight containers for further studies. Stock solutions of crude extracts were prepared by dissolving the dried extracts in dimethyl sulphoxide (DMSO) to obtain a final concentration of 200 mg/ml.

2.2. Test Microorganisms

The bacterial strains used in the study were three Gram-positive, namely, *Streptococcus haemolyticus* (MTCC442), *Bacillus cereus* (MTCC430) and *Staphylococcus aureus* (MTCC87), and ten Gram-negative, namely, *Vibrio parahaemolyticus* (MTCC451), *Vibrio cholera* (MTCC3904), *Salmonella paratyphi* (MTCC735), *Enterobacter aerogenes* (MTCC111), *Salmonella typhi* (MTCC733), *Escherichia coli* (MTCC433), *Proteus vulgaris* (MTCC426), *Klebsiella pneumoniae* (MTCC3384), *Pseudomonas aeruginosa* (MTCC741) and *Serratia marcescens* (MTCC86). All the tested strains are reference strains, and were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh-160036, India.

2.3. Preparation of Inoculums

Suspension of organism was prepared as per McFarland nephelometer standard¹⁹. A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contained approximately 1.5×10^8 colony forming units/ml (CFU/ml). It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 ml of 1.175% w/v of barium chloride and 9.95 ml of 1% v/v (0.18 mol/L) sulphuric acid.

2.4. Antibacterial Assay

The antibacterial activity of different extracts was carried out by disc diffusion method²⁰, recommended by NCCLS, using 25 μl of standardized suspension of test bacteria (1.5×10^8 CFU/ml) spread on Mueller-Hinton agar (MHA, pH 7.3 ± 0.1) plates. The discs (6 mm in diameter) were impregnated with 10 μl (2 mg/disc) of the test compound, followed by air-drying and were placed on seeded agar plates. Negative control was prepared using the same solvent (DMSO) to dissolve the plant extracts. Amoxicillin (30 μg /disc) was used as positive control to determine the sensitivity of bacterial strain. The plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was

carried out in triplicate.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The dilution test was performed to determine minimum inhibitory concentrations (MICs) using the standard procedure as described by Jorgensen *et al*²¹, with slight modifications. One hundred microliters of Mueller-Hinton broth (MHB) were added in each well of a microtiter plate. The stock solutions of each extracts were prepared in DMSO (200 mg/ml) and 100 µl aliquot of stock solution of crude extract was added in to the third well, and subsequently two-fold serially diluted up to eleventh well with MHB. The inoculums suspension (20 µl) of bacterial strain was then added in each well containing crude extract and MHB except second well, which was treated as sterility control. The final concentrations of the extract were 83.3, 41.7, 20.8, 10.4, 5.2, 2.6, 1.3, 0.65 and 0.325 mg/ml. The negative (first well) and positive (last well) controls were also performed using DMSO and amoxicillin respectively. Duplicate wells were run for each concentration of *P. dasycaulon* stem bark extracts. The plates were incubated at 37°C for 24 h, and the turbidity was measured at 620 nm using the microplate

reader (iEMS Reader MF, Labsystems). The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC.

3. Results

The antibacterial activities of *P. dasycaulon* stem bark extracts were examined against thirteen clinical pathogens causing severe respiratory and skin illness in human. The results of the disc diffusion test indicated that crude extracts of *P. dasycaulon* stem bark showed different degrees of growth inhibition, depending on the bacterial strains (Figure 1). As per the investigation, four extracts exhibited antibacterial activity. The chloroform extract showed the highest antibacterial activity followed by acetone, petroleum ether and methanol extracts. Among the thirteen bacterial strains were tested, nine (69.2%) showed susceptibility towards petroleum ether extract. The chloroform and acetone extracts inhibited the growth of eleven (84.6%) bacterial strains tested, while methanol extract inhibited the growth of four bacterial strains tested (31%). Aqueous extract did not show any inhibition of all the bacteria tested (Table 1).

Table 1. Diameter of zones of inhibition (mm) stem bark extracts of *Pittosporum dasycaulon* against test bacteria.

Bacteria	a	b	c	d	e	Amoxicillin
<i>Vibrio parahaemolyticus</i> (-)	12.00±1.00	18.33±0.58	14.33±0.58	13.33±0.58	*	27.66±0.57
<i>Pseudomonas aeruginosa</i> (-)	16.33±1.52	20.33±0.58	17.70±0.58	*	*	33.00±0.00
<i>Bacillus cereus</i> (+)	10.33±0.57	14.33±1.52	11.66±1.53	*	*	35.66±1.15
<i>Enterobacter aerogens</i> (-)	19.00±1.00	23.33±0.58	19.66±0.58	11.33±1.52	*	25.66±0.57
<i>Salmonella paratyphi</i> (-)	13.33±1.15	15.00±1.73	15.00±1.00	09.00±1.73	*	40.33±0.57
<i>Vibrio cholerae</i> (-)	*	12.66±2.08	10.33±1.52	*	*	30.00±1.00
<i>Staphylococcus aureus</i> (+)	08.33±0.57	14.00±2.00	14.00±1.00	*	*	34.33±1.15
<i>Serratiamarcescens</i> (-)	11.66±2.08	13.66±1.53	14.66±1.15	*	*	41.0±0.00
<i>Streptococcus haemolyticus</i> (+)	*	17.00±1.00	11.33±1.53	*	*	36.66±0.57
<i>Proteus vulgaris</i> (-)	17.33±0.57	21.33±0.58	16.33±0.58	08.66±1.15	*	24.33±0.57
<i>Klebsiellapneumoniae</i> (-)	08.66±0.57	14.66±0.57	11.33±1.52	*	*	32.33±1.15
<i>Escherichia coli</i> (-)	*	*	*	*	*	30.33±0.57
<i>Salmonella typhi</i> (-)	*	*	*	*	*	36.00±1.00

Note: Values are presented as mean± standard error of triplicate experiments.

a petroleum ether extract, b chloroform extract, c acetone extract, d methanol extract, e aqueous extract; (+) gram positive, (-) gram negative; * no zone of inhibition observed.

Chloroform extract showed the broadest antibacterial activity by inhibiting the growth of *Streptococcus haemolyticus*, *Bacillus cereus*, *Staphylococcus aureus* (gram-positive), *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Salmonella paratyphi*, *Vibrio cholerae*, *Serratia marcescens*, *Proteus vulgaris*, and *Klebsiella pneumonia* (gram-negative) and the diameter of zones of inhibition were 17.0±1.0, 14.33±1.52, 14.0±2.0, 18.33±0.58, 20.33±0.58, 23.33±0.58, 15.0±1.73, 12.66±2.08, 13.66±1.53, 21.33±0.58 and 14.66±0.57 mm respectively. Acetone extract showed potent inhibitory effect against all

the tested bacteria except *Escherichia coli* and *Salmonella typhi* (Table 1). Petroleum ether extract exhibited inhibitory effect against *Bacillus cereus*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Salmonella paratyphi*, *Serratia marcescens*, *Proteus vulgaris*, and *Klebsiella pneumonia* and the diameter of zones of inhibition were depicted in table 1. *V. parahaemolyticus*, *E. aerogens*, *S. paratyphi* and *P. vulgaris* were susceptible for methanol extract. *V. parahaemolyticus*, *P. aeruginosa*, *E. aerogens* and *P. vulgaris* were found to be more susceptible as compared to other bacteria. In all cases, the activity of the extract was compared with standard

antibiotic amoxicillin (Figure 1). The results revealed that among the thirteen pathogenic bacteria *Salmonella typhi* and *Escherichia coli* were insensitive to extracts of stem bark of *P. dasycaulon*.

Table 2 displays the minimum inhibitory concentrations (MIC) of different extracts against the tested organisms. The MIC values of four extracts were ranged from ≤ 0.325 to 83.3 mg/ml. Petroleum ether extract exhibited MIC ranging from ≤ 0.325 to 41.7 mg/ml against the tested organisms, except *E. coli* and *S. typhi*. The MIC analysis showed that the chloroform extract was highly active in comparison to other

extracts, which inhibited the series of study organisms at a low concentration range ($\leq 0.325 - 2.6$ mg/ml) except *E. coli* and *S. typhi* (41.7 mg/ml). Acetone extract inhibited the growth of all bacteria tested at concentrations ranging from ≤ 0.325 to 83.3 mg/ml. The growth of *S. haemolyticus*, *B. cereus*, *S. aureus*, *V. parahaemolyticus*, *V. cholerae*, *E. aerogens*, *P. vulgaris*, *P. aeruginosa* and *S. marcescens* were inhibited by methanol extract at higher concentrations and MIC values were 5.2 to 83.3 mg/ml, whereas none of the bacteria were inhibited by the aqueous extract of stem bark of *P. dasycaulon* at any concentrations tested.

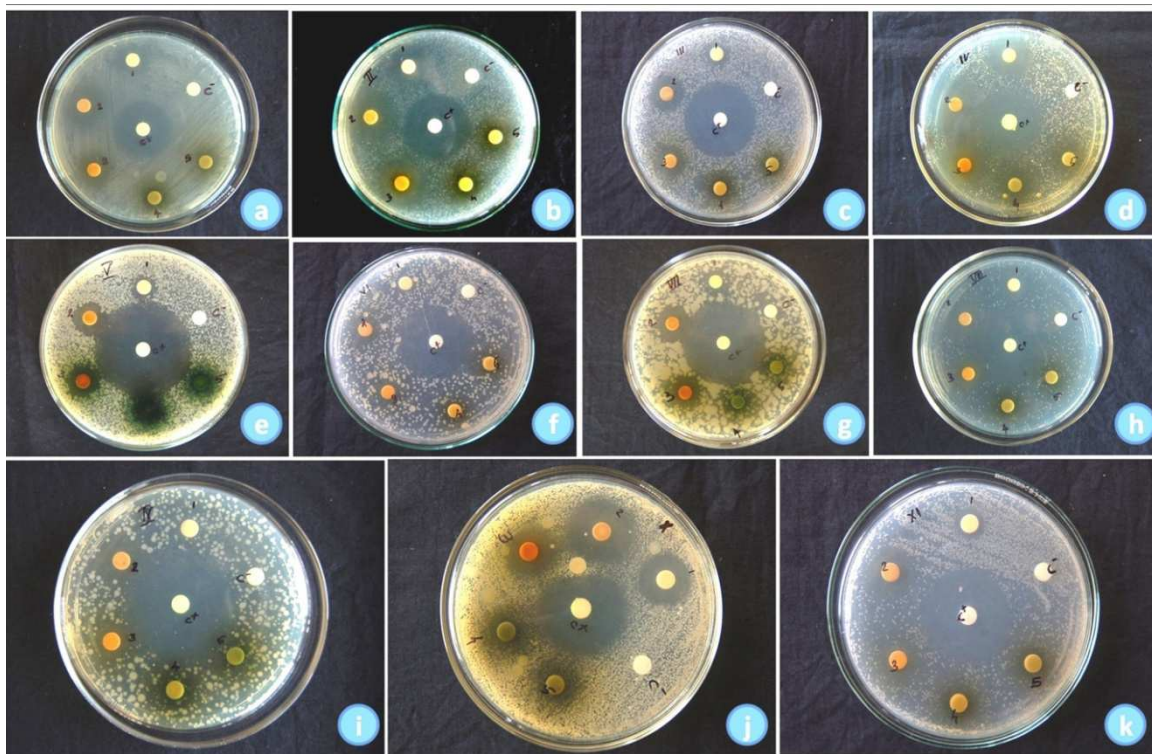


Figure 1. Antibacterial activity of various extracts of *Pittosporum dasycaulon* Miq. stem bark against *Vibrio parahaemolyticus*.

(a), *Pseudomonas aeruginosa* (b), *Bacillus cereus* (c), *Enterobacter aerogens* (d), *Salmonella paratyphi* (e), *Vibrio cholerae* (f), *Staphylococcus aureus* (g), *Serratia marcescens* (h), *Streptococcus haemolyticus* (i), *Proteus vulgaris* (j) and *Klebsiella pneumoniae* (k). 1. Petroleum ether extract, 2. chloroform extract, 3. acetone extract, 4. methanol extract, 5. aqueous extract, C⁺: positive control (Amoxicillin) and C⁻: negative control (Dimethyl sulphoxide).

Table 2. Minimum inhibitory concentration (MIC) of different solvent extracts of *Pittosporum dasycaulon* stem bark against various microorganisms.

Bacteria	a	b	c	d	e
<i>Vibrio parahaemolyticus</i> (-)	0.65	≤ 0.325	0.65	5.20	*
<i>Pseudomonas aeruginosa</i> (-)	0.325	≤ 0.325	0.325	10.40	*
<i>Bacillus cereus</i> (+)	5.20	2.60	2.60	20.80	*
<i>Enterobacter aerogens</i> (-)	≤ 0.325	≤ 0.325	≤ 0.325	5.20	*
<i>Salmonella paratyphi</i> (-)	2.60	0.65	0.65	20.80	*
<i>Vibrio cholerae</i> (-)	41.70	0.65	5.20	83.30	*
<i>Staphylococcus aureus</i> (+)	10.40	0.65	0.65	20.80	*
<i>Serratia marcescens</i> (-)	1.30	0.65	0.65	20.80	*
<i>Streptococcus haemolyticus</i> (+)	10.40	0.325	0.65	20.80	*
<i>Proteus vulgaris</i> (-)	0.325	≤ 0.325	0.325	5.20	*
<i>Klebsiella pneumoniae</i> (-)	5.2	0.325	1.3	41.7	*
<i>Escherichia coli</i> (-)	83.3	20.8	83.3	*	*
<i>Salmonella typhi</i> (-)	83.3	20.8	41.7	*	*

Note: ^apetroleum ether extract, ^b chloroform extract, ^c acetone extract, ^dmethanol extract, ^eaqueous extract; (+) gram positive, (-) gram negative; * no inhibition observed up to 83.3 mg/ml.

4. Discussion

The present study was conducted to obtain preliminary information on the antibacterial activity of different extracts of *Pittosporum dasycaulon* stem bark. The chloroform extract has greater antibacterial activity followed by acetone and petroleum ether extracts while aqueous extract did not show any inhibition of all the bacteria tested. This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair *et al*²². The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium²³⁻²⁴. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators²⁵⁻²⁸.

In our investigation, the most susceptible bacteria against all the active fractions with highest zones of inhibition were *E. aerogens*, *P. aeruginosa*, *V. prahaemolyticus* and *P. vulgaris*, are gram-negative strains. Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other workers²⁹⁻³⁰. In this study, we observed that chloroform, acetone, petroleum ether and methanol extracts were active against many of the Gram-negative bacteria tested along with employed Gram-positive bacteria. The lowest MICs were determined in terms of five Gram-negative bacteria such as *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Proteus vulgaris* and *Klebsiella pneumonia* and one gram-positive bacterium such as *Streptococcus haemolyticus*. The activity of the extracts against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant³¹⁻³³.

5. Conclusions

Today, most pathogenic organisms are becoming resistant to antibiotics^{10, 34}. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *P. dasycaulon*, an ethnomedicinal plant, could become source of promising natural antibacterial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant

extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

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