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Antimicrobial activity of bergenin isolated from *Peltophorum pterocarpum* DC. flowers

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ABSTRACT

Objective: To investigate the antibacterial and antifungal properties of crude methanol extract of *Peltophorum pterocarpum* (*P. pterocarpum*) flower. **Methods:** Disc diffusion method was used for preliminary screening of extracts against microbes. The micro-dilution method was used for the determination of the minimal inhibition concentration (MIC). The isolated compound was identified by crystallography (XRD) technique. **Results:** Bergenin (C-glycosyl benzoic acid) was isolated from methanol fraction of *P. pterocarpum* flowers and confirmed using X-ray crystallography. It was tested against bacteria and fungi. Bergenin showed antifungal activity against *Trichophyton mentagrophytes* (*T. mentagrophytes*) (MIC 250 μg/mL), *Epidermophyton floccosum* (*E. floccosum*) (MIC 500 μg/mL), *Trichophyton rubrum* (*T. rubrum*) (MIC 500 μg/mL), *Aspergillus niger* (*A. niger*) (MIC 500 μg/mL) and *Botrytis cinerea* (*B. cinerea*) (MIC 250 μg/mL). No antibacterial activity was seen. **Conclusion:** The present study was helpful to discovery of new drug and complimentary of alternative medicine.

1. Introduction

Antibiotic drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial substances from medicinal plants. Bacterial infections, especially those produced by *Staphylococcus aureus* (*S. aureus*), are very difficult to manage[1]. New and potent antimicrobial agents, particularly antifungal and anti-*S. aureus* agents are still needed and should be actively sought[2,3].

Antimicrobial properties of certain Indian medicinal plants have been reported based on folklore information[4-7], and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi[8]. The bark of Peltophorum spp. is used to treat dysentery and is externally used as lotion for eye troubles, muscular pains and sores. Part of stem bark prepared in water is applied topically to treat wounds[9]. Antibacterial activity was reported from aqueous and ethanolic extract of *Peltophorum pterocarpum* (*P. pterocarpum*) [10]. Previously flavanol glucoside and flavonol glycosides gallates from Peltophorum africanum (*P. africanum*) [11], Peltophorum dubium (*P. dubium*) [12] and novel cyanomaclurin from *P. africanum*[13] have been reported.

Based on our previous study[9], we selected methanol extract *P. pterocarpum* flowers for further investigation. In this study, we investigated the methanol extract. The fractions and isolated compound were tested for their antimicrobial activity.

2. Materials and methods

2.1. Plant material

P. pterocarpum DC. (Fabaceae) flowers were collected in June 2007 from Loyola College Campus. The plant was identified by Dr. S. Amerjothy, Department of Botany, Presidency College, Chennai, India. The voucher specimen (ERIC-41) has been deposited in the Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India.

2.2. Preparation of plant extracts and isolation of active compound

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Flower extracts were prepared by cold percolation method. The flowers of *P. pterocarpum* were dried under shade and ground into fine powder using electric blender. 500 g of powder was soaked in 1 500 mL of methanol for 48 h with intermittent shaking. The plant extracts were filtered through Whatman No. 1 filter paper and collected in conical flasks. The filtrates were dried at 40 ℃ using vacuum rotary evaporator; the dry weight of the extract yield was 25.2 g. The extract was stored at 4 ℃ for further use. The methanolic extract (10 g) was suspended in 50 mL of MeOH and 150 mL of Double Distilled water, and sequentially partitioned. With 600 mL of hexane yield was 1.4 g. With CHCl3 the yield was 2.7 g. With ethyl acetate the yield was 1.9 g. With Diethyl ether the yield was 1.5 g. Finally the aqueous methanol phases were kept for evaporation; it formed crystal like substances and yielded 200 mg. The crystal was subjected to single crystallography (XRD) at Indian Institute of Technology, Chennai, India for structural determination. The crystal data were recorded using BRUKER-AXS Kappa Apex2 diffractometer.

2.3. Used microorganisms

Bacteria, *Bacillus subtilis* (*B. subtilis*) MTCC 441, *Enterococcus faecalis* (*E. faecalis*) ATCC 29212, *S. aureus* ATCC 25923, S. epidermidis MTCC 3615, Escherichia coli (E. coli) ATCC 25922, *Klebsiella pneumoniae* (*Klebsiella pneumoniae*) ATCC 15380, *Protius vulgaris* (*P. vulgaris*) MTCC 1771, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853 and Erwinia sp. MTCC 2760 and fungi, *Trichophyton rubrum* (*T. rubrum*) MTCC 296, T. rubrum 57/01, *T. mentagrophytes* 66/01, *T. simii* 110/02, Epidermophyton floccosum (E. floccosum) 73/01, Scopulariopsis sp. 101/01 *Aspergillus niger* (*A. niger*) MTCC 1344, *Botyritis cinerea* (*B. cinerea*), *Curvularia lunata* (*C. lunata*) 46/01 and Candida albicans (C. albicans) MTCC 227 were used for the experiment. All cultures were obtained from the Department of Microbiology, 92 Christian Medical College, Vellore, Tamil Nadu, India.

2.4. Antibacterial assay

Antibacterial assay was carried out using disc-diffusion method[14]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai) for bacteria. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the crude extract (5 mg, 2.5 mg and 1.25 mg per disc) with three replicates. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 μg /disc) was used as positive control. The plates were incubated for 24 h at 37 ℃. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

2.5. Antifungal assay and minimum inhibitory concentration

The antifungal activity and Minimum inhibitory concentration (MIC) were performed according to the standard broth microdilution method[15] using Mueller Hinton Broth (Hi-media, Mumbai). The fractions and compound were dissolved in water + 2% dimethyl sulfoxide (DMSO). The initial concentration of fractions was 1 mg/ml and for compound it was 0.5 mg/mL. The initial test concentration was serially diluted two-fold (96-well plate). Each well was inoculated with 5 μL of suspension containing 10^8 CFU/mL of bacteria and 10^4 spore/mL of fungi, respectively. The antifungal agent fluconazole and antibacterial agent Streptomycin were included in the assays as positive controls. For fungi, the plates were incubated 24, 48 or 72 h at 27 ℃ up to 9 d and for bacteria the plates were incubated 24 h at 37 ℃. MIC was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

3. Results

The active methanol extract was partitioned with hexane, chloroform, ethyl acetate, dichloromethane and aqueous methanol. The crystal was obtained from aqueous methanol fraction and subjected to single crystallography. The unit cell parameters were found to be: a = 7.4889 (2) A, b = 13.9271(3) A and c = 14.2778(3) A, $\alpha = \beta =$ and $\gamma = 90^\circ$. The compound was identified

Table 1.
Antibacterial activity of methanol fractions of *P. pterocarpum* flowers.

Bacteria	Zone of inhibition in mm (5 mg/disc)						MIC (μg/mL)	
	(Fr1)	(Fr2)	(Fr3)	(Fr4)	(Fr5)	St (10 μg)	Active Fr5	St
B. subtilis	–	–	–	–	16	13	0.312	25
S. aureus	–	–	–	–	14	12	0.625	6.25
S. epidermidis	–	–	–	–	16	–	0.156	>50
E. faecalis	–	–	–	–	13	12	0.625	25
E. coli	–	–	–	–	–	13	>5	12.5
P. aeruginosa	–	–	–	–	12	13	2.5	25
K. pneumoniae	–	–	–	–	14	11	1.25	6.25
P. vulgaris	–	–	–	–	12	–	>5	nt
E. amylovora	–	–	–	–	–	–	>5	1.56

Fr1 – Hexane; Fr2 – Chloroform; Fr3 – Ethyl acetate; Fr4 – Dichloromethane; Fr 5 –Aqueous and methanol; St – Streptomycin (Antibacterial agent).

Table 2.
Antifungal activity of isolated fractions and compound from *P. pterocarpum* methanol extract.

Fungi	Tested fractions and compound MIC (μg/mL)						
	Fr1	Fr2	Fr3	Fr4	Fr5	Bergenin	Fl
T. mentagrophytes	1000	>1000	>1000	500	500	125	25
E. floccosum	>1000	>1000	>1000	>1000	1000	250	12.5
T. simii	>1000	>1000	>1000	>1000	>1000	>500	<12.5
C. lunata	>1000	>1000	>1000	>1000	1000	125	<12.5
A. niger	>1000	>1000	>1000	1000	500	250	100
B. cinerea	250	1000	1000	>1000	250	500	nt
T. rubrum	500	>1000	>1000	>1000	1000	500	<12.5
M. grisea	500	500	1000	>1000	125	>500	nt
T. rubrum 57	>1000	>1000	>1000	>1000	>1000	>500	25
Scopulariopsis sp	>1000	>1000	>1000	>1000	250	125	<12.5

Fr1 – Hexane; Fr2 – Chloroform; Fr3 – Ethyl acetate; Fr4 – Dichloromethane; Fr 5 – Aqueous and methanol; Bergenin – Isolated compound; FL – Fluconazole (Antifungal agent).

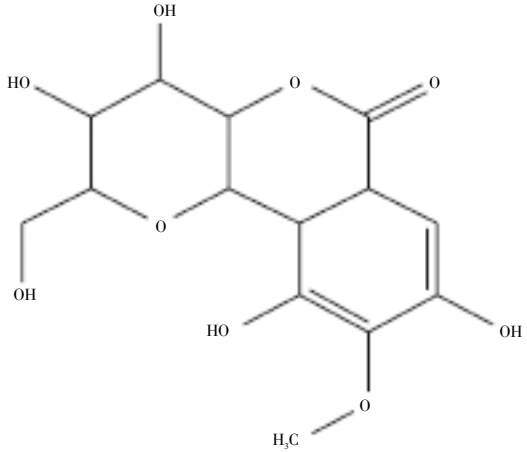

Figure 1. Bergenin derivative of C-glycosyl benzoic acid isolated from *P. pterocarpum* flowers.

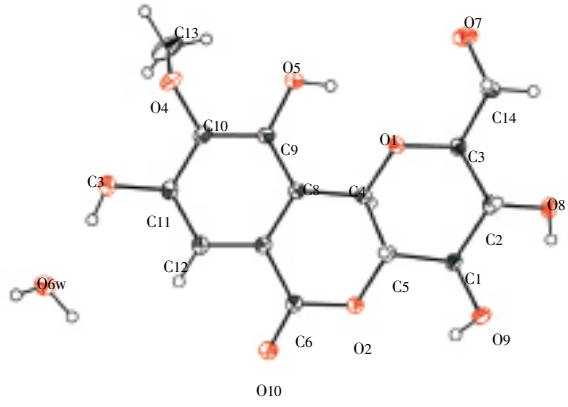

Figure 2. ORTEP diagram of bergenin derivative of C-glycosyl benzoic acid.

as bergenin derivative of C-glycosyl benzoic acid, with molecular weight 346.28, molecular formula $C_{14}H_{18}NO_{10}$, its mp 238 ℃ (Figure 1 & 2).

Aqueous methanol fraction of *P. pterocarpum* flowers exhibited good antibacterial and antifungal activity; the isolated compound showed antifungal activity. Hexane, chloroform, ethyl acetate and dichloromethane fractions were not inhibit the tested bacteria (Table 1). The highest inhibition zone was observed against *B. subtilis* and *S. aureus* at 5 mg/disc concentration. All the tested Gram-positive bacteria were inhibited by aqueous methanol fraction. This fraction also inhibited the growth of Gram-negative bacteria such as *P. aeruginosa*, *K. pneumoniae* and *P. vulgaris* at 5 mg/disc. Gram-negative bacteria *E. coli* and *Erwinia amylovora* (*E. amylovora*) were the most resistant strains. Antifungal activity of isolated fractions and compound were tested and results are given in Table 2.

4. Discussion

Gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances; including antibiotics[16]. Isolated compound did not show any activity against tested bacteria. Fractions and is isolated compound showed antifungal activity against tested fungi. The lowest MIC was observed against *C. lunata* at 125 μg/mL; previously the growth of *C. lunata* was inhibited at 20 μg/mL by the same compound[17].

The isolation of bergenin (c-glycosyl benzoic acid) from this plant reported for the first time. Bergenin is widely distributed in most of the plants. Bergenin was reported from *P. africanum*[18,19]. Bergenin, also known as bergenitol, vakerin and as ardisic acid, is also commonly found in leguminous plants including Caesalpinia[20]. Bergenin is reported in the roots of *Astible thunbergii*[21], aerial parts of *Fluggea virosa* (Rosaceae)[22], *F. microcarpa*[23] and *Ardisia* (Myrsinaceae)[24], roots of *Pentaclethra macrophylla*[25], leaves of *Mallotus japonicus*[26] and bark of *Syzygium cumini*[27]. The maximum activities were exhibited by the aqueous methanol fraction. Isolated compound showed good activity against tested fungi but did not inhibit any tested bacteria. These antimicrobial properties can be used for the therapy of infectious diseases caused by pathogenic microbes.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Hiramatsu K, Hakani H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus*, clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40**: 135-146.

[2] Chambers HF. Methicillin resistance in Staphylococci: Molecular and clinical implications. *Clin Microbiol Rev* 1997; 10: 781-791.

[3] Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. *J Ethnopharmacol* 2007; **112**: 590-594.

[4] Prashanth KN, Neelam S, Chauhan S, Harishpadhi B, Ranjani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethanopharmacol* 2006; **107**: 182-188.

[5] Preethi R, Devanathan VV, Loganathan M. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Adv Biol Res* 2010; **4**: 122-125.

[6] Aqil F, Ahmad I. Antibacterial properties of traditionally used Indian medicinal plants. *Methods Find Exp Clin Pharmacol* 2007; **29**(2): 79-92.

[7] Aqil F, Ahmad I. Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World J Microbiol Biotech* 2003; **19**: 653-657.

[8] Nair R, Chanda S. Activity of some medicinal plants against certain 1148 pathogenic bacterial strains. *Indian J Pharmacol* 2006; **38**: 142-144.

[9] Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med* 2006; **17**: 6-35.

[10] Voravuthukunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S, Supawita T. Effective medicinal plants against Enterohaemorrhagic *Escherichia coli* O157: H7. *J Ethnopharmacol* 2004; **94**: 49-54.

[11] El-Sherbeiny AE, El -Ansari MA, Nawwar MA, El-Sayed NH. Flavonol glycosides and flavonol glucoside gallates from *Peltophorum africanum*. *Planta Medica* 1997; **32**: 165-170.

[12] Salvat A, Antonacci L, Fartunato RH, Suarez EY, Gody HM. Antimicrobial activity of methanol extracts of several plant species from northern Argentina. *Phytomedicine* 2004; **11**: 230-234.

[13] Bam M, Ferreira D, Brandt EV. Novel cyanomaclurin analogue from *Peltophorum africanum*. *Phytochemistry* 1988; **27**: 3704-3705.

[14] Duraipandiyan V, Indwar F, Ignacimuthu S. Antimicrobial activity of confertifolin from *Polygonum hydropiper*. *Pharm Biol* 2010; **48**(2): 187-190.

[15] Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. *J Ethnopharmacol* 2009; **123**: 494-498.

[16] Tortora GJ, Funke BR, Case CL. *Microbiology: an introduction*. San Francisco: Benjamin Cummings; 2001, p. 88.

[17] Prithiviraj B, Singh UP, Manickam M, Srivastava JS, Ray AB. Antifungal activity of bergenin, a constituent of *Flueggea microcarpa*. *Plant Pathol* 1997; **46**: 224-228.

[18] Bessong PO, Obi CL, Line Andreola M, Rojas LB, Pouysegu L, Igumbor E, et al. Evaluation of selected South African medicinal plants for inhibitory properties against human immunodeficiency virus type 1 reverse transcriptase and integrase. *J Ethnopharmacol* 2005; **99**: 83-91.

[19] Mebe PP, Makuhunga P. 11-p-Coumaric acid ester of bergenin from *Peltophorum africanum*. *Phytochemistry* 1992; 31: 3286-3287.

[20] Buckingham J. *Dictionary of natural products on CD-ROM*. London: Chapman and Hall; 2001.

[21] Han LK, Ninomiya H, Taniguchi M, Baba K, Kimura Y, Okuda H. Norepinephrine-augmenting lipolytic effectors from *Astilbe thunbergii* rhizomes. *J Nat Prod* 1998; **61**: 1006-1011.

[22] Pu HL, Huang X, Zhao JH, Hong A. Bergenin is the antiarrhythmic principle of *Fluggea virosa*. *Planta Med* 2002; **68**: 372-374.

[23] Kumar S, Sahai M, Ray B. Chemical constituents of the leaves of *Flueggea microcarpa*. *Planta Med* 1995; **59**: 466.

[24] Kobayashi H, Mejia E. The genus Ardisia: a novel source of health-promoting compounds and phytopharmaceuticals. *J Ethnopharmacol* 2005; **96**: 347.

[25] Folefoc GN, Bisseck JP, Fomum ZT, Bodo B. Constituents from the root of *Pentaclethra macrophylla*. *Biochem Syst Ecol* 2005; **33**: 1280-1282.

[26] Lim HK, Kim HS, Choi HS, Choi J, Kim SH, Chang MJ. Effects of bergenin, the major constituent of *Mallotus japonicus* against D-Galactosamine-Induced hepatotoxicity in rats. *Pharmacol* 2001; **63**: 71-75.

[27] Kopanski I, Schnelle G. Isolation of bergenin from bark of *Syzygium cumini*. *Planta Med* 1998; **54**: 572-595.