

Antibacterial and Antioxidant Activities of 3-O-methyl Ellagic Acid 4'-rhamnoside from Stem Bark of *Polyalthia longifolia* Thw.

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Abstract

The plant *Polyalthia longifolia* (Annonaceae) is an ornamental tree that finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka i.e., *Saraca indica*. However, *P. longifolia* is equated with the name Asoka and often used as an adulterant or substitute of the genuine Asoka bark. The present investigation was carried out with an object to separate and isolate active phytomolecule(s) from stem bark of *P. longifolia* and to screen their antibacterial and antioxidant potential. Column chromatography of the butanol fraction of the hydroalcoholic extract (methanol:water, 1:1) has led to the isolation of a phenolic compound. Structural elucidation was done by IR, ¹H NMR, ¹³C NMR, DEPT, COSY, HSQC, HMBC and mass spectroscopy techniques, and purity was checked by HPTLC and HPLC. Butanol fraction and the isolated compound were screened for antibacterial activity (against facultative aerobic and fastidious aerobic bacterial strains) and antioxidant potential (DPPH method). The compound was revealed to be 3-O-methyl ellagic acid 4'-rhamnoside (**1**), and the purity of the compound was 99.2%. The isolated compound comprises promising antibacterial and antioxidant activities.

Introduction

The plant *Polyalthia longifolia* (Annonaceae) is an ornamental tree, that finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka. Ashoka, a Sanskrit name in Ayurveda stands for the plant *Saraca indica*. However, *Polyalthia longifolia* is equated with the name Asoka and due to its easy availability, often used as an adulterant or substitute of the genuine Asoka bark. As such, no medicinal attributes are accorded to *P. longifolia*. *P. longifolia* is indigenous to the southernmost part of the India and to Ceylon; it has been cultivated in Bombay and other parts of India. It is useful in fever, skin diseases, ulcer, diabetes, hypertension, helminthiasis and vitiated conditions of vata and pitta. It is also used in the treatment of burning sensation, thirst, worm infestations, wound, diarrhoea, scrofulous gland tumors and uterine disorders. The plant contains diterpenoids, alkaloids, tannins and mucilage. The chief components among others are aporphine and azafuorene alkaloids, clerodane and ent-halimane diterpenoids and sesquiterpenes. The present study was designed to isolate phytoconstituent(s) from the hydroalcoholic extract of the bark of the plant and to study their antibacterial and antioxidant activities.

Materials and Methods

Plant Material

The stem bark of *Polyalthia longifolia* (Sonn) Thw. was obtained from Banasthali University campus, Rajasthan, India and identified by Dr. Vinod Kumar Sharma, Department of Botany, Rajasthan University, Jaipur, India (Voucher No.: RUBL 211351). A voucher specimen was preserved in department of pharmacy, Banasthali University, Rajasthan for future references.

Preparation of extract, Isolation of Compound and its Characterization

Air dried and coarse powdered stem bark (2kg) was extracted by cold maceration technique with hydroalcohol (methanol:water, 1:1) at room temperature for twenty four hours, three times successively. Filtered the extracts and pooled, concentrated in rota vapor (Buchi, Switzerland) under reduced pressure, a dark brown viscous mass (178g) was obtained. T

he above hydroalcoholic extract was suspended in water and partitioned with n-butanol. 61 gm of butanol fraction was adsorbed over 90 gm of silica and column chromatographed on a silica gel column (mesh 100-200; Swambe Chemical, India) and eluted with solvent mixtures of increasing polarity and fractions (500ml) were collected and monitored on TLC: chloroform (fraction 1-4), chloroform : methanol [(98:2, fraction 5-9), (96:4, fraction 10-13), (94:6, fraction 14-16), (92:8, fraction 17-18), (90:10, fraction 19-24), (88:12, fraction 25-35), (86:14, fraction 36-43), (84:16, fraction 44-49), (82:18, fraction 50-76), (80:20, fraction 77-89), (78:22, fraction 90-112), (76:24, fraction 113-125), (71:29, fraction 126-130), (66:34, fraction 131-134), (61:39, fraction 135-137), (56:44, fraction 138-140), (51:49, fraction 141-142), (46:54, fraction 143-145), (41:59, fraction 146-147), (36:64, fraction 148-149), (26:74, fraction 150-151), (16:84, fraction 152-153)] and methanol (fraction 154). Fractions (90 to 106) produced an off white coloured compound **1** (yield: 135 mg).

Characterization was done by FTIR, NMR, Mass, HPLC and HPTLC analyses.

Antibacterial and Antioxidant Activities

Antibacterial activity of butanol fraction and compound **1** was studied in facultative aerobic bacterial strains and fastidious aerobic bacterial strains. Antioxidant activity was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Results and Discussion

Characterization of compound 1

The compound isolated by chromatographic techniques was subjected to spectroscopic technique like IR, ¹H NMR, ¹³C NMR, DEPT, COSY, HSQC, HMBC and Mass spectroscopy. Structure elucidation was done on the basis of spectroscopic data as follows:

Compound **1** was obtained as off white colored compound (135 mg, 0.21428 % in butanol fraction), isolated from chloroform: methanol eluents (78:22). The mass spectra displayed a molecular ion peak M⁺ at m/z 462 and M⁺-H at m/z 461 corresponding to formula C₂₁H₁₈O₁₂. The purity of compound **1** assigned by HPLC was 99.2% (Figure 1). The IR spectrum displayed characteristic absorptions for hydroxyl groups (3383 cm⁻¹), α,β unsaturated lactone functions (1738cm⁻¹). The ¹H NMR and ¹³C NMR spectra are depicted in Table 1.

All assignments are in agreement with DEPT, COSY, HSQC and HMBC spectral data. Thus on the basis of spectral data, compound-1 is 3-O-methyl ellagic acid 4'-rhamnoside, having molecular formula C₂₁H₁₈O₁₂ (Figure 2). HPTLC profile of butanol fraction and the isolated compound is depicted in figure 3.

Tab. 1. NMR spectroscopic data for compound-1 (¹H: 400MHz, ¹³C:100MHz, DMSO-d₆)

Position (C/H)	δH	δC	HMBC
1	-	111.4	-
2	-	140	-
3	-	141.1	-
4	-	141.7	-
5	7.5, s, 1H	111.4	C-1(111.4) C-3(141.1) C-7(158.6)
6	-	114.1	-
7	-	158.6	-
OCH3	4.04, s, 3H	60.9	C-3(141.1)
1'	-	111.2	-
2'	-	136	-
3'	-	146.3	-
4'	-	152.6	-
5'	7.7, s, 1H	111.4	C-1'(111.2) C-3'(146.3) C-7'(158.5)
6'	-	112.9	-
7'	-	158.5	-
1''	5.4, d, 1H	100.07	C-4''(152.6), C-2''(70.02)
2''	4.73, dd, 1H	70.02	-
3''	3.5, dd, 1H	69.9	-
4''	3.85, t, 1H	69.9	-
5''	4.9, dq, 1H	71.02	-
6''	1.14(3H, d, 6.1)	17.83	C-5''(71.02)

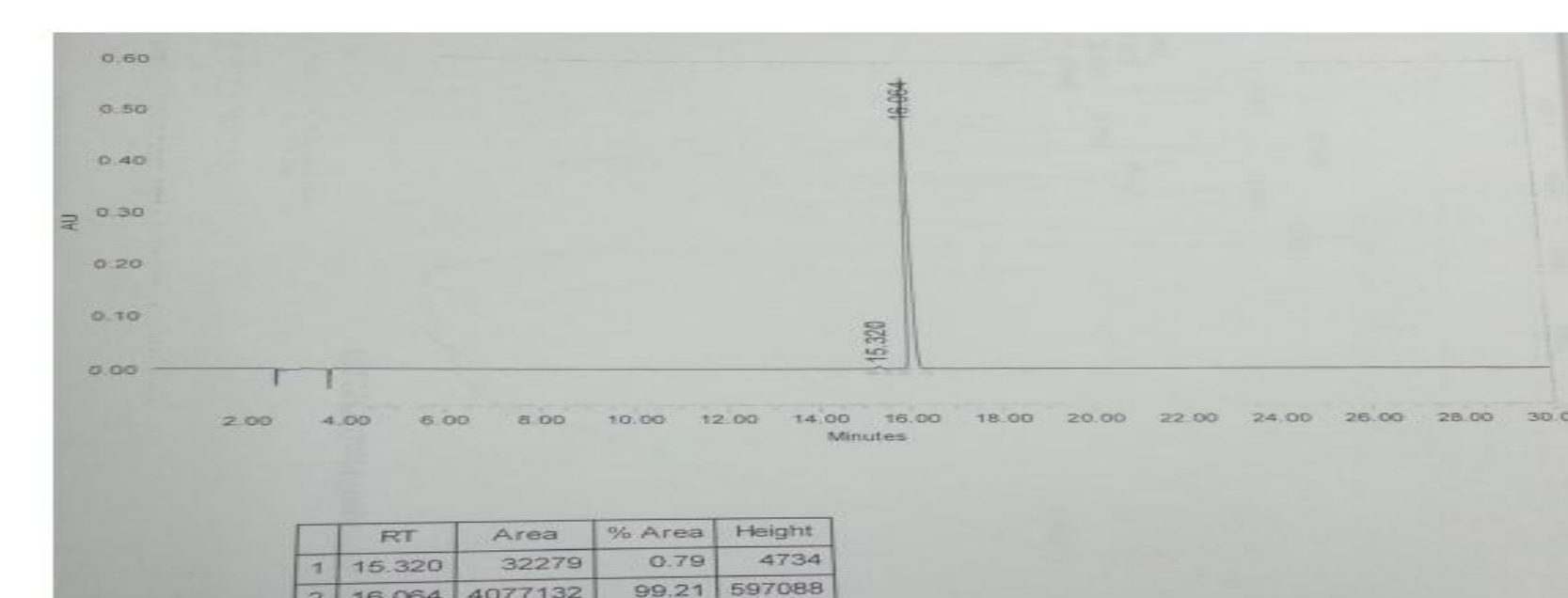


Fig. 1. HPLC chromatogram of compound 1

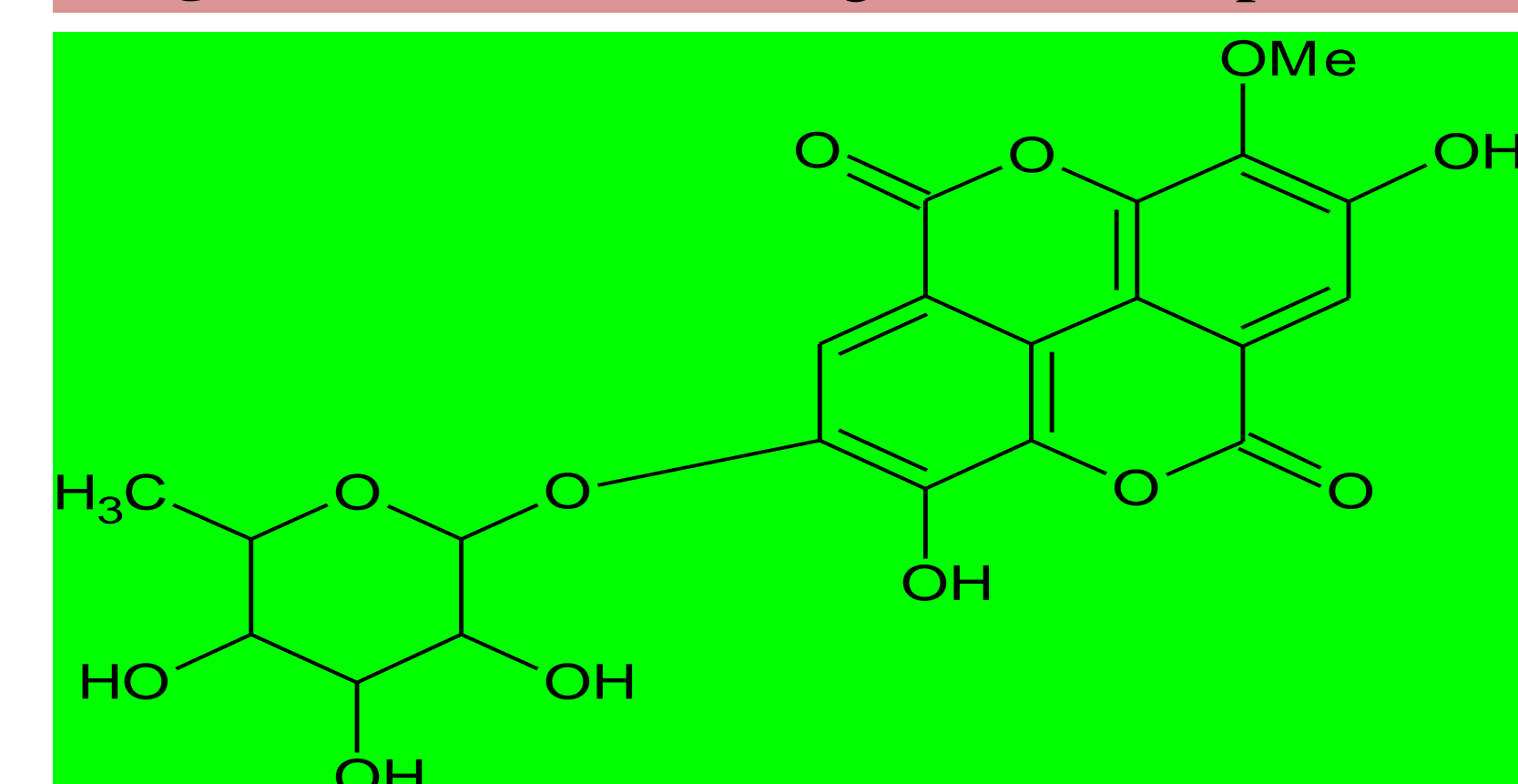


Fig. 2. 3-O-methyl ellagic acid 4'-rhamnoside

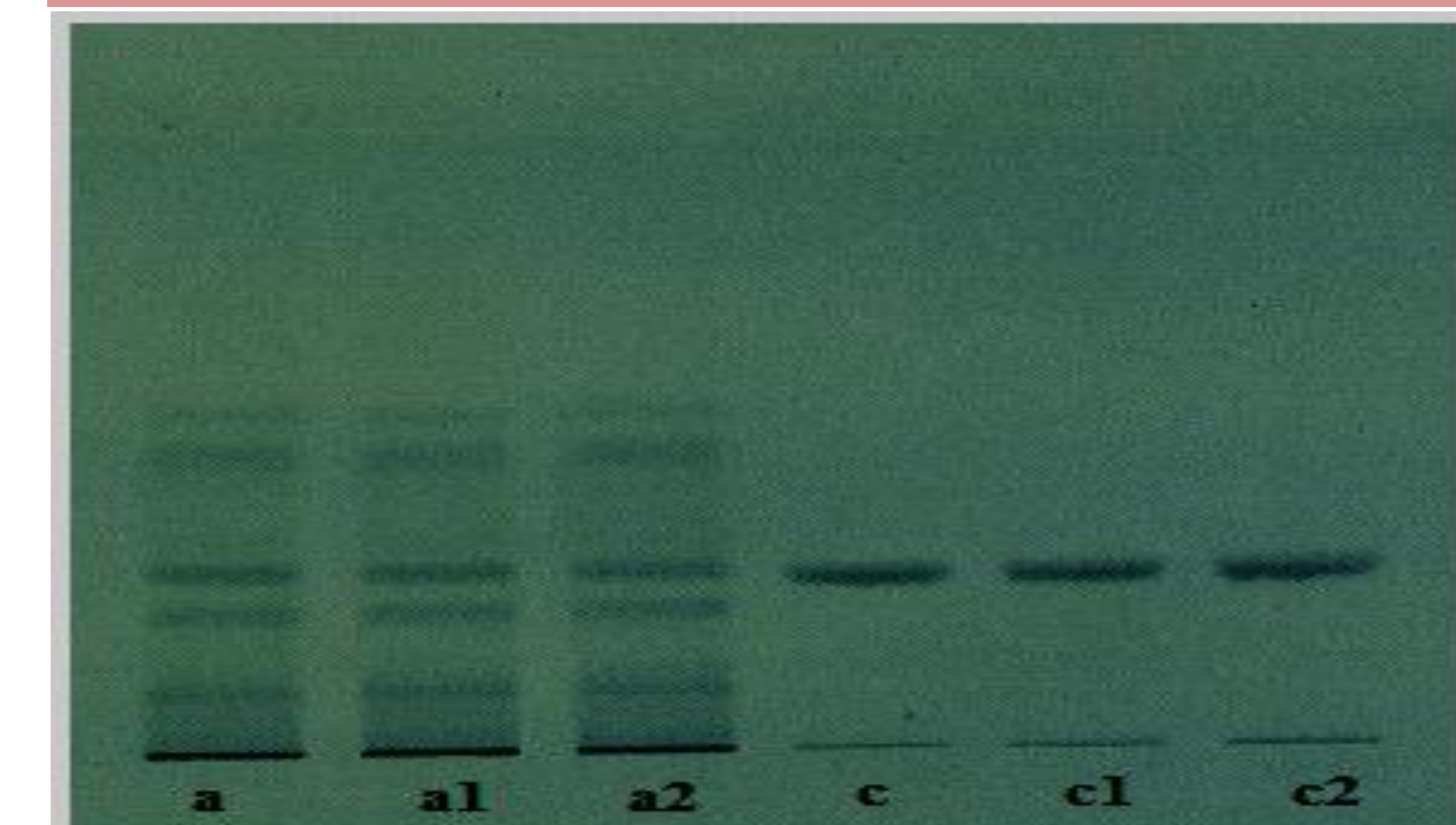


Fig. 3. HPTLC profile: a, a1, a2: butanol fraction; and c, c1, c2: compound 1

Determination of minimum inhibitory concentration

The minimum inhibitory concentration of compound **1** and butanol fraction was found to be in the range of 80-160 µg/ml and 160-320 µg/ml respectively. Compound **1** exhibited higher antibacterial potential against all most all tested bacterial strains than butanol fraction, but the potency is less as compared to standard drugs (Table 2 and 3).

Tab. 2. MIC of standard drug against microorganisms

Bacterial strain	Standard drug	MIC (µg/ml)
<i>S. pneumoniae</i>		0.065
<i>S. pyogenes</i>	Erythromycin	0.125
<i>S. viridens</i>		0.065
<i>S. aureus</i>	Vancomycin	0.25
MRSA	Oxacillin	8.0
<i>P. aeruginosa</i>		0.25
<i>E. coli</i>	Ciprofloxacin	0.015
<i>A. baumannii</i>		0.015

Tab. 3. MIC of butanol fraction and isolated compound

Bacterial strain	Butanol fraction	Compound-1
<i>S. pneumoniae</i>	160	320
<i>S. pyogenes</i>	160	160
<i>S. viridens</i>	160	160
<i>S. aureus</i>	320	320
MRSA	320	320
<i>P. aeruginosa</i>	160	160
<i>E. coli</i>	320	320
<i>A. baumannii</i>	160	160

Antioxidant activity

The highest antioxidant activity of the compound was 57.95% at 40 µg/ml and butanol fraction was 66.05% at 40 µg/ml. Isolated compound exhibited better antioxidant property than the standard drug, vitamin C (Table 4).

Tab. 4. Free radical scavenging activity in DPPH method

Concentration (µg/ml)	Isolated Compounds	Butanol fraction	Vitamin C	% free radical scavenging		
				Compound 1	Butanol fraction	Vitamin C
2.5		25	25	08.24±0.72	12.13±2.26	23.42±0.24
5		50	50	16.32±0.86	22.60±0.78	47.26±0.72
10		100	100	26.56±0.68	29.94±1.19	58.83±0.54
20		200	200	40.48±1.06	44.14±1.48	68.38±0.36
40		400	400	57.95±2.56	66.05±2.87	81.64±0.48

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