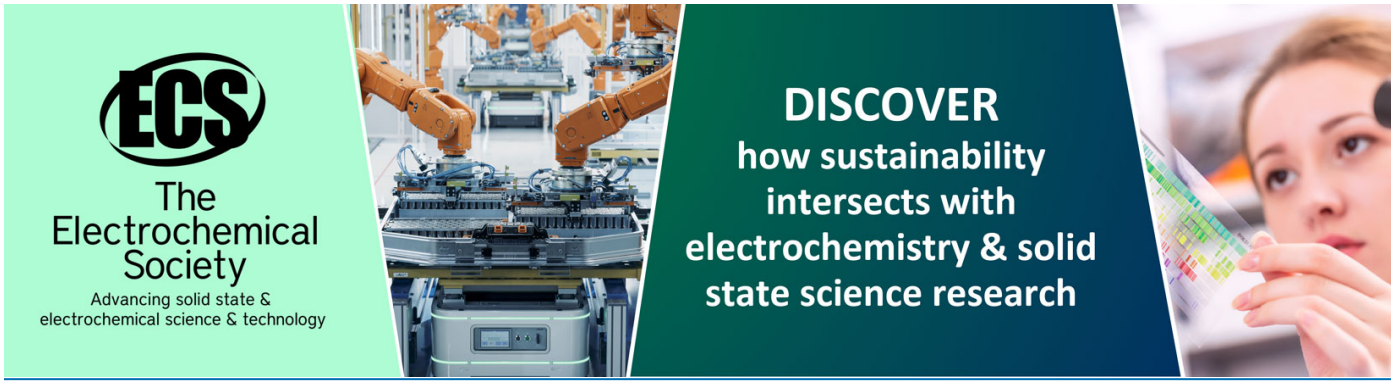
Synthesis and Antibacterial Screening of Novel Derivatives of Embelin



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Synthesis and Antibacterial Screening of Novel Derivatives of Embelin

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Abstract. Embelin, a naturally occurring compound extracted from Embelia ribes is used in Ayurvedic system of medicine owing to its wide spectrum of biological activities. In the present work, we have aimed at improving the efficacy of Embelin by appropriate structural modifications. A few novel derivatives of Embelin have been prepared. The antibacterial screening of these derivatives were carried out and compared with a well known antibiotic, Streptomycin. The derivatives exhibited better activity than Streptomycin and the lead molecule, Embelin.

1. Introduction

Considerable amount of research has been carried out over the past several years towards utilizing natural products for the development of next-generation drugs against several infectious diseases [1]. There has been an exponential rise in the number of diseases with the growth in population. Ayurveda is an ancient system of medicine which involves the use of various herbal preparations. Some of the recent statistics show that the annual turnover of herbal medicinal products manufactured by pharmaceutical companies is several folds higher than that of modern drugs. Identifying the chemical composition of these herbal preparations along with the molecular targets has become highly essential at this juncture. Immense work based on quinones is being carried out these days as it is one among the abundantly occurring natural products. They are believed to be involved in numerous cellular functions thus participating in the electron transfer and hydrogen transfer processes [2]. This in turn leads to the highly reactive oxygen species. Reactive oxygen species modifies and degrades nucleic acids and proteins with in cells [3, 4]. Embelin (figure 1), forms one of the simplest, naturally occurring 1, 4-benzoquinone compound isolated from the dried berries of the plant Embelia ribes, a species in Myrsinaceae family.

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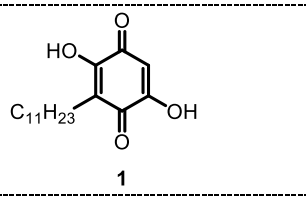

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Figure 1. Structure of Embelin.

The undecyl substituent in embelin confers solubility in the non-polar phase and assists in cell permeability. It exhibits a wide spectrum of biological and physiological activities *viz.*, anthelmintics, antifertility, antitumour, antimicrobial, analgesic, antiinflammatory, anti-implantation and anti-bacterial activity [5-10]. Embelin possesses beneficial effects against oxidative tissue damage [11-13]. It is also known to have wound healing effect which was tested in Swiss Albino Rats [14]. The studies on molecular mechanism of embelin induced apoptosis were carried out by Joy *et al.* [15]. Several derivatives of Embelin have been prepared by various groups [16-18]. They have been prepared by Domino Knoevenagel hetero Diels-Alder reactions by Braun *et al.* [18]. Srinivas *et al.* has reported the antimicrobial and antimitotic activity of a few derivatives of Embelin [19, 20]. Studies on anticancer potential of embelin were reported by Mishra *et al.* [21]. The aromatic amine derivatives of Embelin were reported to exhibit antimalarial activity [22]. This herbal medicine is thus gaining popularity in the recent years mainly due to its natural abundance and multifaceted activities. We herein report, our preliminary investigation on the antibacterial studies of a few derivatives of Embelin.

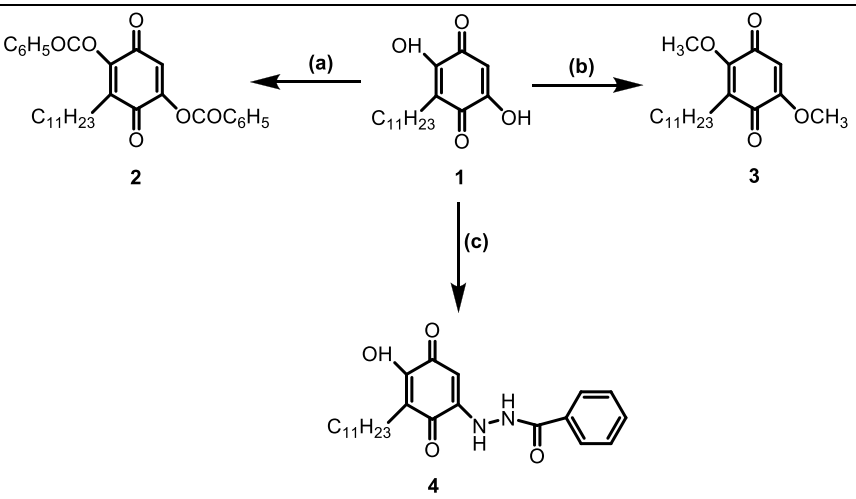
2. Materials and methods

2.1. Extraction and separation of Embelin

Powdered dried berries of Embelia ribes were subjected to Soxhlet extraction using hexane as solvent. Recrystallisation from hexane gave 40% of **1** as bright orange crystals [23]. In the present work, we have derivatised the hydroxyl group of Embelin using aliphatic and aromatic substituents.

2.2. Synthesis of Embelin derivatives

The derivatives of Embelin were prepared (scheme 1) according to standard organic conversions. As shown in the scheme, compound **1** (Embelin) in dry dichloromethane was treated with benzoyl chloride in presence of pyridine under ice-cold conditions to produce compound **2** in 75% yield. Compound **3** was obtained in 67% yield by refluxing compound **1** with dimethyl sulphate in dry acetone in presence of potassium carbonate. To obtain compound **4** in 70% yield, compound **1** was refluxed with benzhydrazide in ethanol. The compounds obtained were purified using column chromatography and characterized using standard spectroscopic techniques.



Scheme 1. Syntheses of compounds **2**, **3** and **4**. (a) Benzoyl chloride, Pyridine, DCM. (b) $(CH_3)_2SO_4$, K_2CO_3, Acetone. (c) Benzhydrazide, Ethanol.

2.3. Antibacterial screening of Embelin derivatives

Screening of compounds **1** to **4** for antibacterial activity was done by the disc diffusion method [24,25]. The antimicrobial activity studies were done in nutrient agar plates with 24h old cultures. A uniform coating of the tested microorganisms were made on nutrient agar- agar plates. Using sterile well borer, wells of specific diameter were made and .the samples were poured into them. The plates were incubated at 37^0C for twenty-four hours and the diameter of the inhibition zones was measured.The *in vitro* antimicrobial potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameter (table 1). The results presented shows promising antimicrobial activity against the microorganisms tested. The minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values were also studied [26]. Table 2 summarises the MIC and MBC values of the derivatives. The results showed that **2**, the dibenzoylated derivative exhibits maximum activity in comparison to rest of the derivatives.

Table 1. Zone of inhibition of compounds **1** to **4** against various bacterial strains.

Organism	**1**	**2**	**3**	**4**	Streptomycin
Salmonella typhi	Nil	16 mm	46 mm	Nil	10 mm
Pseudomonas aeruginosa	8 mm	20 mm	46 mm	8 mm	14 mm
Klebseilla pneumoniae	12 mm	22 mm	47 mm	8 mm	10 mm
Escherichia coli	Nil	15mm	46mm	Nil	11mm
Staphylococus aureus	14mm	28mm	52mm	Nil	12mm
Serratia marcescens	Nil	16mm	38mm	Nil	12mm
Bacillus cereus	Nil	24mm	42mm	10mm	14mm
Bacillus subtilies	Nil	Nil	42mm	Nil	12mm
Proteus vulgaris	12mm	28mm	46mm	10mm	12mm

Table 2. MIC and MBC values of compounds **1** to **4**.

Organism	Compound **1**		Compound **2**		Compound **3**		Compound **4**	
	MIC	MIB	MIC	MIB	MIC	MIB	MIC	MIB
Salmonella typhi	>5	>5	<0.5	<0.5	0.5	1	>5	>5
Pseudomonas aeruginosa	>5	>2.5	<0.5	<0.5	<0.5	<0.5	2.5	5
Klebseilla pneumoniae	5	2.5	<0.5	<0.5	<0.5	<0.5	1	2.5
Escherichia coli	5	2.5	<0.5	<0.5	<0.5	<0.5	1	2.5
Staphylococus aureus	5	2.5	<0.5	<0.5	<0.5	<0.5	1	2.5
Bacillus cereus	5	2.5	<0.5	<0.5	<0.5	<0.5	1	2.5
Serratia marcescens	5	2.5	<0.5	<0.5	0.5	1	1	2.5
Bacillus subtilies	5	2.5	<0.5	<0.5	<0.5	<0.5	5	2.5
Proteus vulgaris	5	2.5	<0.5	<0.5	<0.5	<0.5	>5	>5

3. Experimental section

3.1. Plant materials and chemicals

Embelia ribes (Myrsinaceae) fruit was collected from the local market in Kochi. The fruits thus obtained were dried and powdered mechanically and stored in airtight containers. All the chemicals were of analytical grade and used without further purification, unless otherwise specified. Dichloromethane and acetone were dried according to the reported procedure [23]. Reactions were monitored with analytical TLC on silica gel 60-HF254 pre-coated aluminium plates and visualized under UV (254 nm). Column chromatography was performed on silica gel (100-200 mesh). Standard strains [Microbial Type Culture Collection (MTCC)] of Gram-positive and Gram-negative bacteria were obtained from the Institute of Microbial Technology, Chandigarh, India.

3.2. Experimental methods

NMR spectra (^{1}H and ^{13}C) were recorded on a Bruker Advance DPX- 500 MHz NMR spectrometer. IR spectra were recorded in Shimadzu FTIR spectrometer. Melting points were determined using Toshniwal melting point apparatus.

*3.2.1. Extraction of 2,5-Dihydroxy-3-undecyl-[1,4]benzoquinone (**1**) from Embelia Ribes [15].*

100 g of the powder of Embelia ribes berries were subjected to Soxhlet extraction using hexane as solvent. The extraction was continued till the extract became colourless after which it was filtered and the solvent was removed under reduced pressure. Recrystallization from dichloromethane gave 40% of **1** as bright orange crystals (m. p.: 134 °C).

IR (KBr): ν_{max} 3304, 2919, 2848, 1612, 1461, 1181, 944, 902, 860, 767, 694 cm^{-1}.

*3.2.2. Preparation of 2,5-Dibenzoxy-3-undecyl-[1,4]benzoquinone, **2** [27].*

To a solution of **1** (12.3 mg, 0.04 mmol) dissolved in dry dichloromethane was added 1 ml of pyridine drop wise. During addition, the solution turned dark brownish-red. The solution was stirred for 5 minutes under 0 °C, and then slowly added benzoyl chloride (11.76 mg, 0.1 mmol) into the reaction mixture, dropwise. The solution turned orange-yellow in colour. The reaction mixture was stirred for 1 hour. The crude reaction mixture was extracted with dichloromethane, washed with water, separated and dried over Na_2SO_4, filtered and evaporated under reduced pressure in a rotary evaporator. The residue was chromatographed over SiO_2 using hexane-ethyl acetate (9:1) mixture to get 75% of **2** as yellow solid.

IR (KBr): ν_{max} 2922, 2852, 1746, 1672, 1634, 1598, 1583, 1451, 1236, 1179, 1162, 1132, 1042, 1021, 1001, 935, 906, 858, 836 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 7.50-8.20 (m, 10 H), 7.00 (s, 1H), 2.59 (t, 2H), 1.27-1.56 (m, 18 H), 0.87 (t, 3H).

^{13}C NMR ($CDCl_3$): δ 181.1, 180.4, 167.6, 164.4, 164.2, 163.3, 154.1, 150.4, 137.2, 135.7, 135.5, 135.4, 133.8, 131.5, 131.2, 131.2, 131.1, 130.4, 130.0, 129.3, 129.0, 128.9, 123.1, 32.6, 24.4, 23.3, 14.4.

3.2.3. Preparation of 2,5-Dimethoxy-3-undecyl-[1,4]benzoquinone, ***3*** *[28].*
To a well-stirred solution of **1** (10 mg, 0.03 mmol) in dry acetone (125 ml) was added potassium carbonate (0.09 g) and dimethyl sulphate (0.1 ml, 0.03 mmol) dropwise. The reaction mixture was refluxed for 3 hours. Excess solvent was removed under reduced pressure and the crude product was chromatographed over silica gel (100-200 mesh) using a mixture (1:20) of ethyl acetate and hexane to give 67% of **3** as yellow solid.
IR (KBr): ν_{max} 2915 2848, 1650, 1595, 1453, 1325, 1206, 1156, 1117, 1039, 977, 929, 848, 801, 759, 719, 684 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ 5.73-5.74 (d, 1H), 3.81-4.05 (dd, 6H), 2.41-2.44 (t, 3H), 1.21-1.39 (m, 18H), 0.86-0.89 (t, 3H).
^{13}C NMR ($CDCl_3$): δ 183.5, 182.4, 158.8, 155.8, 130.6, 105.3, 61.2, 58.5, 56.3, 31.8, 29.6, 29.4, 29.3, 29.2, 28.6, 23.0, 22.6, 14.0.

3.2.4. Preparation of Benzoic acid N'-(4-hydroxy-3,6-dioxo-5-undecyl-cyclohexa- 1,4-dienyl)-hydrazide, ***4*** *[29].*
A well-stirred solution of **1** (150 mg, 0.51 mmol) and benzhydrazide (69.46 mg, 0.51 mmol) in absolute alcohol was refluxed for 3 hours. The solid was separated and filtered. The mother liquor was poured over crushed ice. The separated solid on recrystallization from ethanol gave 70% of **4** as a reddish brown solid (m. p.: 189 °C).
IR (KBr): ν_{max} 3307, 2850, 1743, 1664, 1639, 1613, 1559, 1524, 1494, 1479, 1465, 1356, 1325, 1281, 1207, 1193, 1117, 1026, 983, 944, 902, 860, 845, 766, 694 cm^{-1}.
^{1}H NMR (CD_3COCD_3): δ 10.32 (s, -OH), 9.02 (s, 1H), 8.83 (s, 1H), 7.9-8.0 (d, 1H), 7.8-7.9 (d, 1H), 7.6-7.4 (m, H), 6.62 (dd, 6H), 2.41-2.44 (t, 3H), 1.21-1.39 (m, 18H), 0.86-0.89 (t, 3H).

4. Conclusion
In the present work, we have prepared a few derivatives of the natural product Embelin. The derivatives were subjected to antibacterial studies and were found to be very active against several species of bacteria. They were found to be more active than Streptomycin. The parent compound being a bioactive and abundantly available natural product, can find tremendous application in the field of medicine.

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