Antimicrobial Activity of 2,5-Dihydroxy-3-methyl-1,4-benzoquinone from *Embelia schimperi*

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Chromatographic separation of an ethyl acetate extract from *Embelia schimperi* led to the isolation of a new compound identified as 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**) on the basis of spectroscopic and physical data. The plant's crude extract and pure compound **1** were assayed for *in vitro* antimicrobial activity against clinical strains of *Salmonella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Cryptococcus neoformans, Shigella dysentriae* and *Staphylococcus aureus*. Disc diffusion method was used and zones of inhibition, after respective incubation periods, were used to quantify antimicrobial activity. Standard antibiotics namely: augmentin, cotrimoxazole, gentamycin, tetracycline and lyncomycin were used as controls.

The crude extract was inactive while the pure compound **1** showed significant activities against *Salmonella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Cryptococcus neoformans, Shigella dysentriae* and *Staphylococcus aureus* with zones of inhibition ranging from 10–20 mm. The most sensitive microorganism was *P. aeruginosa* while *C. neoformans* was insensitive to both the crude extract and compound **1.**

Key words: *Embelia schimperi*, 2,5-Dihydroxy-3-methyl-1,4-benzoquinone

Introduction

Many plant extracts have been reported to possess antimicrobial activity (Orret, 1997). In traditional medicinal practice, plant preparations have always been used to treat infectious diseases like TB, gonorrhea and skin infections with varying degree of success (Waiyaki, 1997). It is also a well-known fact that most of the drugs that are in use today are of plant origin. In the USA for example, approx. 10% of the major drugs in use have plant extracts as their active ingredients (Mayunga, 2002). The study of biologically active plants with antimicrobial activities, especially those used as herbs, by the local communities, is therefore important and has attracted considerable interest throughout the world (Kofi-Tsekpo, 1992).

In a quest to discover new antimalarial extracts, *Embelia schimperi* was extracted with ethyl acetate. The plant belongs to the family Myrsinacea and can grow to a maximum height of about 7 m (Kokwaro, 1993). The Myrsinaceae is a highland family of trees and/or shrubs that are established all over the world. In Kenya, the species are found in the West and South of Mt. Kenya, Ngong' Hills, Kakamega forests and Western slopes of Mau Ranges around Kericho District. Many communities in Kenya use the species as herb. The Maasai and the Kipsigis tribes living in the Rift Valley Province of Kenya use it to deworm both humans and animals, in cleaning wounds and as a disinfectant (Bogh *et al*., 1996)

The aim of the research was to extract and evaluate extracts from *Embelia schimperi* for their antimicrobial activity.

Materials and Methods

Collection of plant parts

Fresh stem bark of the herbal plant *Embelia schimperi* was collected from the Western slopes of Mau Ranges in Kericho District which is about 300 km west of Nairobi. The plant was identified by the herbarium staff of the Department of Botany, Moi University, where a voucher specimen (No. Ker 02 05 001) was deposited. The collected

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plant parts were air-dried for 5 d and then chopped into small pieces.

Extraction and isolation of compounds

The dried pieces were ground into a fine powder using an electric grinder. 1 kg of the material was soaked in ethyl acetate at room temperature for 48 h. The mixture was then filtered and the solvent evaporated under reduced pressure using a rotary evaporator to afford 50 g of a dark brown gummy solid.

26 g of the crude extract were subjected to column chromatography using a column packed under *n*-hexane with 118 g of dry deactivated silica gel (mesh size 0.063–0.2 mm).

The column was first eluted with pure *n*-hexane followed by a mixture of *n*-hexane/ethyl acetate with increasing polarity. Elution of the column with an *n*-hexane/ethyl acetate mixture (1:9 v/v) led to the isolation of a yellow crystalline compound, **1**. The column was finally washed with methanol. The eluants were collected in portions of 20 ml. The compound was characterized on the basis of spectroscopic (MS, IR and NMR) data and its melting point.

Antibacterial assay

A quantity of 100 μg of compound **1** and crude extract were separately dissolved in 1 ml of distilled water to make 100 μM solutions. To these solutions, 10 sterile filter paper discs were added. The papers were allowed to absorb the solution to saturation point and left to dry in an oven at room temperature. The papers were then aseptically transferred into a lawn of microorganisms *Salmonella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Cryptococcus neoformans, Shigella dysentiae* and *Staphylococcus aureus* inoculated in appropriate media.

Commercial antibiotic discs containing augmentin, tetracycline (Tet, 25 μg), cotrimoxazole (Cot, 25 μg), gentamycin (Gent, 10 μg) and lyncomycin (Lync, 10 μg) were included as positive controls while sterile filter papers saturated with distilled water acted as negative controls. The plates with *Cryptococcus* spp. were kept in the dark at room temperature for 48 h while the rest were incubated at 37 °C for 24 h according to the standard procedure. The zone of inhibition, expressed as inhibition diameter (mm), was measured after respective incubation periods.

Results and Discussion

From an ethyl acetate extract of dried ground stem bark of *Embelia schimperi*, an active antibacterial compound was isolated and identified as 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**), $C_7H_6O_4$, by spectroscopic analysis. The compound was isolated as orange crystals, m.p. 118 °C, recrystallized in chloroform.

Its ^{1}H NMR spectrum exhibited a triplet peak for terminal methyl protons at 0.9 ppm, which upon integration corresponded to three protons, thus suggesting a methyl group. A singlet peak at 5.05–6.5 ppm suggested a quinonoid proton, and another single peak at 7.82 ppm suggested a phenolic hydrogen atom bound to a carbonyl group. The ^{13}C NMR spectrum showed a C-3 peak at 116 ppm and C-6 peak at 102 ppm. The inability to observe four oxygen-bearing carbon atoms at

Table I. ^{1}H and ^{13}C NMR chemical shifts (δ, ppm) for 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**) and embelin (**2**).

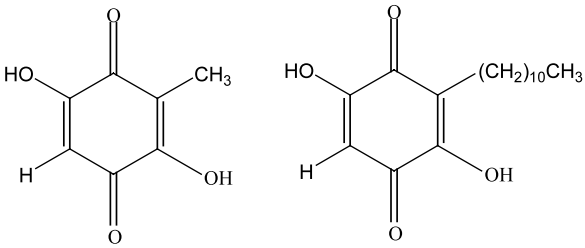
Atom	**2**		**1**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	–	–	–	–
2′	–	–	–	–
3′	117.00	–	116.00	–
4′	–	–	–	–
5′	–	–	–	–
6′	102.20	5.05	102.00	5.05–6.00
1′	31.90	2.45	1.900	0.900
2′	28.50	1.45	–	–
3–9′	29.50	1.30	–	–
10′	22.50	1.24	–	–
11′	14.10	0.88	–	–
2,5-OH	–	7.82 (D_2O exchangeable)	–	7.82 (D_2O exchangeable)

room temperature is not unique for this case and is well known for 2,5-dihydroxy-1,4-benzoquinones due to a rapid tautomeric effect that is called fluxional behaviour. Such tautomerism is also observed in embelin (**2**). A sharp singlet peak at 1.900 ppm suggested a methyl group substituted at the quinonoid ring at C-3.

The ^{13}C NMR chemical shifts are in line with those reported for embelin with peaks observed at C-3 and C-6 only. A major variation was observed in the absence of peaks associated with a long alkyl side chain typified by embelin (**2**) isolated from the Myrsinaceae family. In the case of **1**, the side chain consisted of only of a methyl group, unlike embelin, which has got a $-C_{11}H_{23}$ side chain. The ^{13}C NMR and ^{1}H NMR data are summarized in Table I.

The IR spectrum confirmed the 2,5-dihydroxylation pattern with a single C=O resonance at 1630 cm^{-1} due to equal chelation between the carbonyl groups and the hydroxy proton at C-2 and C-5. The bands at 3520 cm^{-1} showed the presence of the –OH functional group.

The mass spectrum (MS) of **1** showed an M^+, *m/z* peak at 154, a major difference to that of embelin (**2**) which appears at *m/z* 294. There were minor peaks at *m/z* 153 and *m/z* 141. Other marked differences between **1** and **2** were their melting points. Whereas embelin (**2**) has a melting point of 140–142 °C, 2,5-dihydroxy-3-methyl-1,4-benzoquinone has a melting point of 117–118 °C. The chemical structures of the two compounds are shown in Fig. 1.



m/e = 154.022 **1**

m/e = 294.183 **2**

Fig. 1. Strucures of 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**) and embelin (**2**).

All the microorganisms tested were sensitive to the pure compound **1** except for *C. neoformans* which was insensitive to both the crude extract and compound **1** as exhibited by the zones of inhibition which ranged from 10–20 mm. The extracts were most active against *P. aeruginosa* as shown by the largest zone of inhibition of 20 mm, which was comparable to that of the control antibiotic gentamycin, with an inhibition diameter of 21 mm on the same microorganism. Inactivity of embelin (**2**) against *E. coli* at concentrations ranging from 50–400 μg/ml has been reported (Kiprono, 1997). Therefore, unlike embelin (**2**), 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**) can be considered as a potential antibiotic in the management of infections caused by *Salmonella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus.* The antimicrobial activity of compound **1** supports the

Table II. Relative antimicrobial activities of *E. schimperi* crude extract, 2,5-dihydroxy-3-methyl-1,4-benzoquinone (**1**) and conventional antibiotics as controls.

Microorganism	Diameter of zones of inhibition [mm]						
	Crude Extract	**1**	Controls				
			Augmentin	Cotrimoxazole	Gentamycin	Lyncomycin	Tetracycline
P. aeruginosa	0	20	–	–	21*	–	–
S. dysentriae	0	11	15	0	23*	–	–
Proteus spp.	0	12	18*	0	–	–	–
E. coli	0	16	17	15	–	–	18*
K. pneumoniae	0	11	10	0	–	–	–
Salmonella spp.	0	10	18	17*	–	–	–
C. neoformans	0	0	0	0	–	–	–
S. aureus	0	10	18	14	–	19*	–

* Active antibiotic.
– Not done (antibiotic known to be inactive).

fact that the plant is used traditionally for the treatment of chest infections, in cleaning wounds and as a disinfectant.

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