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Antimicrobial Activity of Emblem from *Embelia shimperi* and its Synthetic Derivatives

Chepkwony Kiprono Paul[a*], Ngari Ann Gathigia[a], Chepkemoi Norah[a], Kipkemboi Pius Keronei[a], Andrew Ambogo Obala[b], Kiprop Ambrose[c]

[a]Department of Chemistry, Moi University, P.O. Box 1125, Eldoret, Kenya
[b]School of Medicine, Moi University, P.O. Box 4606, Eldoret, Kenya
[c]Department of Chemical and Process Engineering, Moi University, P.O. Box 3900, Eldoret.

[*]Author for correspondence and reprint requests

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Chromatographic separation of ethyl acetate extract from *Embelia schimperi* led to the isolation of a known compound, Embelin, 2, 5 dihydroxy-3-undecyl-1,4-benzoquinone **(1)** identified on the basis of physical and spectroscopic data. Quantities of compound **(1)** weighing 0.02 g were used to synthesize 2, 5 dihydroxy-5-sulpho-3-undecyl-1, 4-benzoquinone **(2),** 1,2,4,5-Tetra acetoxy-3-undecyl benzene **(3)** and 2, 5 dihydroxyl-1-diethyl imino-3-undecyl-1,4-benzoquinone **(4).** The derivatives were characterized on basis of physical and spectroscopic (MS and FTIR) data. Embelin and its synthetic derivates were assayed against clinical strains of *Pseudomonas aeuruginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans.* Plate titration technique was used and observation of presence or absence of growth after respective incubation periods, were made to gauge antimicrobial activity. Untreated plate acted as the control. Compound **1** and derivative **4** were active against *Staphylococcus aureus* at dilutions 100ppm and 150ppm respectively while compounds **2** and **3** were inactive. Compounds **1, 2, 3** and **4** were inactive against *Pseudomonas aureginosa, Escherichia coli* and *Candida albicans* at all dilutions.

Key words: *Embelia Schimperi,* Embelin, 2, 5 dihydroxy-5-sulpho-3-undecyl-1, 4-benzoquinone, 1,2,4,5-Tetra acetoxy-3-undecyl benzene, 2, 5 dihydroxyl-1-diethyl imino-3-undecyl-1,4-benzoquinone, *Pseudomonas aureginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans*

Introduction

In traditional medicine, plant preparations have always been used to treat infectious diseases such as malaria and skin infections with a varying degree of success. (Waiyaki, 1997). Use of *Embelia schimperi* and *Embelia ribes* Plant extracts as dewormers and wound cleaners has been reported in many parts of Africa and Asia (Ogawa and Natori, 1968). Kiprono, 1997, reported biological activity of embelin from *Embelia schimperi* against a number of microorganisms. Recent studies by Gabriela *et al.,* 2003 indicated antifungal and antibacterial activities in embelin from *Oxalis erythrorhiza.* Research on

bioactive plants with ethno pharmacological uses, has attracted a lot of interest all over the world. Today in USA and Canada, 25% of prescriptions drugs are plant based compounds. Many of these drugs were discovered following leads provided by indigenous knowledge systems (Ciba foundation, 1994). The current study was to isolate embelin and use it to synthesize derivatives with enhanced antimicrobial activity.

Materials and methods

Collection of plant parts

Embelia schimperi berries were 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 in the Department of Botany, Moi University where a voucher specimen was deposited. The collected samples were air dried for five days.

Extraction and isolation of compounds

The dried berries were ground into fine powder using an electric grinder. A half (1/2) kg of the material was soaked in ethyl acetate at room temperature for 48 h. The mixture was filtered and the solvent evaporated under reduced pressure using a rotary evaporator to afford 25 g of dark brown solid. A quantity of 10 g of the crude extract was subjected to column chromatography using a column packed under *n*-hexane with dry de-activated silica gel. 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 (1:10 v/v) led to isolation of bright orange crystalline embelin (**1).**

Embelin **(1)** was characterized on the basis of physical and spectroscopic data (MS and FTIR) and identified as 2, 5 dihydroxy-3-undecyl 1, 4 benzoquinone

Synthesis of embelin derivatives

Quantities of embelin weighing 0.02 g were used to synthesize three derivates in the following reactions:

Sulphonation reaction

Embelin was dissolved in ethyl acetate and treated with concentrated H_2SO_4 acid in a drop-wise manner until the colour of the solution changed from orange to dark brown. The solution was warmed in a water bath for five minutes at 50^oC and left to cool.

Acetylation reaction

Embelin was suspended in 5 ml of acetic anhydride. Pyridine was added drop wise until embelin dissolved to form a pale yellow solution. The solution was covered and left to stand overnight after which excess solvents were removed.

Alkylamination reaction

Embelin was first dissolved in chloroform and treated with 2 ml of triethylamine/ethanol. The mixture was warmed in a water bath for 1 minute until a purple solution was formed. The solution was left to stand for five days after which excess solvents were removed.

The sulphonation, acetylation and alkylamination were purified by preparative TLC and recrystallization. Suphonation reaction afforded 0.017g of greenish brown crystals of compound **2** (67% yield). Acetylation reaction afforded 0.0047g of orange crystals of compound **3** (15% yield).

Alkylamination reaction afforded 0.0085g of purple crystals of compound **4,** (37% yield). The derivatives were characterized on the basis of physical and spectroscopic data (MS and FTIR).

Antimicrobial assay

A range of three dilutions (100ppm, 150ppm and 200ppm) of the test compounds **1, 2, 3** and **4** were prepared. 1 ml of test compounds was transferred to the sterile Petri dishes which had been labeled starting with the weakest dilution.

Using a 25ml pipette, 19ml of MH diagnostic media was transferred to each plate with test compound and mixed well. An electric rotator facilitated the mixing. The pates were dried in the incubator with their lids tilted for 30 minutes after the media had set.

The dry plates were stored at 4^{o}C for 1week after which ditches were cut on the media to give four portions.

Each portion on the plate was inoculated with one test microorganism giving four microorganisms on one plate. The inoculation cultures were 24 hold. Observations were made after overnight incubation for bacteria and 48 hours for fungi

Results and discussion

The physical characteristics of embelin and its synthetic derivatives such as colour, melting point and solubility are summarized in table 1 below:

The spectroscopic data on MS and FTIR helped to elucidate the structures of embelinderivatives. The entire test compounds 1, 2, 3 and were inactive against *E.coli*. *Pseudomonas auruginosa.*, and *Candida albicans.* at all the dilutions tested. However, compounds 1 and 4 were active against staphylococcus a. at 100ppm and 150mmp respectively. The inactivity of Embelin against *E.coli* had earlier been reported by Kiprono 1997.

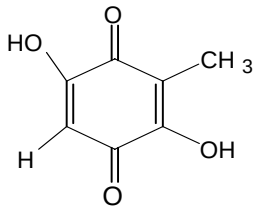
The loss of antimicrobial activity in embelin derivatives can be attributed to increase in molecular weight in compound 2 and 3.

Steric effects could have led to decrease in activity in compound 4 due to branched alkyl groups on nitrogen.

The activity of 1 against *Staphylococcus aureus* supports the fact that the plant is used traditionally to clean wounds. The mild activity of compound 4 against *Staphylococcus*

aureus indicates that embelin derivates with less bulky functional groups and a shorter alkyl side chain could have more antimicrobial activity. This is supported by earlier studies by

Ooko *et al.*, 2008, where a new bioactive compound (5) from *Embelia schimperi* showed high antimicrobial activity against a number of microorganisms.
The compound 5 has a methyl group at C-3 instead of the long undecyl chain in embelin.



(5)

Table 3: Antimicrobial activities of Embelin and its synthetic derivatives

Microorganisms	**Conc.**	**Compounds**				**Control**
		1	2	3	4	
E. coli	100ppm 150ppm 200ppm	- - -	- - -	- - -	- - -	- - -
Pseudomonas a.	100ppm 150ppm 200ppm	- - -	- - -	- - -	- - -	- - -
Staphylococus a.	100ppm 150ppm 200ppm	+ + +	- + +	- - -	- - -	- - -
Candida a.	100ppm 150ppm 200ppm	- - -	- - -	- - -	- - -	- - -

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